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Catecholaminergic Systems in Stress: Structural and Molecular Genetic Approaches

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Kvetnansky R, Sabban EL, Palkovits M. Catecholaminergic Systems in Stress: Structural and Molecular Genetic Approaches. *Physiol Rev* 89: 535–606, 2009; doi:10.1152/physrev.00042.2006.—Stressful stimuli evoke complex endocrine, autonomic, and behavioral responses that are extremely variable and specific depending on the type and nature of the stressors. We first provide a short overview of physiology, biochemistry, and molecular genetics of sympatho-adrenomedullary, sympatho-neural, and brain catecholaminergic systems. Important processes of catecholamine biosynthesis, storage, release, secretion, uptake, reuptake, degradation, and transporters in acutely or chronically stressed organisms are described. We emphasize the structural variability of catecholamine systems and the molecular genetics of enzymes involved in biosynthesis and degradation of catecholamines and transporters. Characterization of enzyme gene promoters, transcriptional and posttranscriptional mechanisms, transcription factors, gene expression and protein translation, as well as different phases of stress-activated transcription and quantitative determination of mRNA levels in stressed organisms are discussed. Data from catecholamine enzyme gene knockout mice are shown. Interaction of catecholaminergic systems with other neurotransmitter and hormonal systems are discussed. We describe the effects of homotypic and heterotypic stressors, adaptation and maladaptation of the organism, and the specificity of stressors (physical, emotional, metabolic, etc.) on activation of catecholaminergic systems at all levels from plasma catecholamines to gene expression of catecholamine enzymes. We also discuss cross-adaptation and the effect of novel heterotypic stressors on organisms adapted to long-term monotypic stressors. The extra-adrenal nonneuronal adrenergic system is described. Stress-related central neuronal regulatory circuits and central organization of responses to various stressors are presented with selected examples

of regulatory molecular mechanisms. Data summarized here indicate that catecholaminergic systems are activated in different ways following exposure to distinct stressful stimuli.

I. INTRODUCTION

A. Catecholamines: Historical View

Catecholamines (CA), the neurotransmitters and hormones of the adrenomedullary, sympatho-neuronal, and brain catecholaminergic systems, were discovered already at the beginning of 20th century. Chemical structures of the main physiologically relevant CA are shown on Figure 1.

The concept of a sympathetic-like system is very old and was already mentioned in the second century by the Greek physician Galen. In 1852, Bernard demonstrated regulation of vascular tone by the sympathetic nervous system (31, 32). In 1895, Oliver and Schafer (463, 464) published the first report about the cardiovascular effects of adrenal extracts. Two years later, Abel and Crawford (1) identified adrenaline (ADR) as the endogenously active substance from the adrenal gland with pronounced effects on the cardiovascular system. Soon thereafter, Takamine (637) isolated ADR in crystalline form and reported its chemical structure. Thus ADR was the first catecholamine and also the first hormone to be identified.

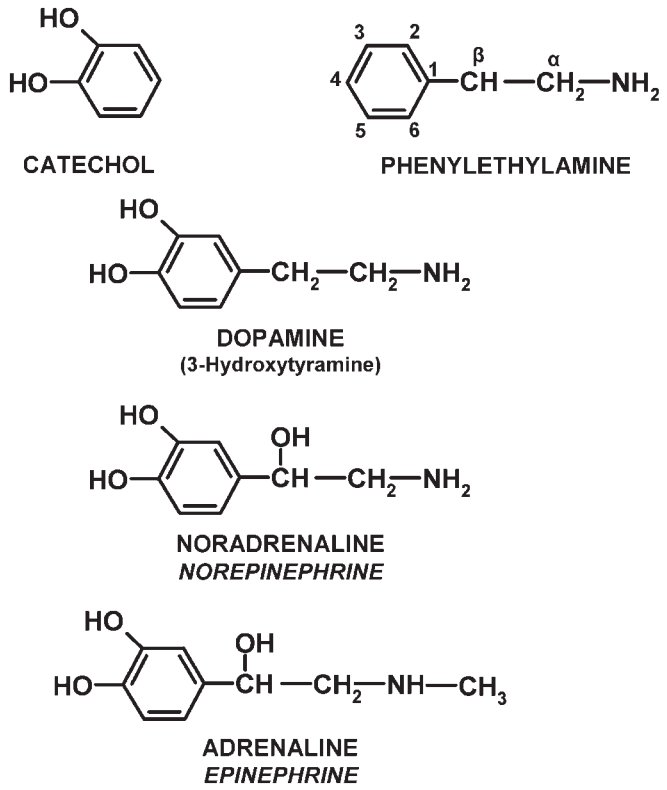

FIG. 1. Structures of main catecholamines occurring in mammals. Phenylethylamine is the parent compound. Numbers indicate the carbons in the ring; α and β letters identify the carbons in the side chain. European literature uses the names adrenaline and noradrenaline, while in American literature, epinephrine and norepinephrine are used.

Another catecholamine, noradrenaline (NA), was discovered in the middle of the 20th century (1946) by Ulf von Euler (675) as the neurotransmitter in sympathetic nerves and as the precursor of adrenaline.

The first catecholaminergic system (ADR, NA) in the brain was discovered by Vogt (673). In 1958, Arvid Carlsson identified dopamine (DA) as an additional catecholamine in the brain (75, 76).

This review is devoted to physiological, biochemical, and molecular genetic changes of peripheral and brain catecholaminergic systems under stress.

B. Stress Theory: Current Status

Comprehending the physiology and pathophysiology of the autonomic nervous system depends on understanding the roles of the sympatho-neural and sympatho-adrenomedullary systems in stress and distress. This in turn depends on defining stress and distress in scientific terms. Changes in activity of catecholaminergic systems after influence of various stimuli from outside or inside the organism (later called stressors) had already been studied 100 years ago. In spite of that, the definition of stress is still not satisfactorily stated. Thousands of papers have been dedicated to the topic of CA changes during stress, and a great deal of important knowledge has been obtained (97, 332, 399, 470, 703). However, we still do not know exactly what stress is nor what are the detailed mechanisms of activation of catecholaminergic systems under various specific stressors. In addition, it has recently been shown that single, chronic, or repeated stress exposures are all able to affect gene expression of some hormones and neurotransmitters activated by stress. The molecular genetic aspects of stress responses of catecholaminergic systems are the main topics of this review. At the outset we would like to say a few words about the development of the stress theory.

The physiologist Walter Cannon conducted a series of seminal experiments on the importance of adrenal medullary secretion of ADR during various types of stimulation in animals and humans. Based on these studies, he introduced the terms *homeostasis* and *fight-or-flight* to the scientific literature, and these terms remain in use today (68–70). However, he never used the term *stress*. Cannon thought that the adrenal medulla and sympathetic nervous system functioned as a unit and proposed that ADR is not only the active principle of the adrenal gland

but also is a neurotransmitter of the sympathetic nervous system (71). In 1946, however, von Euler (675) correctly identified NA as the sympathetic neurotransmitter. The notion of a unitary sympatho-adrenal system, however, continues in medical thinking, despite persuasive evidence for separate pathways and differential changes in sympathetic nervous and adrenomedullary activities with various stressors (201, 341, 472, 474).

The Canadian scientist Hans Selye, born in the city of Komarno, Slovakia, introduced the term *stress* and highly popularized the stress theory as a medical and scientific idea (571–573). According to his theory, "Stress is the nonspecific response of the body to any demand upon it." "Distress" was defined as stress that is unpleasant or harmful to the body (572). Excessive, repeated, or inappropriate stress responses were viewed as maladaptive, and Selye described it with the phrase "diseases of adaptation." In 1936, Selye described a pathological triad: adrenal enlargement, gastrointestinal ulceration, and thymicolymphatic involution, which should be elicited by any stressor (573). In contrast to Cannon, who concentrated on the adrenal medulla, Selye focused mainly on the pituitary-adrenocortical axis as the key effector of the stress response. He considered the adrenal cortex to be the organ of integration during stress (571). Selye also introduced the term *general adaptation syndrome* with its three successive phases: the alarm reaction, stage of resistance, and exhaustion stage. In our long-term stress experiments, we have never seen the exhaustion stage.

Mason (395) criticized Selye's doctrine of nonspecificity. Anxiety and fear were understood as the main factors contributing to nonspecific responses upon exposure to various stressors. Chrousos and Gold (96) modified the doctrine of nonspecificity by proposing that above a threshold intensity, any stressor would elicit the "stress syndrome." They defined stress as a state of disharmony or of threatened homeostasis that evokes both specific and nonspecific responses. They included genetic factors, which interact with the environment and previous experience, as important determinants of individual stress responses (96).

More than a half century elapsed before Selye's doctrine of nonspecificity underwent experimental testing by our National Institutes of Health group, which failed to confirm it (479). We published data that were inconsistent with Selye's stress theory and refuted the existence of a unitary "stress syndrome." By now, researchers have largely abandoned both Cannon's and Selye's notions of stereotyped, nonspecific neuroendocrine responses regardless of the type of stressor. More modern theories view stress as a sensed threat to homeostasis (202, 404), where the response has a degree of specificity, depending among other things on the particular challenge to homeostasis, the organism's perception of the stressor, and its ability to cope with it (197, 201).

Goldstein recently introduced a new definition of stress (196–198, 201). Central to his stress theory is that the body possesses numerous homeostatic comparators, which have been called "homeostats." Different homeostats can regulate the activity of the same effector system. For instance, the osmostat and volustat share the vasopressin effector (525). The definition of stress formulated by Goldstein is as follows: "Stress is a condition in which expectations, whether genetically programmed, established by prior learning, or deduced from circumstances, do not match the current or anticipated perceptions of the internal or external environment, and this discrepancy between what is observed or sensed and what is expected or programmed elicits patterned, compensatory responses". According to this new view, "distress is a form of stress with additional defining features–consciousness, aversiveness, observable signs, and pituitary-adrenocortical and adrenomedullary activation." Distress could worsen some pathophysiologic processes. For instance, because of adrenomedullary activation in patients with coronary artery stenosis, distress could elicit cardiovascular stimulation and produce an excess of myocardial oxygen consumption, precipitating myocardial infarction or lethal ventricular arrhythmias. Moreover, long-term distress could augment both the risk of a mood disorder and the risk of worsening coronary disease.

Recently, the term *allostasis* has been introduced into stress research (402, 403, 610). Allostasis is the process of adaptation of the body upon the exposure to various stressors. When mediators of allostasis, like cortisol or ADR, are released in response to stressors or to life-style factors, they promote adaptation and are generally beneficial. However, when these stress mediators are not turned on adequately during stress, when they are not turned off when the stress is over, or when they are overused by many stressors, there are cumulative changes that lead to "allostatic load or overload" (401, 402). Thus long-term physical or mental consequences of stress would depend on long-term effects of allostatic load. Maintenance of allostatic states requires energy.

Stressor-specific responses of CA systems have been described (341, 474, 479). By the term *stressor*, we mean a stimulus that disrupts homeostasis. In general, stressors can be divided into four main categories (474): *1*) physical stressors, e.g., cold, heat, radiation, noise, vibration, chemical stressors, pain, and immobilization (IMO); *2*) psychological stressors that affect emotional processes and may result in behavioral changes such as anxiety, fear, frustration, in animals handling, restraint, etc.; *3*) social stressors, reflecting disturbed interactions among individuals, e.g., unemployment, marital separation, death of partner, in animals dominancy, etc.; and *4*) stressors that challenge cardiovascular and metabolic homeostasis, e.g., exercise, orthostasis, upright tilt, hypoglycemia, hemorrhage, etc. In terms of duration, stressors may be either *1*) acute stressors (single, intermittent, time-limited expo-

sure) or *2*) chronic or repeated stressors (continuous long-term prolonged exposure, intermittent long-term exposure).

The ability to determine stressor-specific responses of CA systems became available after development of a sensitive radioenzymatic assay for separate measurement of low basal plasma NA and ADR levels and subsequent differing changes after exposure to various stressors. A new view of the concept of stressor specificity began to emerge, in which NA levels, and thereby overall sympathetic nervous activity, would play a key role, such as during exercise, orthostasis, cold exposure, blood loss, locomotion, altered salt intake, water immersion, etc. Adrenaline levels, and thereby specific sympatho-adrenomedullary hormonal system activation, would respond to global or metabolic threats, such as hypoglycemia, hemorrhagic hypotension, exercise beyond an anaerobic threshold, asphyxiation, emotional distress, shock, etc. There are also different patterns of sympatho-neural, sympatho-adrenomedullary, and hypothalamo-pituitary-adrenocortical responses to different stressors (198, 201, 474). An important feature of successful coping with stress is that physiological systems are not only turned on efficiently by a particular stressor but are also turned off again after cessation of the stressor to conserve resources.

Recent data fully support the view of the high specificity in responses of the organism to various stressors not only at the level of plasma CA (341, 474, 479) but also at the level of gene expression and transcription factors of enzymes involved in CA biosynthesis (317, 328, 338, 370, 549, 555, 556, 696). With the use of more detailed functional immunocytochemistry, pathways of specific stressors are being elucidated.

There are, however, many critical questions that still have to be answered. For example, what are the molecular mechanisms for transition from short-term beneficial to long-term adaptive or maladaptive response of the organism? How does adaptation to one type of stressor alter the response to a subsequent stressor of the same (homotypic) or different (heterotypic, novel) type of stressor? The mechanisms of these phenomena have to be clarified.

II. PHYSIOLOGY, BIOCHEMISTRY, AND MOLECULAR BIOLOGY OF CATECHOLAMINES

Catecholamines influence virtually all tissues and many functions. Together with other neuronal and hormonal systems they significantly participate in regulation of a multitude of physiological processes. Catecholamines are highly involved in regulation of secretion of many peptide and steroid hormones and may also alter hormone synthesis through effects on gene transcription. For details on CA function, see review articles (197, 199, 473, 705).

It is important to understand correctly the function of CA systems under both basal and stressful conditions. In contrast to Cannon's original concept of a unitary sympatho-adrenal system (68, 69), today it is clear that there are several catecholaminergic systems that can be differently regulated by various stressors. According to the new concept, there are at least three distinct peripheral CA systems, each with different effectors, regulation, and roles (197, 198). The peripheral catecholaminergic systems are sympatho-adrenomedullary system, sympatho-neural system, and DOPA-dopamine autocrine/paracrine system. Brain catecholaminergic systems are divided into noradrenergic system, adrenergic system, dopaminergic system, and L-DOPA neurons.

Many original views especially on CA release, transport, and metabolism have recently been revised. Therefore, in this review, we first describe the new aspects of the basic physiological and biochemical processes in the CA systems under normal and stressful conditions. We then describe and discuss the stress-induced molecular genetic changes of these systems.

A. Catecholamine Biosynthesis

The catecholamine biosynthetic pathway is summarized in Figure 2. Catecholamines are synthesized from the amino acid precursor L-tyrosine. There are two primary sources of tyrosine, from the diet and from hydroxylation of the amino acid phenylalanine in the liver.

Upon entry into an adrenal chromaffin cell, sympathetic or brain catecholaminergic nerve terminals, tyrosine is converted to dihydroxyphenylalanine (DOPA) by the soluble cytoplasmic enzyme tyrosine hydroxylase (TH; EC 1.14.16.2). TH is an iron-containing, biopterin-dependent amino acid hydroxylase. It utilizes tyrosine, tetrahydrobiopterin (BH4), and molecular oxygen to generate DOPA, dihydrobiopterin, and water. The cofactor BH4 is resynthesized from dihydropterin by the enzyme dihydropteridine reductase. Since BH4 is present in subsaturating levels, TH activity depends on its availability (reviewed in Ref. 444). For the de novo biosynthesis of BH4, the enzyme GTP-cyclohydrolase I catalyzes the rate-limiting step (reviewed in Refs. 444, 456, 651).

Under most conditions, TH is intricately regulated in the short and long term. There are a number of excellent reviews on TH activity (172–174, 281, 282, 312, 443, 550, 553). In the short term, TH enzymatic activity is regulated by feedback inhibition; thus TH is inhibited by catechols (DOPA, NA, DA). TH is also regulated by allosteric regulation and phosphorylation. TH can be phosphorylated by a variety of kinases at several serines (positions 8, 19, 31, and 40) in the NH_2-terminal domain. The phosphorylation

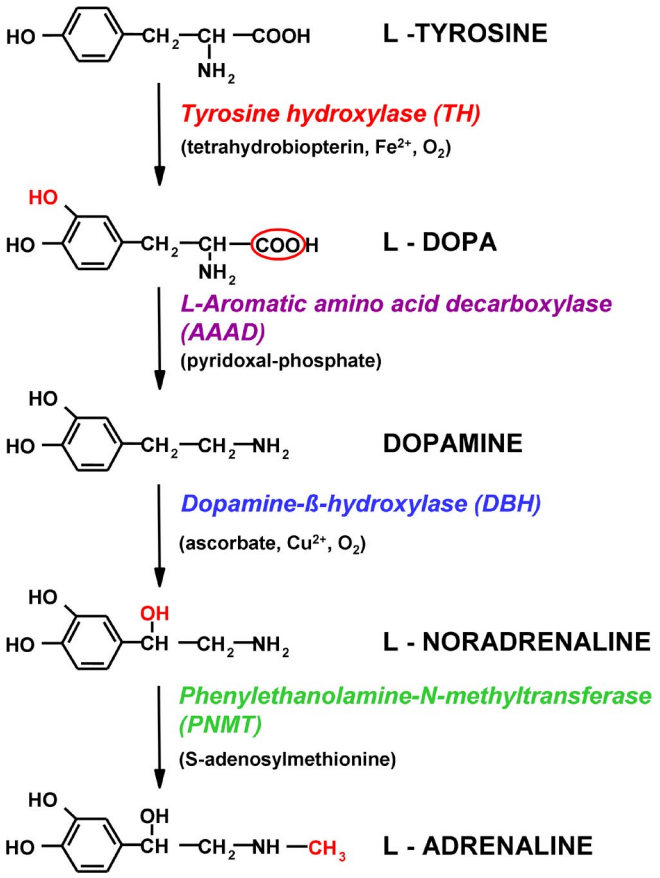

FIG. 2. Pathway for catecholamine biosynthesis and its enzymatic steps. The steps of conversion from L-tyrosine to L-noradrenaline are typical for sympathetic and some brain neurons, and the conversion of L-noradrenaline to L-adrenaline is typical for the adrenal medullary cells and some peripheral and brain neurons.

has been a topic of extensive research (reviewed in Ref. 137). In the medium to long term, TH can be regulated by enzyme stability, transcriptional regulation, RNA stability, alternative RNA splicing, and translational activity. Change in TH gene expression is a major mechanism whereby the catecholaminergic system responds to stress and is discussed in considerable detail in later sections of this review.

In humans, several isoforms of TH can arise from alternative splicing of a single primary transcript (441, 445) (see sect. II*E*). Primates exhibit only two of these isoforms, and lower animals have only one (253). The significance of these isoforms, the physiology of the alternative splicing, and whether it is affected by stress is not clear.

DOPA is converted into DA by a nonspecific enzyme, aromatic L-amino acid decarboxylase (AAAD; EC 4.1.1.28). The activity of AAAD depends on levels of its cofactor, pyridoxal phosphate.

Dopamine is taken up from the cytoplasm into storage vesicles and converted into NA by dopamine-β-hydroxylase (DBH; EC 1.14.17.1), an enzyme found in soluble and membrane-bound forms within storage vesicles. Both forms are encoded by the same mRNA (168, 363). DBH activity utilizes copper, ascorbic acid, and molecular oxygen. A portion of DBH is released by exocytosis with NA and ADR, and DBH is present in plasma and cerebrospinal fluid (CSF) (683, 684).

NA is then converted into ADR by the soluble cytoplasmic enzyme phenylethanolamine *N*-methyltransferase (PNMT; EC 2.1.1.28) that uses *S*-adenosyl-methionine as the cofactor. PNMT is inducible by glucocorticoids. PNMT is mainly localized in the adrenal medulla; however, sympathetically innervated organs and some brain areas are also able to synthesize small amounts of ADR. Separate populations of adrenal chromaffin cells contain NA and ADR as the final products of CA biosynthesis (687). PNMT gene expression was found also in some nonneuronal cells in the heart, skin, etc. (247, 248, 288, 333, 522, 713).

The biosynthesis of CA is greatly increased under stress (350). Activities of CA biosynthetic enzymes other than AAAD were found to be highly elevated under long-term stress situations (324, 326, 351, 649). Detailed mechanisms of increases in CA biosynthesis, gene expression, activity, and protein levels of CA biosynthetic enzymes in the adrenal medulla as well as sympathetic and brain catecholaminergic neurons in stress are discussed in detail in later sections of this review.

B. Release of Catecholamines

Currently, more than 30 biologically active substances have been localized in adrenal chromaffin, sympathetic neuronal, and brain catecholaminergic cells, and a number of them are released following depolarization of the cell membrane. In addition, various biologically active neuropeptides are colocalized with acetylcholine within sympathetic preganglionic nerve terminals [e.g., substance P, neuropeptide Y (NPY), vasoactive intestinal polypeptide (VIP), enkephalins, PACAP, and others], and they appear to act as neuromodulators or cotransmitters of cholinergic transmission (124, 705).

The process of CA release is similar in the adrenal medulla and the sympathetic nerve endings. Acetylcholine released from sympathetic preganglionic nerve terminals binds to nicotinic cholinergic receptors and leads to a depolarization of cell membrane, resulting in an increase in membrane permeability to sodium. This initiates a series of events that lead to an increase in the influx of calcium. Then, CA storage vesicles fuse with the chromaffin or sympathetic neuronal cell membrane and, via exocytosis release their contents of CA, together with chromogranins, other neuropeptides, ATP and a fraction of the soluble DBH, via exocytosis (see reviews in Refs. 197, 268,

446, 612, 705). The exact mechanism of Ca^{2+}-evoked exocytosis is, however, not clear.

Several proteins play an important role in the process of exocytosis, e.g., synapsin, synexin, synaptophysin, syntaxin, etc. The membrane protein synexin has been suggested to be a transducer that mediates fusion of the vesicle with the cell membrane (512). Some of these proteins show inhibitory effects on CA secretion, e.g., catestatin (290). Adrenomedullary cells secrete CA directly into the bloodstream.

NA and ATP are stored in both small synaptic and large dense-core vesicles, but neuropeptides are stored only in the large ones. Release of NPY, therefore, does not parallel that of NA and ATP, as exocytosis from small and large vesicles is regulated differently (95, 382, 717). Neuropeptides (such as VIP, PACAP) play a role in stimulation of CA release (VIP), in modulation of responses to acetylcholine (enkephalins, substance P), and they also exert systemic effects. NPY may be the sympathetic trophic factor (718) and recently has been described as the factor responsible for stress-induced obesity (313, 314). After exocytosis, the vesicle membrane is retrieved from the plasma membrane and recycled into newly formed vesicles.

Sympathetic nerve endings can release NA also by calcium-independent, nonexocytotic mechanisms, e.g., by reverse transport through the neuronal uptake carrier (315, 569, 612). However, this process occurs in vivo mainly in extraordinary circumstances such as during ischemia (315, 569). Catecholamines at varicosities on sympathetic fibers could diffuse to surrounding tissue by volume transmission (631).

Following exocytosis, CA that escape reuptake and local metabolism diffuse into circulation and constitute the circulating pool of CA. Plasma CA turn over very rapidly. The half-time of disappearance is ~2.5 min (162). Under resting conditions, low levels of CA are released into the blood from the adrenal medulla and sympathetic nerve terminals. During stressful stimulation, however, a huge amount of ADR (~95%) and a significant amount of NA (which may comprise up to 30% of the total circulating NA) may be released from the adrenal medulla. The remaining 70% of NA is released from sympathetic nerve terminals and enters blood capillaries from the site of release at the neuroeffector junction.

Thousands of papers have described changes of plasma CA levels under different specific stress conditions (132, 133, 327, 339, 341, 347, 352, 474, 479). Our stress model of IMO triggers discharge of ADR from the adrenal medulla and NA mainly from the sympatho-neural system. Plasma ADR levels reach peak values that are ~40-fold greater than in control undisturbed rats at ~20 min IMO and then decline to about one-third the peak levels (347). Plasma levels of NA are increased about sixfold throughout the IMO (347). Because decapitation of animals produced by itself an 80-fold increase in plasma ADR and an 8-fold increase in plasma NA levels, these values were obtained from blood collected via permanently inserted arterial catheter. In decapitated rats, IMO shows only threefold increase in plasma CA (347). Even minor disturbances like handling or transfer of animals produce highly significant increases in plasma ADR and NA levels (347). Many stressors, including IMO or gentle handling, rapidly increase not only plasma CA levels, but also the CA precursor DOPA and the CA metabolites (DHPG, DOPAC, MHPG, etc). Plasma DA levels and the DA metabolite homovanilic acid (HVA), however, remain unchanged (320, 327). This indicates not only a rapid stress-induced increase in plasma CA levels but also rapid increases in CA synthesis, release, and metabolism.

Adrenal medullectomy completely prevents the stress-induced increases in plasma ADR but reduces plasma NA levels only by about 30%. Combined adrenal medullectomy and sympathectomy almost completely abolishes plasma NA. Therefore, during stress, the increment in plasma ADR is derived almost completely from the adrenal medulla, whereas most plasma NA (about 70%) is derived from sympathetic nerves (352).

There is a very close interaction of the sympatho-adrenal (SAS) and hypothalamo-pituitary-adrenocortical (HPA) systems (323, 340). Biosynthesis of CA and activation of CA biosynthetic enzymes (including their gene expression) is regulated by neuronal and humoral mechanisms (318, 344, 551). An intact HPA axis is essential for the activity of PNMT, the enzyme responsible for ADR synthesis (318, 330, 344, 551, 689, 691, 698).

Regulation of ADR and NA release is highly stressor-specific. Adrenal medullary ADR release is mainly induced by hypoglycemia, glucopenia, IMO, emotional stressors, etc. (341, 479). Conversely, cold or pain (formalin) exposure does not activate ADR release but highly stimulates NA release from the sympathetic terminals (341, 479).

When IMO stress is applied daily for several weeks, the baseline levels of plasma CA are significantly elevated; however, the stress-induced increment is reduced compared with animals after the first stress exposure (339). Reduced plasma CA response to subsequent exposure to the same stressor is seen in a wide variety of stressors (339, 614). Even repeated handling (for 14 days) led to reduction of plasma ADR as well as ACTH levels, while NA levels increase to the same extent as after the first handling procedure (128). When handling-adapted animals are handled by a different person, ADR levels are again elevated. This dissociation of plasma ADR and NA responses is another example of differing control of sympatho-adrenomedullary and sympatho-neural systems by specific stressors.

Rats exposed to repeated IMO have significantly increased plasma DBH activities (339, 684). The physiolog-

ical meaning of this finding is not clear. An exaggerated response of plasma CA has been found in rats adapted to a homotypic stressor after the exposure to a heterotypic novel stressor. This is a new, important phenomenon that must be investigated in future studies. Novel stressors are discussed in section IV*A3*.

C. Inactivation and Uptake of Catecholamines

The biological effects of CA released into the synaptic cleft are terminated very rapidly by uptake back into the sympathetic nerve endings and/or to effector cells, or by conversion of CA to inactive metabolites. The mechanism of inactivation of catecholamines by both neuronal reuptake (21) and by enzymatic degradation (18) was discovered by Julius Axelrod.

Sympathetic nerve endings take up CA from the extracellular fluid by a process distinct from the intraneuronal uptake of CA by the storage granules. Neuronal uptake is known as "uptake 1" (see Ref. 144) and the uptake by nonneuronal tissues as "uptake 2" (263).

Uptake 1 (U1) serves at least to recapture locally released NA or circulating NA to save it by intraneuronal storage for reuse. Uptake 1 is energy requiring and carrier mediated. The carrier can transport CA against large concentration gradients. This uptake plays a less important role in the inactivation of circulating ADR. Uptake 1 increases in parallel with increase NA release during exposure to stressors (145, 147). About 90% of released NA is reuptaken to neurons (145, 147, 148, 155).

The neuronal uptake is mediated by noradrenaline transporter (NET) and dopamine transporter (DAT) proteins. Transport by NET and DAT is a Na^+- and temperature-dependent process that displays high affinity, but relatively low capacity for CA. Since U1 functions as a first-order kinetic process, the rate of NA reuptake increases in parallel with increases in NA release (145, 147). Among CA transporter substrates there are differences: NA is translocated by NET about twofold more effectively than ADR. This explains why sympathetic nerves take up NA more efficiently than ADR. Dopamine is a much better substrate for DAT than NA or ADR (144). Sympathetic nerves do not take up *O*-methylated CA metabolites, such as normetanephrine and methoxyhydroxyphenylglycol (MHPG) (146).

Molecular identification of the structure of NET and DAT followed their cloning in the early 1990s from several species including rats. These two transporters show close homology and share several structural features: 12 putative transmembrane domains, several similarly configured intracellular and extracellular loops or regions with respective phosphorylation and glycosylation sites (see review in Refs. 49, 144). The ADR transporter that was cloned from sympathetic ganglia of the frog (12) shows close homology to NET and DAT and translocates ADR more efficiently than NA and DA. However, whether an ADR transporter is expressed also in mammals has not been established.

In brain, TH and DAT are considered characteristic markers for dopaminergic neurons. While DAT is expressed mainly in brain dopaminergic cells, neither NET nor DAT expression is restricted to central or peripheral noradrenergic neurons (144). Uptake of NA by NET also takes place in some extraneuronal cell types that express the same NET that is expressed in noradrenergic neurons. Extraneuronal sites of NET expression are in the chromafin cells of adrenal medulla, in lung, placenta, etc. Extraneuronal DAT expression was found in the gastrointestinal tract, pancreas, kidney, etc. (see review in Ref. 144). Information on brain CA transporters in stress situations is presented in section III*E*.

Extraneuronal uptake 2 (U2) is an active process of transport into nonneuronal cells. The extraneuronal monoamine transporter of U2 (EMT), initially described by Iversen (263), has little if any stereospecificity and has low affinity (higher K_m) and specificity for CA. U2 favors ADR over NA, shows a higher maximum rate of CA uptake (higher V_{max}), and is not a Na^+- and Cl^--dependent process (see Refs. 144, 197). U2 is sensitive to inhibition by CA *O*-methylated metabolites normetanephrine, metanephrine, and by corticosteroid (50). U2 is responsible for formation of CA metabolites in liver, kidney, and lung and is highly sensitive to inhibition by glucocorticoids (657).

It now appears that there are at least three nonneuronal CA transporters functioning at extraneuronal locations. The classic transporter is corticosterone-sensitive EMT, the U2 transporter, recently also referred as OCT3 "organic cation transporter." Another two extraneuronal organic cation transporters, which also transport CA, were identified as OCT1 and OCT2. Among the CA, these transporters have the highest substrate specificity for DA. Similar to neuronal CA transporters, EMT has 12 putative transmembrane domains but has a different primary structure and represents a different family of transporters (see review in Ref. 144).

Vesicular monoamine transporters (VMAT): varicosities in peripheral sympathetic neurons or in catecholaminergic neurons in the brain contain cytoplasmic vesicles. These vesicles actively store synthesized or recaptured cytoplasmic CA by specific carrier proteins called VMATs (276). Cloning studies revealed the existence of two isoforms of this transporter: VMAT-1 (the "neuroendocrine" isoform) and VMAT-2 (the "neuronal" isoform) (143, 570). Neurons, whether at the periphery or in the brain, express only VMAT-2. In contrast, adrenal medullary chromaffin cells express both isoforms with VMAT-1 major in rodents and VMAT-2 major in humans. Paracrine SIF cells of sympathetic ganglia express predominantly the VMAT-1 isoform (143). All catecholamines (DA, NA, and ADR) are, in

general, better substrates for VMAT-2 than for VMAT-1 (197).

VMAT gene has 12 predicted transmembrane domains. The localization of the COOH-terminal tail to the cytoplasm is the only established structural feature. VMAT is member of the TEXAN family of transporters (see Ref. 143).

The energy for vesicular uptake by VMAT is provided by the proton gradient established by the H^+ transporter. This is in contrast to transport of neurotransmitters across the plasma membrane (NET), which is sodium dependent.

Fast inactivation of CA released into the synaptic cleft is a prerequisite for fine control over the effector system. Contrary to the usual depictions, vesicular stores of CA do not exist in a static state simply waiting for exocytotic release. Rather, they exist in a highly dynamic equilibrium with the surrounding cytoplasm, with passive outward leakage of CA, counterbalanced by inward active transport under the control of VMAT (152).

Monoamine transporters play an important role in metabolic and physiological functions of CA removed by neuronal or extraneuronal uptake, which are then transported into storage vesicles or metabolized by monoamine oxidase (MAO) in the cytoplasm of neurons or by catechol-*O*-metyltransferase (COMT) in nonneuronal cells.

In spite of the fact that this process is highly activated by stress, the studies on stress-induced changes in activity and gene expression of CA transporters located in the peripheral neurons are quite rare. Very few reports deal with NET transporter protein and gene expression during oxidative stress in PC12 cells (393, 394). These results support a functional role of oxidative stress in mediating the neuronal NA uptake associated with reductions in NA uptake binding sites and NET protein production, without changes in NET gene expression. The effect of oxidative stress on NET is a posttranscriptional event (394).

Cardiac NA uptake is reduced in cardiomyopathy and is associated with a decrease in NET receptor. This process can be reproduced in PC12 cells by high extracellular NA. Administration of NA decreases glycosylated NET in both membrane and cytosolic fractions and increases cytosolic unglycosylated NET protein. Antioxidants prevent the downregulation of NET proteins. Accordingly, the downregulation of membrane NET by NA is mediated by decreased *N*-glycosylation of NET proteins secondary to induction of endoplasmic reticulum stress pathways by NA-derived oxidative metabolites (393).

Eisenhofer et al. (152) have shown increased rates of NA release and reuptake versus the unchanged rate of NA leakage from storage vesicles under conditions of exercise. These data indirectly show increased activation of NET and VMAT during stress.

Thus, surprisingly, peripheral CA transporters have not been extensively studied under stress conditions. Stress-induced changes in transporter levels and their gene expression in the brain are discussed in section III*E*.

D. Degradation and Vesicular Leakage of Catecholamines

Catecholamines are subjected to chemical degradation by 3-*O*-methylation (COMT; EC 2.1.1.6), oxidative deamination (MAO; EC 1.4.3.4), and by conjugation as sulfate and glucuronide. Neuronally released CA are inactivated by U1 in combination with enzymatic degradation by MAO, a mitochondrial flavoprotein located in the outer membrane of presynaptic neurons. Circulating CA are inactivated by U2 and enzymatic degradation by COMT, located in effector cells (Figs. 3 and 4).

After neuronal reuptake, cytoplasmic NA can undergo metabolism catalyzed by MAO to form dihydroxyphenylglycol (DHPG) or translocation back into the storage vesicles via the VMAT (149, 200). The later constitutes the predominant pathway.

MAO catalyzes deamination of amines, with production of aldehydes that are metabolized to carboxylic acids or alcohols (Fig. 3). MAO-A subtype has a higher affinity for NA and ADR and is highly localized in brain neurons. MAO-B is responsible for degradation of DA. Both MAO-A and MAO-B have been localized in liver. MAO significantly participates in regulation of NA storage in the nerve terminals (442, 592).

COMT is primarily an extraneuronal enzyme, but some of the enzyme may also be localized intraneuronally. Adrenal chromaffin cells express abundant COMT (153), which explains why all plasma metanephrine derives from *O*-methylation of CA within the adrenal medulla (154). This fact is used in the detection of pheochromocytoma, the tumor that synthesizes CA and expresses COMT (150, 360, 473). The enzyme utilizes *S*-adenosylmethionine as a cofactor. COMT metabolizes circulating catechols mainly in the liver and kidney. Figure 3 depicts details of CA metabolism.

Phenolic hydroxyl groups of CA can be conjugated to sulfates or glucuronides as another mechanism of CA inactivation. Glucuronide is the principal conjugate in rats, and sulfate predominates as the conjugate in humans.

Eisenhofer and co-workers (151, 152) have recently demonstrated a new view of CA metabolism in normal and stress conditions. Importantly, most metabolism of CA takes place within the same cells in which the amines are synthesized. This mainly occurs secondary to leakage of CA from vesicular stores into the cytoplasm. These stores exist in a highly dynamic equilibrium, with passive outward leakage counterbalanced by inward active trans-

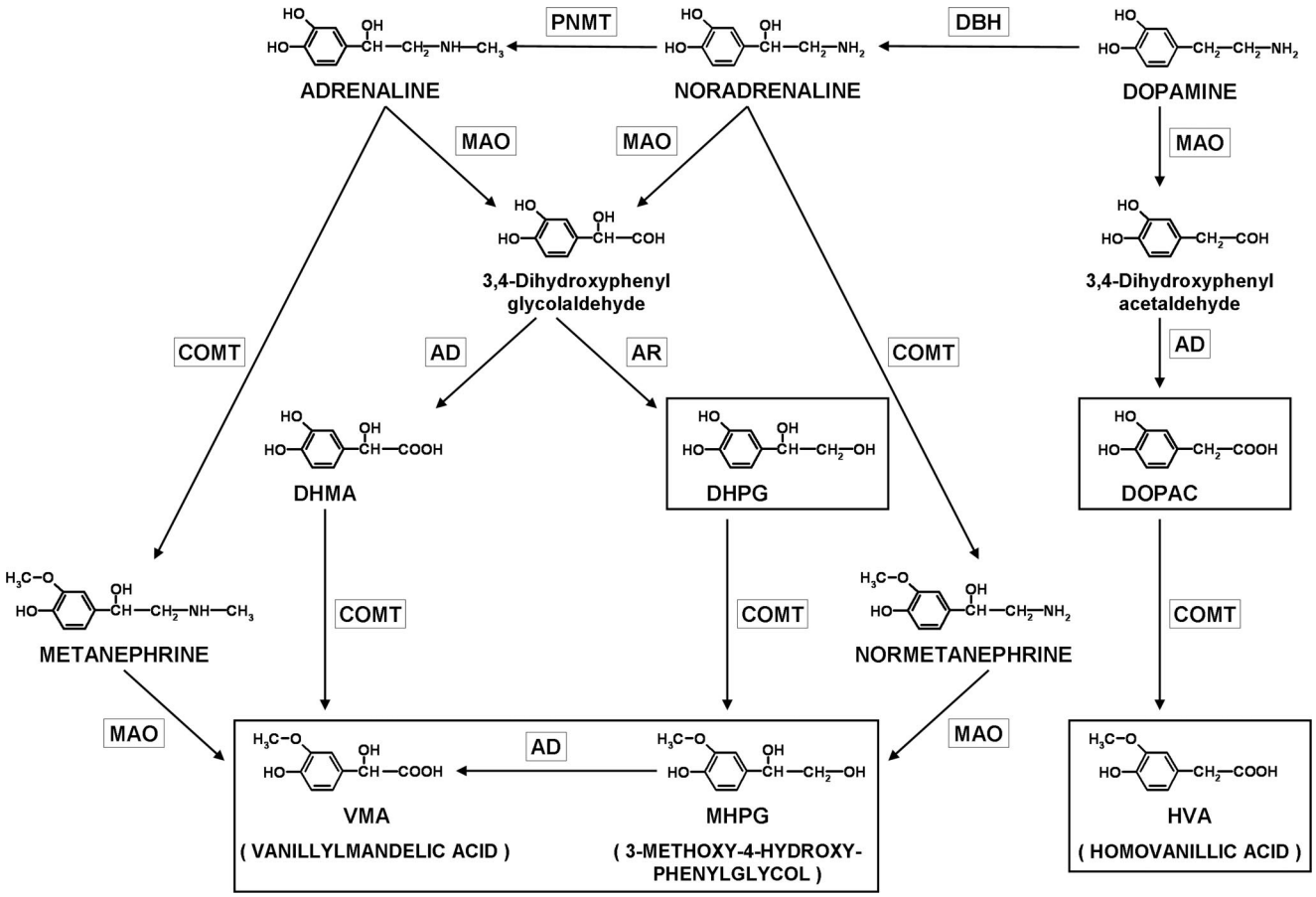

FIG. 3. The main pathways of catecholamine metabolism. The end products of noradrenaline (NA) and adrenaline (ADR) metabolism in effector cells are 3-methoxy-4-hydroxyphenylglycol (MHPG) and vanillylmandelic acid (VMA), and of dopamine metabolism homovanillic acid (HVA). In sympathetic nerve terminals, the end product of NA and ADR metabolism is 3,4-dihydroxyphenylglycol (DHPG), and 3,4-dihydoxyphenylacetic acid (DOPAC) is the end product of the dopamine metabolism. The aldehyde intermediates exist only transiently and are rapidly metabolized to corresponding glycols by the enzyme aldehyde reductase (AR) or to acids by the enzyme aldehyde dehydrogenase (AD). The majority of the metabolites are conjugated at the position of phenolic hydroxyl group with sulfate or glucuronide. MAO, monoamine oxidase; COMT, catechol-*O*-methyltransferase; DHMA, 3,4-dihydroxymandelic acid.

port controlled by VMATs. In sympathetic nerves, the aldehyde produced from NA by MAO is converted to DHPG and not to 3,4-dihydroxymandelic acid (DHMA). Subsequent extraneuronal *O*-methylation leads to production of 3-methoxy-4-hydroxyphenylglycol (MHPG) and not to vanillylmandelic acid (VMA) (Figs. 3 and 4). This acid is instead formed in the liver by oxidation catalyzed by alcohol and aldehyde dehydrogenases. Compared with intraneuronal deamination, extraneuronal *O*-methylation of NA and ADR represents a minor pathway of CA metabolism.

Most of the MAO metabolite DHPG, produced under resting conditions, comes from NA leakage from vesicles. In the resting human heart, ~73% of NA turnover is due to intraneuronal metabolism of NA leaking from storage vesicles (150, 151, 153).

Under conditions of exercise (at 50% of maximal work capacity), rates of NA release and reuptake exceed the rate of NA leakage from storage vesicles, which as a passive process operates independently of exocytotic release and remains relatively constant (Fig. 5). Vesicular leakage may therefore be viewed as a stress response coping mechanism, where the large and constant contribution of vesicular leakage to CA turnover reduces the requirement during sympathetic activation for relative increases in CA synthesis to match those in release. Because the ability to increase TH activity is limited, this leakage mechanism provides sympathetic nerves with a capacity for a more extended range of sustainable release rates than would otherwise be possible (151, 152).

E. Molecular Genetics of Catecholaminergic Systems

1. Catecholamine biosynthetic enzymes

A) TH. Human TH is encoded by a single gene localized to chromosome 11, comprising 14 exons and 13

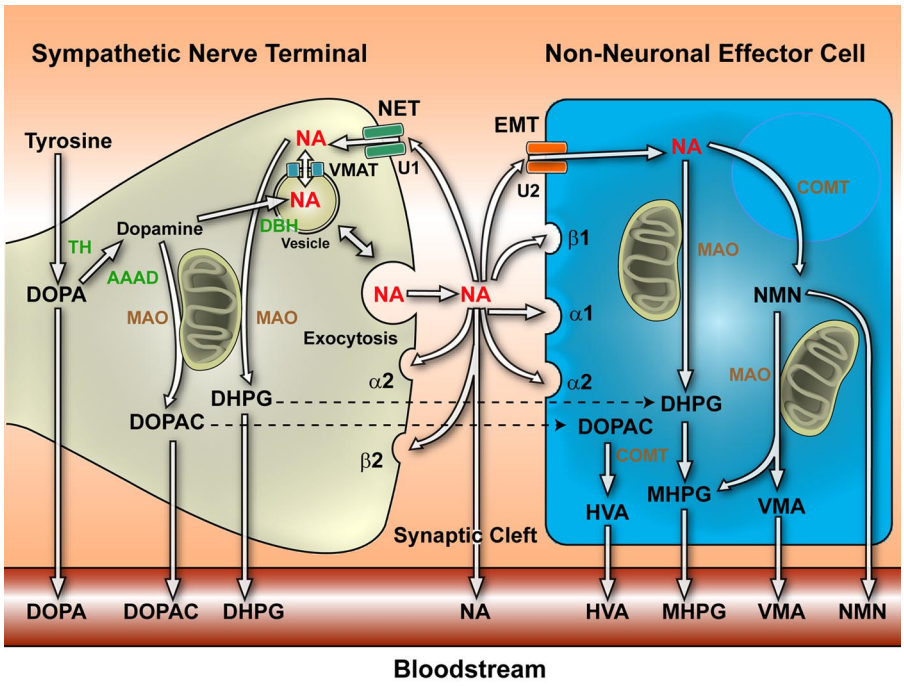

FIG. 4. Schema of events at the sympathetic nerve terminals and nonneuronal effector cells. Catecholamine biosynthesis begins with uptake of tyrosine into the sympathetic neurons. Tyrosine is converted to DOPA by tyrosine hydroxylase (TH), and DOPA is then converted to dopamine (DA) by L-aromatic amino acid decarboxylase (AAAD). Cytoplasmic DA is taken up into vesicles and hydroxylated by dopamine-β-hydroxylase (DBH) to noradrenaline (NA). In dopaminergic neurons, DA is the end product of catecholamine biosynthesis and is stored in vesicles or may be metabolized by monoamine oxidase (MAO) to dihydroxyphenylacetic acid (DOPAC) which is released into blood. Similar events are also present in the chromaffin cells of the adrenal medulla where NA is further converted by PNMT to ADR. Neuronal stimulation causes exocytotic release of NA from vesicles into the synaptic cleft. Released NA can bind to receptors, be taken back up into nerve terminal (uptake 1; U1) or into nonneuronal cells (uptake 2; U2), or diffuses into blood. By far, the predominant fate of the released NA is its reuptake into the nerve terminals by U1, via the noradrenaline transporter (NET). Uptaken cytoplasmic NA is taken back into vesicles by vesicular monoamine transporter (VMAT), or converted by MAO deamination to dihydroxyphenylglycol (DHPG). This metabolite diffuses into blood or traverses the nonneuronal cell membrane and is converted to 3-methoxy-4-hydroxyphenylglycol (MHPG) by catechol-*O*-methyltransferase (COMT). NA uptaken by extraneuronal monoamine transporter (EMT; U2) into nonneuronal cells can be metabolized by COMT to form normetanephrine (NMN) or can form DHPG and MHPG or in humans vanillylmandelic acid (VMA), the end product of NA and ADR metabolism. [Modified from Kvetnansky et al. (340).]

introns within 12.5 kb (519). The human gene undergoes alternative splicing, and at least eight TH mRNAs with variations in the sequence coding for the NH_2-terminal domain of the protein have been described. These involve the inclusion or omission of a 12-bp sequence encoded by the 3′-terminal portion of the first exon or the 81-bp sequence of the second exon (210, 280, 357, 460). Additional mRNA variants by alternative splicing of exon 3 were reported and overexpressed in progressive supranuclear palsy patients (134). Variants with omission of exon 4 in normal adrenal medulla were described (462). Splicing of exons 8 and 8+9, outside the NH_2-terminal region, were recently observed in patients with neuroblastomas (495). While some differences in functional characteristics have been found among these isoforms, it is not yet clear whether regulation of either isoform expression or alternative splicing is an important mechanism for the regulation of TH during physiological manipulations. Since rodent TH does not appear to undergo alternative splicing, this mode of regulation is not pertinent to most of the experiments on stress.

The promoter of TH, given its major regulatory role in CA biosynthesis, has been studied in great detail. Most of these studies have concentrated on the proximal region of the promoter within the first few hundred base pairs upstream of the start site. This region is sufficient for specific expression in catecholaminergic cells and for response to many external stimuli in cell culture. However, more upstream regions of the promoter are required for TH expression in catecholaminergic cells in vivo. Lengthening the 5′ -flanking sequences to 4.5 kb or longer produced CNS TH expression in transgenic mice with minimal ectopic expression (26, 369, 421).

A diagram of the proximal rat TH promoter is shown in Figure 6. It contains several verified and putative regulatory elements. A perfect consensus cAMP response element (CRE) at −45 and a noncanonical AP1 element at −205 are particularly important for basal expression of TH. Mutations of these motifs in a 5.3-kb promoter constructs prevented expression in catecholaminergic neurons of adult transgenic animals (659). In cell culture, this site was found to be the most crucial for regulation of TH

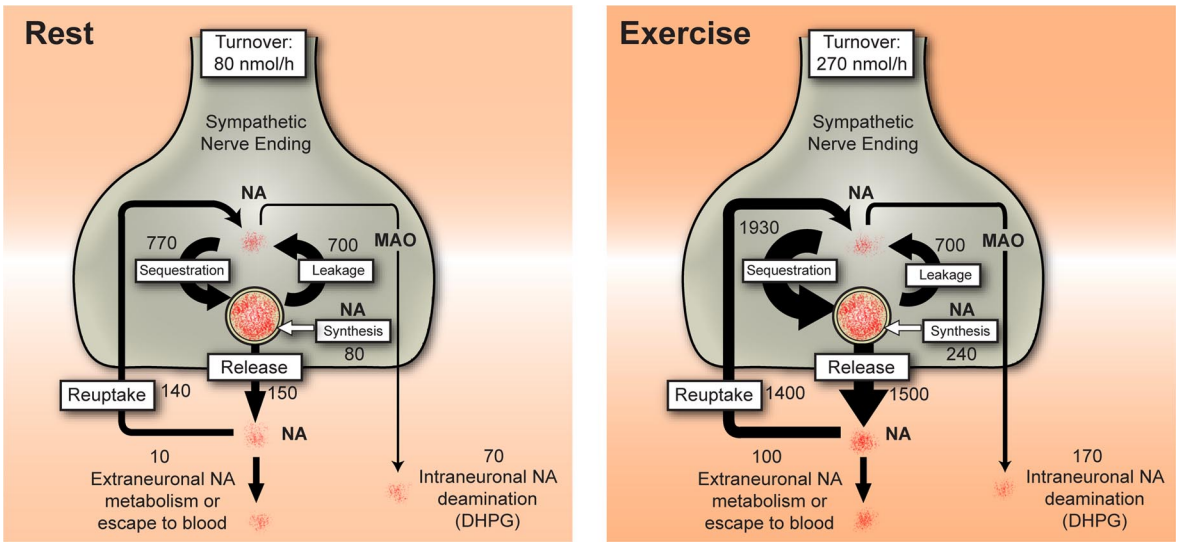

FIG. 5. Noradrenaline turnover in human cardiac sympathetic nerve endings under resting conditions and during exercise (50% of maximum work capacity). Individual processes contributing to NA turnover are expressed in nmol/h. Efficiencies of neuronal reuptake and vesicular sequestration are very similar during exercise-induced increase in NA release. The constant contribution of leakage (700 nmol/h) to turnover of NA at rest and during exercise is important. Due to such a constant leakage, the observed 10-fold increase in NA release with exercise results from only a 3.4-fold increase in NA turnover (270 vs. 80 nmol/h), which approximately matchs with the 3-fold increase in NA synthesis (240 vs. 80 nmol/h). [Modified from Eisenhofer et al. (152).]

transcription not only by changes in basal expression, but also in response to stimulation by cAMP, hypoxia, and changes in intracellular calcium (238, 298, 362, 440). It also overlaps with an imperfect estrogen response element (ERE) (386) and thus may also play a role in sex differences in the response of TH transcription to stress. The CRE motif can bind not only CREB as homodimers, but also heterodimers containing other ATF family members such as ATF-1 and ATF-2 (212, 440, 625). As described in detail later, changes in phosphorylation of CREB, which is rapidly increased in response to stress, are likely to function at this motif.

The TH promoter contains a functional Sp1/Egr1 motif (494, 702). Overexpression of Egr1 is sufficient to trigger activation of TH gene expression in cell culture (493). This motif is likely also involved in the response to stress.

The AP1-like motif on the TH promoter is implicated in regulation of TH transcription, for example, by phorbol esters and nerve growth factor, and in some cases modulating basal expression in conjunction with the E box. The AP1 motif can interact with the Sp1/Egr1 motif in the regulation of TH transcription, as mutation of the AP1 motif or inserting additional nucleotides between the Sp1/Egr1 and AP1 motifs, reduced the response to Egr1 (447, 493).

Other sites on the TH promoter which might be involved in regulating transcription include AP2-like motifs, hypoxia inducible site (HIF), E box/dyad element, octamer(Oct)/heptamer (Hept), BBE, NBRE and dyad (D/2) motifs. The NBRE motif is critical for the transactivation of the TH promoter by Nurr1, while the bicoid-type binding element (BBE) is needed for the regulation of TH by Ptx3. Both Ptx3 and Nurr1 are pivotal for development of the midbrain dopaminergic system (80, 297).

While TH gene transcription is markedly regulated by glucocorticoids, at least in isolated cell cultures (180), a glucocorticoid response element has so far only been identified in the mouse TH promoter ~2,400 upstream from the start site (−2435 to −2421) (215) and at about −5.7 kb of the rat promoter at a novel CRE/AP1 motif (526a). An androgen response element in the proximal TH promoter (region of −1562 to −1328) has also recently been identified (270).

The human TH promoter revealed high homology to the rat TH promoter especially in the proximal region containing the TATA box, BBE motif and a CRE, and in a region −2323 to −2384 (299). In addition to the regulatory motifs on the promoter, the human TH gene contains a microsatellite that is related to levels of TH gene expression. The human TH01 microsatellite is a $(TCAT)_n$ motif localized in the first intron of the human TH gene (513). It has been shown that a polymorphic version of this microsatellite can act as a transcription regulatory element (411). Polymorphisms in this motif have now been associated with altered CA turnover (682), essential hypertension (587), susceptibility to high altitude edema (221), nicotine dependence (10), and a number of cardiovascular measurements including basal and poststress heart rate (711) and a greater hemodynamic response to stressors in humans (28). The relationship between TH polymorphisms and the human stress responses is described in detail in the recent papers (527, 528).

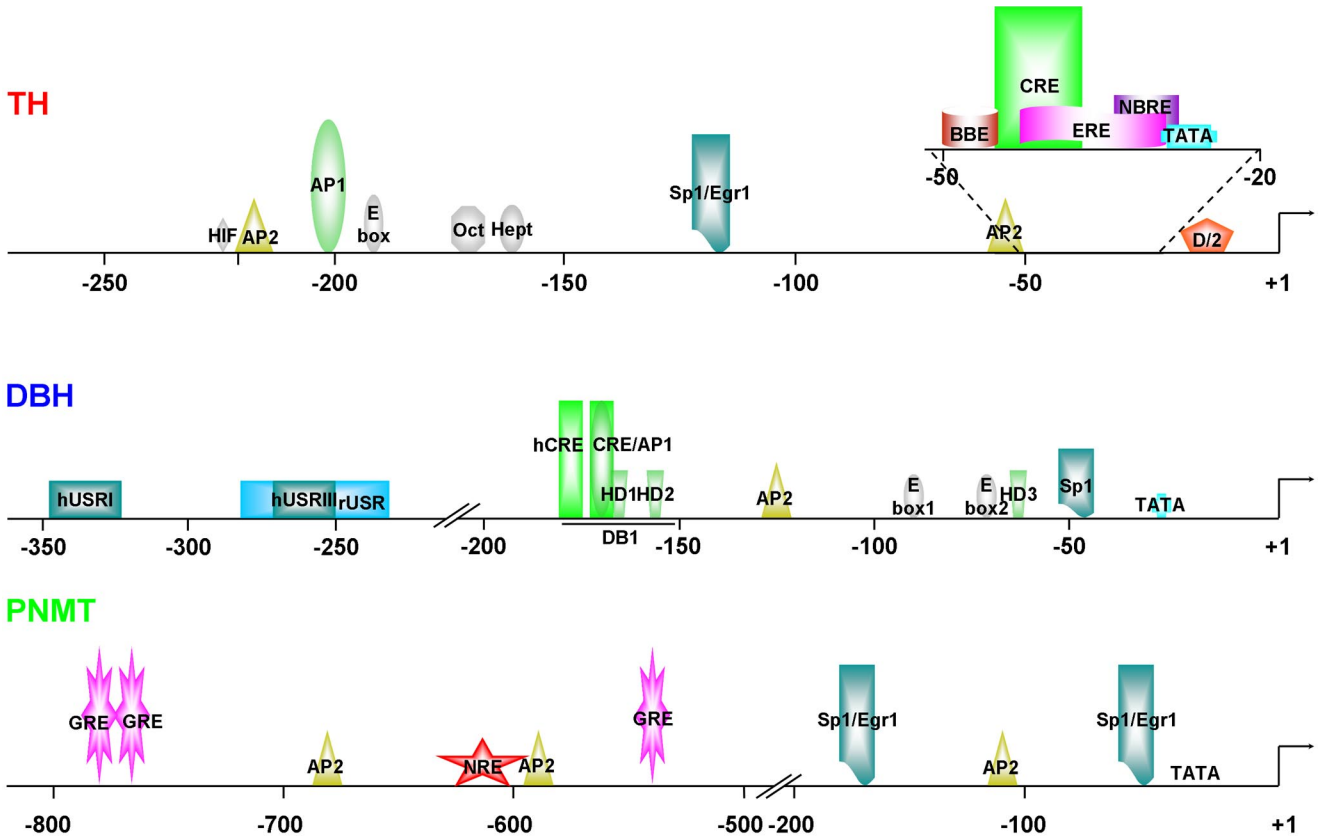

FIG. 6. Diagram of regulatory elements on proximal TH, DBH, and PNMT promoters. See text for description of the promoter elements. The elements implicated in the response to stress are enlarged.

B) DBH. The human DBH gene is present on chromosome 9q34 (106). It contains 12 exons spanning 23 kb. A number of polymorphisms have been reported in the human DBH gene (reviewed in Ref. 108). A C to T transition 1021 bp upstream of the transcription start codon is strongly associated with very low DBH plasma activity. It is postulated that this polymorphism diminishes DBH gene transcription. However, the mechanism remains to be determined. Genetic variations in the DBH gene have been linked to several psychiatric disorders, such as reactive schizophrenia, psychotic depression, alcoholism, attention deficit hyperactivity disorders, a modulation of smoking and other reward-seeking behaviors, and susceptibility to autism and Parkinson's disease (10, 108, 224).

Human and rat DBH mRNAs were shown to be coded for by two mRNA species, which appear to differ in the poly(A) addition site (303, 405). DBH is a glycosylated protein and the only CA-synthesizing protein localized within the neurosecretory vesicles, where it is present in soluble and membrane forms (611). A fraction of DBH is released by exocytosis and is present in plasma and CSF. Transfection of DBH cDNA demonstrated that both membrane-bound and soluble forms of DBH can be derived from a single mRNA (363). Extensive studies have led to the conclusion that DBH is anchored to the membrane due to nonremoval of a signal sequence (168, 638) and in some cases also by a second mechanism independent of the signal sequence (246).

The DBH promoter is sufficient to enable specific expression in noradrenergic tissues and cell culture. A number of functional motifs in the proximal DBH promoter, that are involved in the basal and induced regulation of DBH gene expression, were identified by DNA footprinting and regulation of reporter activity in catecholaminergic cells lines (Fig. 6). A region containing one or more negative regulatory (silencer) elements was shown to be involved in inhibiting human (hUSR) (258, 296) or rat (rUSR) (589) DBH gene expression in nonneuronal cells. A Sp1 motif was identified and shown to interact with Sp1 transcription factor, without cell type specificity (295). The AP2 motif is implicated in high-affinity AP2 binding preferentially in noradrenergic cells, and in the maintenance of neurotransmitter phenotype in noradrenergic and adrenergic neurons and neuroendocrine cells (294).

There are three homeodomain (HD) motifs that bind Arix/Phox2a and NBPhox/Phox2b (295, 701, 710), which appear to act in concert to regulate noradrenergic traits in both the central and peripheral nervous systems. Two of these elements (HD1 and HD2) are contained within a mul-

tifunctional region (DB1) that is important for basal as well as cAMP- and phorbol ester-mediated induction of DBH transcription (588). In addition to two homeodomain sites for Arix/Phox2a, the DB1 motif has an overlapping or adjacent CRE/AP1 motif (627). In the human DBH promoter, this motif may bind AP1 transcription factors and/or CREB and overlaps with a potential Yin Yang 1 (YY1) site (259, 575). While the human DBH promoter contains two adjacent CRE-like sites, there is one nucleotide change in the more distal CRE (hCREh) in the rat sequence, which likely renders it ineffective. This is an issue, as the majority of experiments on regulation of DBH gene expression by stress (see later sections) are performed in rodents.

The DB1 motif of the DBH promoter has been the topic of considerable research, given its importance in both basal and regulated DBH gene expression and the proximity between the Arix/phox homeodomain motifs, and overlap between the CRE/AP1 and HD1. There is functional synergism among different transcription factors in the regulation of DBH transcription, and Arix/Phox2a interacts synergistically with cAMP to regulate DBH transcription. There is some controversy regarding the binding of AP1 proteins and/or CREB family members to this motif. However, it was shown that binding of AP1 family members, including c-Fos and c-Jun, to the CRE/AP1 motif are necessary for transcriptional synergism of Arix with protein kinase A pathway, and appears to involve recruitment of the coactivator CBP (626, 627). AP-1 proteins, and especially c-Jun (rather than CREB), were shown to bind to this site in the DBH promoter in nuclear extracts from rat adrenal medulla (554). With repeated stress, Fos-family members, but not c-Fos, are involved in the interaction (452).

DBH gene expression is also regulated by glucocorticoids, and several putative sites have been identified in the rat DBH promoter (252, 407). A site involved in negative regulation by Egr1 has recently been identified between the DBH and USR sites in the rat promoter (90a). The DBH promoter also contains motifs involved in estrogen responsiveness (577), which are in the process of being mapped.

c) PNMT. The human PNMT gene is localized on chromosome 17q21-q22 (279), an area that shows positive linkage to risk for hypertension. It spans only 2.5 kb and has only three exons. Resequencing of the PNMT gene in a large sample has identified a number of polymorphisms (273). Luciferase constructs for common PNMT promoter haplotypes indicated significant differences in their ability to drive transcription. Selected polymorphisms in the PNMT promoter at −353 and −148 were used for association studies for several disorders including Alzheimer's disease, multiple sclerosis, and obesity. The genetic polymorphisms at −353 are implicated in the development of essential hypertension in certain ethnic groups, sporadic early-onset Alzheimer's disease, and multiple sclerosis (109, 391, 392).

The 2-kb size human PNMT promoter is sufficient to direct tissue-specific expression in the adrenal medulla (24). In addition to its developmental and tissue-specific expression, the PNMT gene is also regulated by several factors (Fig. 6).

Glucocorticoids play a major role in the regulation of the expression of the PNMT gene (692). A glucocorticoid responsive element (GRE) was initially identified at −533 of the rat PNMT promoter (539). Subsequently, two further upstream overlapping GREs at −759 and −773 of the rat promoter were found to be the primary sites involved in the regulation of the PNMT gene by glucocorticoids (634). The PNMT promoter also contains several Sp1/Egr1 and AP2 motifs. Importantly, Egr1 and AP2 transcription factors synergistically interact with glucocorticoid activation at the two overlapping GREs (634, 693). Experiments with the most proximal of the Sp1/Egr1 motifs indicate the importance of Sp1 binding for tissue-specific PNMT expression and that MAZ may compete with Sp1 in this regard (230). The Sp1/Egr1 motifs also appear to be important for regulation of PNMT expression by protein kinase A and protein kinase C (635, 636, 696).

An additional element (NRE) on the PNMT gene required for response to stimulation by nicotine has recently been reported (165).

2. Catecholamine-degrading enzymes

a) MAO-A AND MAO-B. The molecular genetics of MAO-A and MAO-B have been reviewed (2, 442, 594). The genes for both MAO-A and MAO-B are on the X-chromosome. Both genes consist of 15 exons with an identical intron-exon organization, suggesting that they are derived from a common ancestral gene. The MAO-A and MAO-B genes are closely linked and organized in opposite directions, tail to tail 24 kb apart. They also share ~70% amino acid sequence identity. However, they have different promoter organizations (reviewed in Refs. 88, 594). The MAO-A proximal promoter was shown to consist of three binding sites for the transcription factor Sp1 in reverse orientations and to lack a TATA box. It is activated by Sp1 and repressed by a novel R1 (RAM2/CDCA71) repressor (89). Three glucocorticoid/androgen response elements were also found within the first 2 kb of the human MAO-A promoter. The MAO-B proximal promoter contains a TATA box and two clusters of Sp1 binding sites, separated by a CACC box (88, 594). It was shown that the more distal of the MAO-B Sp1 clusters can also bind to Egr1. Thus phorbol ester treatment of cell cultures expressing both MAO-A and MAO-B induced Egr1 expression and increased MAO-B, but not MAO-A gene expression.

In the human brain, MAO-A is present in catecholaminergic cells while MAO-B is expressed in serotonergic neurons and astrocytes. Thus regulation of MAO-A gene ex-

pression is likely more pertinent to control of the physiology of catecholaminergic cells. A functional polymorphism in the MAO-A gene involving a 30-bp repeat ~12 kb upstream of the coding region, which is present in 3, 3.5, 4, or 5 copies has been described. This polymorphism was shown to affect transcriptional activity of the MAO-A gene (557). The apparent linkage of this polymorphism in the MAO-A promoter to some aspects of neuropsychiatric disorders is of great interest, but beyond the scope of this review.

B) COMT. Human COMT is localized on chromosome 22q11.21. The COMT gene lies in a chromosomal region of interest for psychosis and bipolar spectrum disorder, and a common polymorphism within the gene alters the activity of the enzyme. As a consequence, COMT has been one of the most studied genes for psychosis (reviewed in Ref. 105). Genetic variations in the COMT gene have been associated with altered prefrontal cortex function and higher risk for schizophrenia, but the specific alleles and their functional implications are controversial.

Two COMT polypeptides, soluble and membrane bound, are expressed from one gene by two separate promoters. One promoter, P2, functions constitutively, whereas the other, the proximal P1 promoter, is regulated in a tissue-specific manner. The P1 promoter expresses a 1.6-kb transcript which codes for the *S*-COMT. The P2 promoter controls the expression of an alternatively spliced 1.9-kb transcript, which is able to direct synthesis of both forms of COMT (35, 383).

3. Transporters in catecholaminergic cells

A) VMAT. The biochemistry and molecular biology of VMAT are reviewed (53, 228). Two homologous but distinct genes express VMAT. The human VMAT-1 gene maps to chromosome 8p21.3 and the VMAT-2 gene to chromosome 10q25. Central, peripheral, and enteric neurons express only VMAT-2. VMAT-2 alone is expressed in histamine-storing enterochromaffin-like cells of the mucosa of the stomach. VMAT-1 and VMAT-2 are coexpressed in chromaffin cells of the adrenal medulla (160).

Several gain-of-function haplotypes in the VMAT-2 gene that display significantly increased transcriptional activity were tested for association with Parkinson's disease and found to confer a protective effect that was selective for females (194).

The expression of VMAT-2 mRNA and promoter activity is increased by elevated intracellular calcium and also phorbol esters in enterochromaffin lining cells (681). The VMAT-2 promoter lacks TATA or CAAT box sequences. Both human and rat VMAT-2 promoters contain CRE at about −40 bp, NFκB sites in the first intron, and two Sp1 sites within the first 120 bp upstream of the transcription start site (191, 700). Other further upstream potential regulatory motifs have been identified, including sites that may function as AP2 and glucocorticoid regulatory motifs (681).

B) NET. The human NET is localized on chromosome 16q12.2. It spans 45 kb and consists of 14 exons (190, 518). Many studies have analyzed polymorphism in the NET gene and potential association with disease given the importance of NET in fine regulated control over many NA-mediated behavioral and physiological effects and also because NET is a target of several drugs therapeutically used in the treatment or diagnosis of various disorders among which depression, attention-deficit hyperactivity disorder, and feeding disturbances are the most common.

Despite the clinical and physiological significance of NET, relatively little is known about the transcriptional mechanisms governing its regulation. The first intron is needed for high-level reporter gene activity. An E-box motif residing at the junction of the first exon and intron was shown to be critical for the promoter enhancing activity as well as splicing of the first intron (292).

The structural organization of 9 kb of the human NET gene has been characterized. The NET promoter (4.0 kb of the 5′-upstream sequences) contains sufficient genetic information to drive reporter gene expression in an NA cell type-specific manner (293). Two positive and one negative domains in the human NET promoter have been identified to be potentially important for its regulation. The most proximal domain (between −128 and −80) contains several *cis*-regulatory elements in tandem, including a homeodomain core motif which can interact with Phox2a and HoxA5 and is proposed to be critically important in controlling noradrenergic specific NET gene expression (293).

C) DAT. The human DAT gene is localized on chromosome 5p15.3. Changes in human DAT gene transcription level have been reported in patients affected by a number of psychiatric, addictive, and neurodegenerative disorders (27). The human DAT promoter region has been used for functional analysis (307, 558, 677). It does not contain canonical TATA and CAAT boxes, while it displays high GC content and several Sp1 binding sites in the proximal promoter regions. The two GC boxes (−130 and −60) were shown to be important *cis*-acting elements in mediating DAT expression in dopaminergic cell line. Indeed, Sp1 and Sp3 transcription factors are strong activators of DAT transcriptional activity and are likely to play an important role in regulating DAT expression in dopaminergic neurons (677). Human zinc protein 161 was recently found to activate human DAT promoter activity (358).

One or more nonselective negative or silencing elements were observed between −798 and −2750 bp. Several putative NBRE motifs were identified, and DAT promoter directed gene transcription was increased by Nurr1, consistent with its involvement in dopaminergic specific

expression (558). The first intron has been implicated in restricting human DAT gene expression to neuronal cells (307).

The human DAT gene contains a variable number of tandem repeats in its 3′-untranslated region (UTR). The linkage and association between this polymorphism of DAT1 and various neuropsychiatric disorders have been reported. The effect of this polymorphism in the 3′-UTR region of DAT1 on gene expression suggests that the 3′-end polymorphism regulates DAT expression (183).

III. CENTRAL CATECHOLAMINERGIC SYSTEMS IN STRESS

A. Specificity of Brain Catecholamine Neurons in Stress

The brain catecholaminergic systems utilizing NA, ADR, DA, and L-DOPA neurons represent anatomically and functionally specific systems that play key roles in the central organization of stress response. These neurons possess a number of anatomical characteristics distinct from any other types of neurons in the brain: *1*) most of the catecholaminergic neurons or cell groups do not respect the anatomical borders of brain nuclei; *2*) CA neurons have a highly divergent axonal arborization with a high number of axon collaterals and thousands of terminals (synapses or axonal varicosities); *3*) CA neurons establish a high number of nonsynaptic nerve terminals and release catecholamines in a nonsynaptic manner (termed extra-synaptic neurotransmission); *4*) CA neurons also express a great variety of neuropeptides as cotransmitters; *5*) CA neurons express specific membrane transporters for the intensive reuptake of catecholamines from the synaptic cleft or from the extracellular space around the axonal varicosities; *6*) CA neurons have a high ability to regenerate their damaged axons; and *7*) CA neurons display strong, rapid, and selective response to stressful stimuli.

In a heterogeneous distribution, CA nerve fibers and terminals are present in all of the areas in the CNS. Catecholamines expressing neuronal perikarya, however, are located only in restricted brain regions from where their axons arise and run in compact nerve bundles (Table 1), but more frequently in loosely arranged projections to reach and influence, in a synaptic or nonsynaptic manner, the activity of almost every neuronal cells in the CNS. Applying a variety of neuroanatomical techniques, such as histofluorescence, immunohistochemistry, or in situ hybridization histochemistry, allows localization of CA neurons in the brain. For the visualization of the catecholaminergic system with emphasis on the differentiation between DA, NA, and ADR, the expression and the presence of their biosynthetic enzymes TH, DBH, PNMT, and AAAD should be demonstrated at cellular, axonal, and terminal levels.

Catecholaminergic neurons in the brain have been classified by Dahlström and Fuxe (118) into 12 cell groups (A1–A12). Later, further CA cell groups (A13–A16, C1–C3) have been described by Fuxe and Hökfelt (185), Björklund and co-workers (43, 44), and Hökfelt et al. (242).

Stressful stimuli accelerate both release and turnover of brain CA, which may result in depletion of stored CA. Brain noradrenergic and adrenergic neurons are directly involved in central processing of stress responses, while information about the exact role of central dopaminergic neurons in stress response is still controversial. Besides synthesis and release, the effect of CA during stress depends on their receptors and transporters that are expressed in particular brain regions. Adrenaline binds to the same receptors as NA, and adrenergic cells may use

TABLE 1. *Major noradrenaline-, adrenaline-, and dopamine-containing neuronal pathways from neurons in various catecholaminergic cell groups*

Pathways	NA Cell Groups	ADR Cell Groups	DA Cell Groups
Noradrenaline/adrenaline			
Ventral noradrenergic bundle	A1–A2	C1–C2	
Dorsal tegmental bundle	A6	C2–C3	
Dorsal periventricular system	A1–A2–A6	C1–C2–C3	
Spinal projections	A5–A6–A7	C1–C2	
Cerebellar projections	A4, A6		
Dopamine			
Mesocortical pathway			A9–A10
Mesostriatal pathway			A8–A9
Mesolimbic pathway			A10
Tuberoinfundibular DA system			A12
Spinal projections			A11
Incerto-hypothalamic pathway			A13
Periventricular DA systems			A14
Olfactory DA system			A16

NA, noradrenaline; ADR, adrenaline; DA, dopamine.

NA transporters for their reuptake from the synaptic cleft. Two types (α and β) and several subtypes of adrenergic receptors have been classified and localized in different brain nuclei. Their changes, as well as the consequences of their changes on the activity and stress response of brain CA neurons depend on the type, quality, severity, and duration of stressful stimuli. Dopamine neurons express their own transporters and act through specific DA receptors.

Acute stress leads to transient alterations in brain CA system accompanied by transient activation of the HPA axis. After a short period of time, depending on the character and the intensity of stressful stimuli, homeostasis can be restored, and the activity of CA neurons returns to "unstressed" levels. Chronic, long-lasting, or repeated stressors may induce permanent changes and keep CA neurons in a highly active status (390, 543, 680). However, during prolonged stress for several weeks, the CA stores could be exhausted, the extent of the increase in CA turnover may not compensate the transmission, or the activity of CA neurons may be reduced, which leads to a deficit in CA in nerve terminals (378, 664). In the HPA axis, the expression of corticotropin-releasing hormone (CRH) mRNA and the corticosteroid reaction are reduced. However, vasopressin mRNA, which reacts minimally to the acute stress in the paraventricular nucleus (PVN), shows increased activity in chronic stress situations (78). Furthermore, animals exposed to chronic stress exhibit enhanced HPA and sympathetic nervous system responses to novel stressors (39, 188, 317, 328, 338, 496). Long-term, repeatedly applied stressors can result in an enhancement of TH mRNA levels in response to novel stressors (328, 543).

Brain stem NA neurons are directly involved in response to the majority of stressors. The activation of the HPA axis through CA innervations of PVN neurons, however, is stressor specific (187, 472, 474). The induction and adaptation of brain Fos expression in response to acute stressors are varied from region to region and are dependent on the intensity and duration of stressful stimuli.

B. Central Noradrenergic System: Topography and Responses to Stressors

Noradrenaline-synthesizing perikarya in the brain exist only in the pons and the medulla oblongata (Fig. 7*A*) where they have been classified into seven (A1–A7) cell groups. NA cells in the A1, A2, A4, and A6 (locus coeruleus) cell groups (together with C1–C2 ADR neurons) belong to the "ascending catecholaminergic system" which represents the afferent arm of the central sympa-

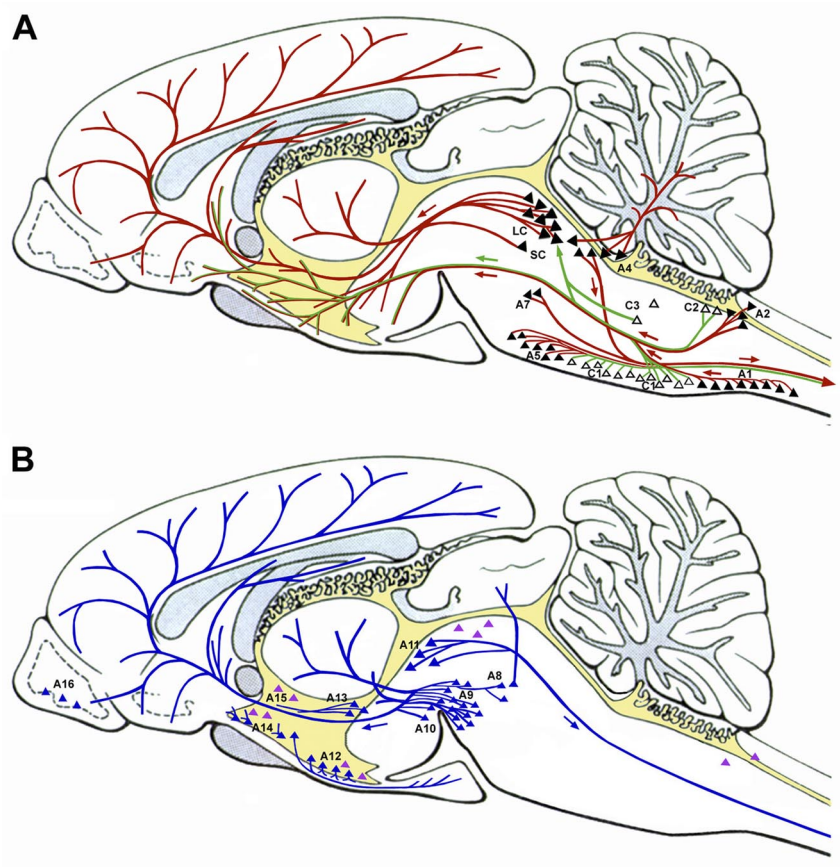

FIG. 7. Catecholaminergic neurons and their major projections in the rat brain (drawings on the midsagittal plane of the brain). *A*: noradrenaline (solid triangles and lines in red) and adrenaline (open triangles and lines in green). *B*: dopamine (triangles and lines in blue) and L-DOPA neurons (pink triangles). A1, A2, A4, A5, and A7, noradrenergic neurons; C1, C2, and C3, adrenergic neurons; A8-A16, dopaminergic neurons; LC, locus coeruleus; SC, subcoeruleus area.

thetic system projecting to the forebrain and the cerebellum. These CA neurons provide neural inputs to the so-called integrative centers (hypothalamus, limbic cortex, and nuclei) and influence the activity of the HPA axis (Fig. 8). NA cells in the A5, A7 cell groups, in the ventral-caudal portion of the locus coeruleus and the subcoeruleus area (together with rostral C1 ADR neurons) belong to the "descending catecholaminergic system" (Fig. 8), projecting to the spinal cord including the sympathetic preganglionic neurons in the thoracic segments. These neurons represent the efferent arm of the central sympathetic system that influence the sympathetic outflow, the activity of the peripheral sympathetic system.

1. A1 noradrenergic cell group

The A1 cell group consists of the most caudal noradrenergic cells in the caudal portion of the ventrolateral medulla (CVLM) between the level of the obex and the medulla/spinal cord junction (Figs. 7*A* and 9). It is comprised of large multipolar TH-positive neurons. The majority of the cells distribute in the lateral reticular nucleus while some of the cells extend further caudally, into the cervical spinal cord. Rostrally, A1 cells intermingle with the caudal extension of the C1 adrenergic neurons. Here, a group of CA cells are present in a very superficial position, along the ventral surface of the caudal medulla (14, 118, 217, 243, 660).

Almost all of the A1 neurons express NA transporters (104, 240), and a high percentage of these neurons coexpress NPY (164) and $GABA_B$ receptor R1 and R2 subunits (62).

These cells receive neuronal inputs from both viscerosensory (nucleus of the solitary tract) and somatosensory (spinoreticular tract) neurons (85, 367, 490, 597). Descending hypothalamic (paraventricular) fibers also innervate A1 neurons (380, 656). Ascending (efferent) fibers arising in A1 neurons distribute in the hypothalamus (114, 491, 567) and the amygdala (274, 534). The hypothalamic paraventricular neurons are reciprocally and bilaterally connected with A1 neurons. Fibers in both directions join the ventral noradrenergic bundle (Fig. 7*A*, Table 1). Surgical transections of this CA bundle or lesioning of the caudal ventrolateral medulla result in marked decreases in the NA content of the paraventricular and arcuate nuclei, as well as in the median eminence (486, 491), and they strongly reduce the stress-elicited NA release from the paraventricular nucleus (475, 476).

A1 noradrenergic cells in the CVLM are sensitive to nociceptive stimuli and react with an immediate expression of c-*fos* mRNA and Fos protein (81, 485, 487, 574). Similarly, IMO also evokes very strong c-Fos activation, while cold stress or hemorrhage have moderate to minor effects on A1 neurons (61, 135, 485, 487). Insulin-induced hypoglycemia fails to activate c-*fos* expression in these neurons (485, 487). Footshock and audiogenic stress can also elicit Fos activation in A1 NA neurons (226, 365, 507). Strong increases in TH and NET mRNA levels are observed in A1 neurons after single or repeated immobilization (543).

2. A2 noradrenergic cell group

The majority of TH- and DBH-positive neurons are located along the entire territory of the nucleus of the solitary tract (NTS) and the dorsal motor vagal nucleus (Figs. 7*A* and 9). NA cells are concentrated in the caudal (commissural) subdivision of the nucleus. Rostral to the level of the obex, scattered NA cells distribute close to the fourth ventricle, while other A2 cells extend caudally into the central grey of the cervical spinal cord. Noradrenergic neurons overlap with adrenergic cells (C2 cells) at the rostral portion of the NTS (14, 118, 243, 277). TH-positive,

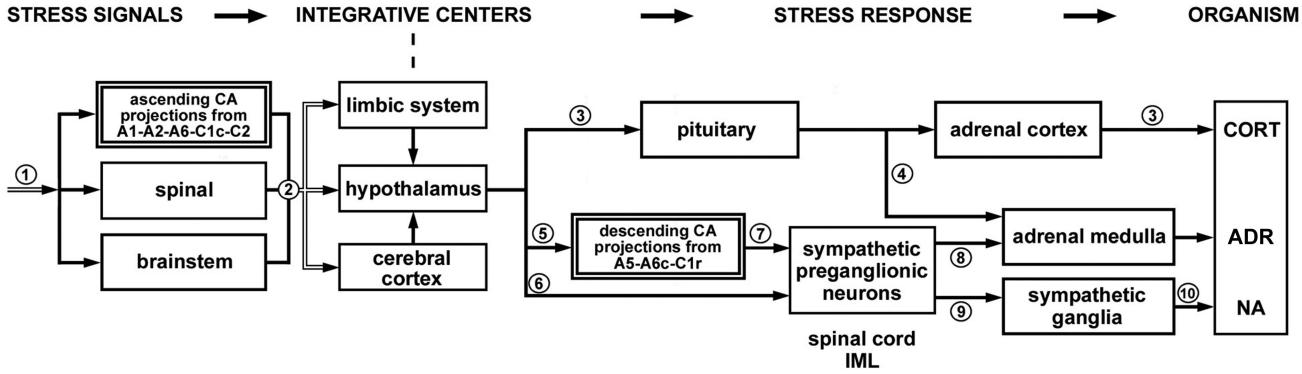

FIG. 8. A block diagram for the illustration of the central organization of stress responses. *1*, Viscero- and somatosensory stressful stimuli; *2*, ascending pathways from spinal and medullary non-CA and medullary CA neurons to integrative centers in the hypothalamus, limbic system, and the cerebral cortex; *3*, hypothalamo-pituitary-adrenal axis; *4*, circulating ACTH influences ADR synthesis in adrenal medullary chromaffin cells; *5*, hypothalamic projections to descending CA neurons in the pons-medulla; *6*, premotor sympathetic projections from the hypothalamus; *7*, premotor sympathetic neurons from the descending CA cell groups to the IML; *8*, sympatho-adrenomedullary system; *9*, sympatho-neural system; *10*, varicosities in the sympathetic postganglionic nerve terminals. CORT, corticosterone; IML, intermediolateral cell column.

but DBH- and PNMT-negative, neurons have been described as dopaminergic neurons in the dorsomedial medulla. However, these cells lack AAAD (267), which indicates that they may use L-DOPA, rather than DA as transmitter.

Over 95% of A2 neurons express NA transporter (NET) mRNA (104, 240, 376). A fairly high percentage of these cells also express NPY (164), and almost all of the A2 TH-positive cells coexpress $GABA_B$ receptor R1 and R2 subunits (62).

The NTS serves as the primary autonomic center that receives viscerosensory inputs from the spinal cord, and cranial nerves project to the nucleus through the sensory trigeminal tract (412, 621). The spinal afferents cross within the spinal cord, and they project to the NTS bilaterally (Fig. 9). Vagal afferents establish synaptic contacts on ipsilateral A2 neurons (621). Through descending projections, prelimbic, infralimbic, amygdala, and hypothalamic neurons innervate the NTS (208, 251, 584, 669, 676). Paraventricular projections to A2 cells (380, 566, 629, 656) may serve as a feedback route from CRH-containing neurons that receive dense noradrenergic innervation from the A1 and A2 cell groups. Descending paraventricular fibers cross over in the commissural subdivision of the NTS and terminate in the nucleus bilaterally (656).

Neurons in the A2 cell group project to the hypothalamus where they densely innervate both magno- and parvocellular neurosecretory neurons and the median eminence (116, 243, 486, 488, 491, 567, 644). The ascending A2 fibers join the ventral noradrenergic bundle at medullary level (Fig. 7*A*) and ascend without any major decussations to the forebrain (486). NA efferents from A2 neurons also distribute in the amygdala (709).

A2 neurons, but only in the most caudal part of the NTS, project to the spinal cord (439, 561). TH-positive neurons in the commissural part of the NTS innervate A1 and noncatecholaminergic neurons in the caudal ventrolateral medulla (85, 490).

A2 noradrenergic neurons show moderate response to both physical and psychological stressful stimuli, as indicated by c-Fos activation (81, 485, 487, 507). Hemorrhage also induces Fos immunoreactivity in A2 neurons (135, 485, 487). Both single and repeated immobilization, as well as cold, but not insulin, can induce TH mRNA expression in these neurons (543).

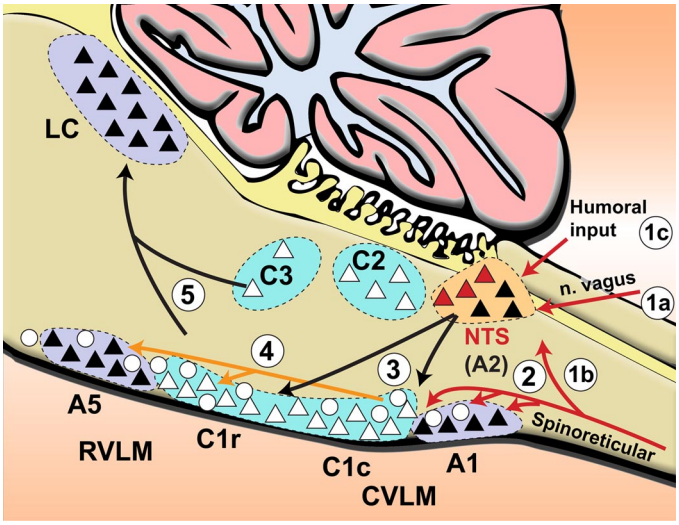

FIG. 9. Intramedullary neuronal circuit. *1*) Inputs to the nucleus of the solitary tract: *a*) through the vagus nerve, *b*) ascending signals from the spinal cord (spino-solitary tract = fibers ascend in the spinoreticular tract and leave it for the NTS), *c*) inputs from the circulation through the area postrema (no blood-brain barrier). *2*) Spinal cord inputs to the caudal ventrolateral medulla (CVLM), to A1 noradrenergic (solid triangles) and C1 adrenergic (open triangles) and noncatecholaminergic (circles) neurons (spinoreticular tract). *3*) Neurons from the NTS project to neurons in the CVLM and the rostral ventrolateral medulla (RVLM). *4*) Inhibitory inputs from the CVLM to the RVLM (to A5 and C1 premotor sympathetic neurons). *5*) Projections from C1 and C3 adrenergic neurons to the locus coeruleus (LC).

3. A4 noradrenergic cell group

At the level of the pontine-medullary border, a few TH- and DBH-positive but PNMT-negative neurons are located in the roof of the fourth ventricle. They are topographically closely related to the ependymal lining and are considered to be a dorsolateral continuation of locus coeruleus (LC) neurons (Fig. 7*A*). These cells represent the A4 noradrenergic cell group that participates in the noradrenergic innervation of the cerebellum (118, 243).

4. A5 noradrenergic cell group

TH- and DBH-positive, PNMT-negative noradrenergic neurons occupy the rostral portion of the ventrolateral medulla (RVLM) and the caudal ventrolateral part of the pons between the internal roots of the facial nerve and the superior olivary complex (Figs. 7*A*, 9, and 10, *A* and *B*). NA cells here overlap with the most rostral cells of the C1 adrenergic cell group (14, 660).

All of the A5 neurons express NET (104, 240), and many of them coexpress NPY (164), cocaine- and amphetamine-regulated transcript peptide (CART), $GABA_B$ receptor R1 and R2 receptor subunits (62, 63), glutamic acid decarboxylase mRNA (615), and vesicular glutamate transporter (DNPI/VGLUT2) mRNA (617).

A5 neurons receive neuronal inputs from the hypothalamic paraventricular nucleus (244, 566, 585, 656, 660). They may receive axon collaterals from descending fibers, which arise in limbic cortical areas and the amygdala through the parabrachial nuclei (64, 251, 561, 584). Peptidergic nerve terminals immunostained for vasopressin, NPY, thyrotropin releasing hormone, and calcitonin gene-related peptide (CGRP) form a fine network around A5 neurons.

The A5 noradrenergic neurons belong to the descending catecholaminergic system (Fig. 8). The spinal

cord is the major target of the A5 noradrenergic projections (64, 182, 375, 619, 686). Descending axons from A5 neurons travel in the ventral and dorsolateral funiculi (182) and preferentially project to target-specific sympathetic preganglionic neurons that form rostrocaudally extending columns in the thoracic spinal cord (524). The catecholaminergic innervation of the intermediolateral cell column (IML) is quite dense, especially in the thoracic segments 5–9 where the majority of the sympatho-adrenergic cells are located (Fig. 10, *C* and *D*). Axons of catecholaminergic neurons establish synaptic contacts with sympathetic preganglionic neurons in the IML (92), including those labeled retrogradely from the adrenal medulla (708). The spinal projection neurons of the A5 cell groups are regulated by GABAergic neurons (354) that are located in the ventrolateral medulla, in or immediately caudal to the A5 cell group.

The RVML makes up the sympathetic vasomotor center and mediates baroreceptor reflexes (57). The vasomotor A5 neurons are tonically inhibited by GABA and glycine neurons (see Ref. 624). A5 neurons also integrate cardiovascular responses originating in the hypothalamus (57).

It should be noted that some noradrenergic fibers of A5 cell origin innervate the dorsal horn of the spinal cord and may modulate nociception (103).

Immobilization, pain, and foot-shock, but not cold, insulin-evoked hypoglycemia, or hypovolemic hemorrhage, may elicit moderate to strong c-Fos activation in these neurons (485, 487, 507, 574). Repeated IMO very effectively elevates TH expression, but novel stressors are not able to elicit any further increase (543).

5. *A6 noradrenergic neurons: locus coeruleus and subcoeruleus area*

The LC consists of a large compact group of cells with four subdivisions and six cell types (distinguished by their shape, size, and location within the nucleus, as well as their projections) in the dorsolateral pontine tegmentum under the lateral edge of the fourth ventricle (for details, see Refs. 17, 378, 384). The LC establishes the major NA cell pool in the central nervous system; almost half of the NA perikarya are present there. Although the LC represents the A6 cells group in anatomical terms, NA cells are extended more rostral to the LC. NA cells along the most caudal portion of the periaqueductal gray matter also belong to the A6 cell group. Ventral to the LC, large-sized NA cells consist of the subcoeruleus area that interconnects the LC with the ventrolaterally located A5 cell group.

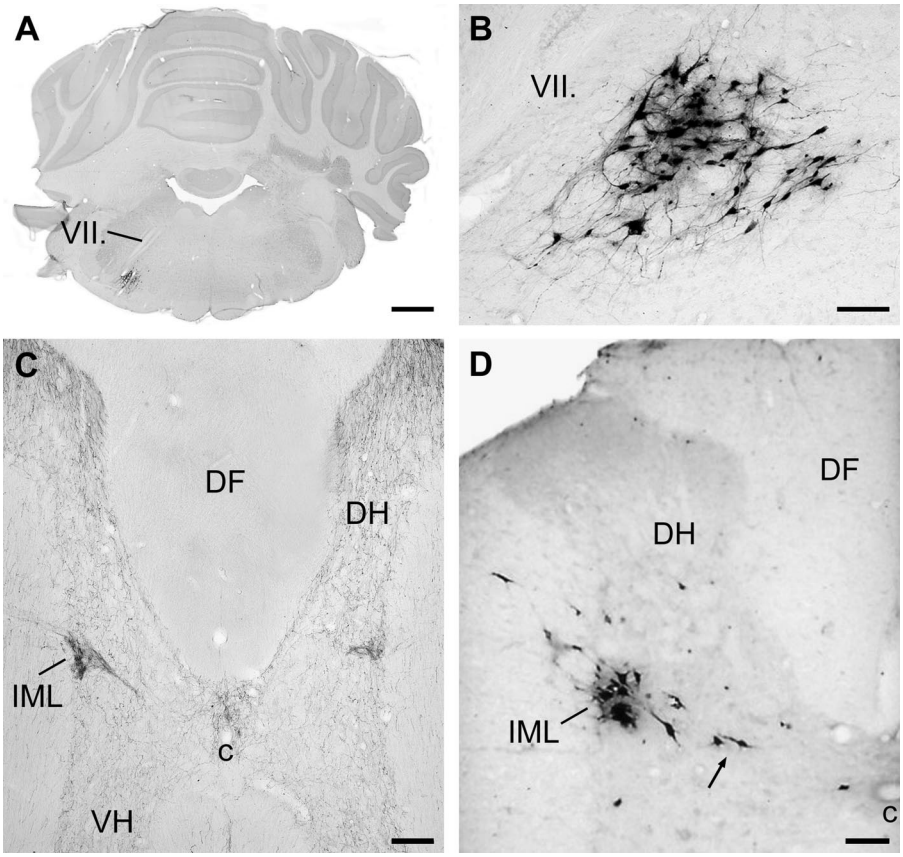

FIG. 10. Noradrenergic sympathetic premotor neurons in the A5 cell group (*A* and *B*) and TH-positive nerve terminals in the spinal cord (*C* and *D*). *A*: coronal section through the pontomedullary junction showing immunohistochemical staining of A5 neurons 3 days after a unilateral injection of retrograde *trans*-synaptic tracer, pseudorabies virus into the thoracic intermediolateral cell column (IML). Cells were labeled only on one side, ipsilateral to the site of the injection. *B*: high-power magnification of virus-labeled A5 neurons. *C*: thoracic spinal cord segments 6 (*C*) and 9 (*D*). There is a strong TH-positive network of fibers and terminals in the IML and around the central canal. Delicate TH-positive fibers are present in the dorsal horn (especially in the marginal layers), and with somewhat less density in the ventral horn. *D*: retrogradely labeled cells in the IML 3 days after pseudorabies virus inoculation of the adrenal gland. A few additional labeled cells are also seen in the neighboring areas, especially in the central gray medial to the IML cells (arrow) and in the lateral funiculus. c, Central canal; DF, dorsal funiculi; DH, dorsal horn; VH, ventral horn; VII, internal fibers of the facial nerve. Scale bars: 1.0 mm (*A*), 200 μm (*B* and *D*), and 100 μm (*C*).

NA transporters are synthesized by all of the TH-positive neurons in the LC (240). Subsets of noradrenergic neurons coexpress neuropeptides like enkephalin, galanin, NPY, neurotensin, and VIP (see Ref. 17). Almost all of the LC neurons express $GABA_B$ receptor R1 and R2 subunits (62).

The LC receives rich neuronal afferentations from several brain areas (see Refs. 17, 384, 483). A great variety of putative neurotransmitters and neuropeptides have been localized in the LC or in the "pericoerulear region" (see Refs. 17, 665). That is the region around the LC where the majority of the dendrites of LC neurons extend outside the nucleus, and where the vast majority of the LC afferents terminate. GABA-, glutamate-, and glycine-immunoreactive fibers synapse on LC neurons, both on their intra- or extranuclear dendrites (604). The inhibitory GABAergic axons arise in the prepositus hypoglossal, the glutamatergic axons in the paragigantocellular nucleus of the medulla oblongata (158). CRH-containing axons establish synaptic contacts on dendrites of both TH-positive and TH-negative neurons, mainly in the rostral pole of the nucleus (665, 666). They arise in neurons of the hypothalamic PVN (656) and the central nucleus of the amygdala (665).

Viscerosensory signals reach the LC through the NTS (84). Direct somatosensory afferents arise from cells in the marginal zones of the dorsal horn in the spinal cord and from the sensory spinal trigeminal nucleus (84). The LC also receives afferents from several hypothalamic nuclei (paraventricular, dorsomedial, arcuate, medial preoptic nuclei) (380, 645, 656, 666).

The existence of ADR-containing nerve terminals in the LC was first reported by Hökfelt et al. (242), and a detectable amount of ADR was measured in the microdissected LC (668). Adrenaline axons could be of importance for the inhibitory control of LC noradrenergic neurons. The adrenergic innervation of the LC arises in the RVLM (C1 cell group) and the C3 adrenergic cell group (15, 158, 213, 510). Adrenergic axons of these neurons form the ipsilateral ascending longitudinal bundle (Fig. 9) in the rostral medulla (15). This adrenergic bundle constitutes a part of the "medullary catecholaminergic regulatory circuit" (see sect. III*F1*).

All major forebrain areas (cerebral cortex, olfactory bulb, hippocampus, striatum, thalamus, the basal forebrain, cerebellum) are innervated by LC neurons (see Refs. 17, 166, 179). Neurons in the different subpopulations of the LC project to well-outlined individual targets (377, 378). Histofluorescence, tract-tracing, and electron microscopic studies demonstrated that NA neurons in the LC neurons participate in the innervation of the hypothalamus; however, their contribution seems to be minor compared with those from A1 and A2 neurons (475, 477, 491). By an electron microscopic study, nerve terminals of LC origin were demonstrated in the paraventricular nucleus and the median eminence (488, 491).

The majority of the noradrenergic efferent fibers belong to the ascending catecholaminergic system (Fig. 8). After a short run, LC fibers collected by the dorsal noradrenergic bundle in the pontine tegmentum join the ascending ventral noradrenergic bundle to innervate the cerebral cortex, hippocampus, basal ganglia, thalamus, and a part of the hypothalamus (Fig. 7*A*). Through a separate bundle of fibers, LC axons run into the cerebellum that receives moderate noradrenergic innervation.

Some of the LC and subcoeruleus noradrenergic neurons participate in the descending catecholaminergic system, influencing peripheral CA release, as well as the viscero- and somatomotor responses to stress (Fig. 8). Descending axons to the spinal cord join noradrenergic axons from A5 and A7, and descending adrenergic axons from C1 neurons in the RVLM (Fig. 7*A*), enter the spinal cord in the dorsal portion of the lateral funiculus. From here, the destination and the distribution pattern of LC noradrenergic axons in the spinal cord differ from other descending catecholaminergic fibers: they mainly run downwards in the lamina I and II, and innervate these laminae in the dorsal horn, the intermediate zone, lamina X (182), and IML neurons in the thoracic spinal cord (102, 385, 459). There is evidence for the LC innervation of adreno-medullary sympathetic preganglionic neurons: 4 days after injections of neurotropic virus (pseudorabies) into the adrenal, retrogradely infected cells were found in the LC (619), just as after subcutaneous virus injection into the hindlimb (482).

The LC is very responsive to stress. There is a long list of different types of stress shown to increase firing rate of noradrenergic neurons in the LC (reviewed in Ref. 609). Many types of stress increase TH activity, protein and mRNA levels in the LC (reviewed in Ref. 551). Given the important role of this NE area in responding to stress, a detailed discussion of the stress triggered alterations in gene expression in the LC is presented in section III*G*.

6. A7 noradrenergic cell group

These cells are embedded into the lateral part of the pontine reticular formation, close to the lateral lemniscus, and rostral to the Kölliker-Fuse nucleus (243). A complete overlap between NET mRNA and TH-positive neurons has been demonstrated here (240). A7 NA neurons are densely innervated by local inhibitory GABA neurons (458). They receive neuronal inputs from cells in the periaqueductal central grey and the ventromedial medulla.

The A7 cells provide a descending noradrenergic projection to the spinal cord (102, 182, 353, 458, 686) belonging to the descending catecholaminergic system (Figs. 7*A* and 8). Reportedly, their axons travel in the ventral and

dorsolateral funiculi and mainly terminate in the ventral horn and innervate motoneurons (182, 385). NA neurons in this cell group react to stressful stimuli like other NA neurons that locate in the A5 cell group (485, 487, 507, 574).

C. Central Adrenergic System: Topography and Responses to Stressors

Some of the brain stem catecholaminergic neurons utilize ADR and express NET as a presynaptic membrane transporter. Using immunohistochemistry, Hökfelt et al. (242) first demonstrated the existence of a brain adrenergic system in 1974. Two years later, the content of ADR was measured in a variety of brain nuclei in rats (668). Detailed studies revealed that the wide distribution of adrenergic nerve fibers and terminals arise in neuronal perikarya located in three adrenergic cell groups (C1–C3 cell groups) of the medulla oblongata (243).

The brain stem ADR-synthesizing neurons receive direct inputs from neurons in viscerosensory relay neurons in the nucleus of the solitary tract and/or from the superficial layers of the dorsal horn. In a well-recognized topographical pattern, C1–C3 neurons project to forebrain areas including all of the stress-sensitive nuclei in the hypothalamus and the amygdala, and they contribute to the adrenergic innervation of the periaqeductal gray matter (including the dorsal raphe nucleus), the LC and the spinal cord (14, 114, 243, 489, 510, 541, 563). Adrenaline-containing nerve terminals of brain stem origin synapse directly on sympathetic preganglionic neurons in the thoracic spinal cord (419).

The vast majority of medullary ADR-synthesizing neurons, including those that innervate CRH neurons in the PVN, express glucocorticoid receptors (563).

1. C1 adrenergic cell group

Forming a chain of separate groups (217, 243), C1 ADR cells occupy a part of the ventrolateral medulla from the level of the obex up to the pons/medullary border. In both rostral and caudal directions, adrenergic neurons intermingle with noradrenergic neurons of the A5 and the A1 cell groups, respectively (Figs. 7*A* and 9). Some of the C1 adrenergic neurons are located very close to the ventral surface of the medulla (14, 114, 277, 278).

The majority of C1 adrenergic neurons coexpress $GABA_B$ R1 and R2 subunits (62), glutamic acid decarboxylase mRNA (615), vesicular glutamate transporter (DNPI/VGLUT2) mRNA (617), CGRP (63), and preproenkephalin mRNA (616). Some of the C1 neurons (~13% of the total C1 cells) express NET mRNA (104).

The C1 neurons receive viscerosensory inputs through the NTS (567) and somatosensory inputs from the spinal cord through the spinoreticular tract. Enkephalin- and GABA-containing nerve terminals of medullary origin establish synaptic contacts with C1 neurons (420).

The C1 adrenergic neurons belong to both the ascending (caudal part of the cell group; C1c) and the descending (rostral part of the cell group; C1r) catecholaminergic systems (Figs. 7*A* and 8). Efferent fibers from the C1c ADR neurons run together with noradrenergic fibers within the ventral noradrenergic bundle up to the ipsilateral forebrain (Fig. 7*A*). Unilateral brain stem transections eliminate adrenergic fibers and nerve terminals almost completely in the PVN, the lateral hypothalamus, and the central nucleus of the amygdala, at the side of the transection (489).

Adrenergic neurons in the rostral part of the cell group provide innervations of sympathetic preganglionic neurons of the IML in the thoracic spinal cord (419, 424, 537, 660). It has been demonstrated that some of these sympathetic preganglionic cells innervate the adrenal medulla (685).

ADR neurons in the rostral C1 cell group react sensitively to immobilization stress and pain, as it is indicated by rapid and strong Fos activation in the nuclei of these neurons (574). Foot-shock also activates Fos expression in the CVLM, both in noradrenergic A1 and adrenergic C1 neurons (365, 507). Other stressors, like hemorrhage, also significantly induce Fos (23, 135) and subsequently PNMT expressions (366) in C1 neurons.

2. C2 adrenergic neurons

A relatively small number of ADR neurons distribute sparsely to the dorsomedial medulla. The territory of C2 ADR neurons partly overlaps with that of A2 NA cells, but the majority of ADR cells are more rostrally located, mainly in and around the medial subdivision of the NTS and some of them among the neurons of the dorsal motor vagal nucleus (Figs. 7*A* and 9). About 10% of C2 neurons express NET mRNA (104). The VMAT2 mRNA expressing cells in this area, other than those that express NET, may represent adrenergic (C2) neurons (240).

The C2 adrenergic neurons belong to the ascending catecholaminergic system (Fig. 8). Fibers from the C2 cell group join the ascending ventral noradrenergic bundle (Fig. 7*A*) and innervate hypothalamic and amygdala nuclei (114, 489).

Hemorrhage, immobilization, and audiogenic stress have been demonstrated to act on C2 neurons (226, 329, 545, 546).

3. C3 adrenergic neurons

These cells represent the most rostral adrenergic neurons in the lower brain stem (Figs. 7*A* and 9). They occur mainly in the paramedian reticular nucleus and among the fibers of the medial longitudinal fascicle. The periventricular portion of the C3 cells project to the hy-

pothalamic PVN, while cells in the ventral portion project to the spinal cord (424, 531) and innervate adrenal-projecting sympathetic preganglionic neurons in the IML (685). In addition, a certain percentage of C3 cells innervate the locus coeruleus (15, 158, 510) and the preganglionic parasympathetic neurons in the dorsal motor vagal nucleus (278, 374).

D. Central Dopaminergic System and L-DOPA Neurons in Stress

The general knowledge about the role of brain-born DA in the central mechanism of stress response is controversial. In fact, all of the brain areas that significantly participate in stress have strong or very strong dopaminergic innervations. In contrast, most of the dopaminergic neurons in the brain (except DA cells in the hypothalamic arcuate nucleus) fail to respond to acute stressor with c-Fos expression.

Some of the TH-positive, DBH- and PNMT-negative neurons, both in the forebrain and the brain stem, do not express AAAD, the enzyme converting L-DOPA to dopamine (267). These cells may use L-DOPA, rather than DA, as their neurotransmitter (409) and do not express DAT mRNA (241). They occur in the lateral part of the NTS and the dorsal motor vagal nucleus (among A2 neurons), the pontine tegmentum, and the ventrolateral subdivision of the arcuate nucleus (267), and they are scattered in the anterior and posterior hypothalamus, among A11 and A15 neurons (Fig. 7*B*).

Dopaminergic neurons in the brain are organized into three major systems (Fig. 7*B*): mesencephalic (A8, A9, A10), diencephalic (A11, A12, A13, A14, A15), and olfactory (A16) DA systems (42, 118, 166, 243, 628). Dopaminergic fibers from these cell groups run either in well-organized and target-specific bundles (Table 1) or participate in other neuronal pathways innervating practically the entire CNS.

The mesencephalic DA cell groups comprise three systems: mesostriatal (nigrostriatal), mesolimbic, and mesocortical (for detailed distribution of DA cells and their fine projection patterns, see Refs. 42, 243).

Hypothalamic dopaminergic axons of A12 and ventrocaudal A14 origin establish an extraordinarily dense network of fibers and varicosities in the median eminence and a fine distribution throughout the posterior lobe of the pituitary. Varicose DA axons do not form synaptic connections here, but they are in close topographical contact with noncatecholaminergic axons, ependymal cells (tanycytes) in the pericapillary space (4). These dopaminergic terminals in the median eminence, along with noradrenergic terminals from the lower brain stem (491), support the hypothesis that in certain condition, including acute stress response, CA released from the nerve terminals may influence the release of other substances from the neighboring peptidergic (releasing or release-inhibiting hormone-containing) terminals in the median eminence. Dopamine released into the pericapillary space diffuses through fenestrated capillaries and transported via hypophysial portal blood into the anterior lobe. Thus CA may exert their effects on the hypothalamic neurosecretory system in two target areas, namely, innervating neurohormone-producing cells in the hypothalamus and influencing of the release of neurohormones from the nerve terminals in the median eminence and the pituitary gland.

Several conflicting results have been reported about the possible involvement of brain DA neurons in stress response (see Refs. 176, 233, 620). Acute stressful stimuli, like immobilization, cold, hypoglycemia, or hypovolemic hemorrhage, do not elicit c-Fos activations in DA neurons in the brain. In contrast, 60 min after formalin-induced painful stimuli some of the TH-positive tuberoinfundibular DA neurons (A12 cells) express c-Fos (474). These DA neurons innervate the posterior pituitary.

The A13 DA neurons in the medial part of the zona incerta that project to the hypothalamic paraventricular nucleus and the central nucleus of the amygdala (91, 138) do not express c-*fos*, while the other immediate early genes show rapid activation in response to acute stressors (112, 474).

DA neurons may influence the activity of sympathetic preganglionic neurons in the IML. Besides NA and ADR, DA also participates in the dense innervation of the IML cell column. These descending DA fibers arise, at least mainly, in DA neurons of the A11 cell group.

It should be noted that repeated immobilization stress significantly elevates TH mRNA levels in the VTA and the substantia nigra in rats (576, 578). Reportedly, exercise training doubled TH mRNA levels in these dopaminergic cell groups of young adult but not in 24-mo-old rats (661). Conversely, chronic cold stress leads to a reduction of spontaneous activity in the VTA (427).

In chronic stress-induced situations (social challenges, conditioned stimuli, audiogenic stress) as well as in psychopathological conditions like depression and schizophrenia, the functional significance of brain DA systems is unquestionable. It appears that the mesocortical DA system is more sensitive to stress than the mesolimbic or the mesostriatal DA systems: DA neurons are activated in the VTA in response to above-mentioned stimuli (11, 169, 226, 428, 457, 620). The mesocortical and the mesolimbic DA pathways that arise in A10 DA cell group have been implicated in memory, learning, and emotional processes. Mesocortical DA also plays an adaptive role in the extinction of conditioned fear behavior and prevents excessive physiological stress reactivity (620). DA release is elevated in the medial prefrontal cortex in response to acute and chronic stress, even handling or short mild pain in-

duce increase in extracellular DA levels in this cortical area (3, 171, 620).

The regional patterns of brain DA activation after certain physical and psychological stressors in the limbic cortical areas might relate to behavioral changes in response to stressful stimuli.

Thus conflicting results in stress-induced changes in DA levels and gene expression of CA enzymes and DAT suggest that the effects depend on the severity and controllability of the stressor as well as the genetic background and life history of the animals.

E. Catecholamine Transporters in Stress

As indicated in section II*C*, the removal of CA by active transport from the synaptic cleft back into presynaptic neurons via NET and DAT is the primary mechanism for termination of the action of released CA. VMATs package monoamines into synaptic and secretory vesicles. After synthesis, VMATs are localized in the perikarya and proximal dendrites while NET and DAT are transported by NA or DA axons from the perikarya to the nerve terminals, respectively. Thus both NET and DAT are considered characteristic markers for NA and DA neurons in the brain. Indeed, there is a complete overlap of NET mRNA, TH-positive, and DBH-positive neurons in all of the brain stem noradrenergic cell groups (240, 241, 376).

NET is expressed only in a small percentage of neurons that are both TH- and PNMT-positive, suggesting that ADR cells may have their own unique transporter (104, 240, 241). In most DA cells (especially in the substantia nigra/ventral tegmental area), DAT mRNA completely overlaps with TH mRNA-positive neurons. In other brain areas (in the hypothalamus and the pons), there is a lack of one to one correspondence (101, 240, 241). Transections of catecholaminergic axons result in a complete disappearance of CA from the nerve terminals and temporary overexpression and accumulation of CA in the perikarya and in the transected axons proximal to the cells. In contrast, transections of NET fibers of LC origin, or DAT fibers from A9, A10, or A13 dopaminergic cells drastically diminish transporter expressions in the perikarya of these cell groups after a very short period of time (240, 241). This type of alteration in the expression of NET or DAT in axon-transected neurons clearly indicates that intact axons contact with the target, i.e., some sort of feed-back signals are required for the synthesis of CA membrane transporters in the brain.

Stress-induced changes in levels and gene expression of CA transporters in the brain neurons were described in numerous reports. Hemisection of the median forebrain bundle causes coordinate changes in both DAT and TH mRNA. However, under stress situations (immobilization), differential increases in these two mRNAs are produced in different DA neurons (240, 241). This suggests stimulus-specific regulation of TH and DAT mRNA expression. A mouse model was developed in which the DAT gene was inactivated by homologous recombination (193) and mice lacking DAT show hyperactive behavior.

Pain-induced (subcutaneously injected 4% formalin) stressful stimuli increased DAT mRNA expression parallel with TH mRNA expression in the A14 cell group (periventricular nucleus) in the hypothalamus, but failed to exert such effects on tuberoinfundibular A12 DA neurons in the arcuate nucleus. The A14 neurons represent the periventricular-hypophysial dopaminergic system, and axons of these neurons terminate on melanotrophs in the intermediate lobe of the pituitary. Thus the pain-induced stress response in these neurons could be the consequence of increased plasma ADR level, which, in turn, releases α-MSH from the pituitary by activation of β2-receptors on melanotrophs (240, 241).

In contrast to pain, acute immobilization stress increased both TH and DAT mRNA in the tuberoinfundibular A12 dopaminergic neurons in the dorsomedial subdivision of the arcuate nucleus. In addition to these neurons, elevated DAT mRNA expression was detectable in spinal cord-projecting A11 and incerto-hypothalamic A13 dopamine neurons, but not in the midbrain (A8-A10) dopaminergic system (240, 241).

Enhanced DAT mRNA together with TH mRNA levels in the ventral tegmental area of midbrain has been found in mice with repeated aggression experience of social victories (winners) compared with social defeats (losers) or to control mice (169). Thus a positive correlation between DAT mRNA and TH mRNA levels was shown.

Brain DAT binding sites were also studied in tree shrews subjected to psychosocial stress for 28 days (260). Chronic stress reduced the number of binding sites in the caudate nucleus and the putamen without affecting their affinity. This stress, however, did not influence the binding parameters in the nucleus accumbens, the substantia nigra, or the ventral tegmental area. The chronic exposure to psychosocial stressors decreased DAT binding sites in motor-related brain areas, suggesting that the reduction in locomotor activity in subordinated tree shrews is related to the downregulation of DAT binding sites.

Rusnak et al. (543) suggested a stressor-specific and brain area-specific regulation of TH, NET, and VMAT-2 gene expression. The data indicated that repeated exposure of rats to a homotypic stressor induced an adaptation of NA neurons, whereas a single exposure of such animals to heterotypic novel stressors produced an exaggerated response of the system at the mRNA level of TH (in the locus coeruleus) and NET (in A1 and A2 cell groups) in brain areas.

NET mRNA levels were unaltered, while TH mRNA levels were increased in LC of two strains of rats (Wistar and Wistar-Kyoto) after exposure to an acute restraint

stress (559). Zafar et al. (707) measured NET in several limbic brain regions. While acute restraint stress had no effect on NET binding sites, repeated restraint decreased NET sites in the amygdala, hypothalamus, and the locus coeruleus.

Behavioral responses to social stress in NET knockout mice were also studied (216). NET gene disruption inhibited depression-like behavior in chronically stressed mice tested in a situation heterotypic to the original cause of chronic stress. The authors suggest that the behavioral effects of NET gene disruption were overruled by experience and learning in the homotypic situations but manifested fully in the heterotypic situations.

Chronic stress can precipitate depressive symptoms in humans (219) and prolonged exposure of rats to various stressors results in many neuroendocrine and behavioral characteristics observed in human depression (55). Chronic cold stress increased the plasmalemmal distribution of NET and the coexpression of TH in NA axons in the prefrontal cortex (422). The proportion of NET nearly doubled in chronically cold stressed versus control rats. These data represented the first demonstration of activity-dependent trafficking of NET and expression of TH under physiological stress conditions and can have important implications for understanding the pathophysiology and treatment of stress-related affective disorders (422).

Exposure to repeated swim stress resulted in significant reduction in VMAT-2 density in nucleus accumbens and some of the striatal subregions (716). The downregulation of VMAT-2 in these dopaminergic regions may serve as an adaptation mechanism in the response to prolonged stress and may be relevant to chronic stress-induced depression.

Recently, the localization of the extraneuronal monoamine transporters in rat brain have been described (189, 214).

Data available clearly show that the inactivation of released catecholamine in response to stress is essential for synaptic signal transduction. In certain conditions, NET and DAT efficiently remove released EPI, NE, and DA from the synaptic cleft. Several observations support the hypothesis that physiological and behavioral responses to stressful stimuli are coordinated with catecholamine transportation (release, reuptake, degradation) in the brain. For better knowledge of the coordination of action, further investigations are required to elucidate the changes of major parameters, like timing and brain pattern of transport mechanisms, the coordination of the rate of synthesis and metabolism of brain catecholamines versus catecholamine transporters before and during stress, and the possible participation of extraneuronal monoamine transporters in the inactivation of the released catecholamines.

In summary, stress-induced changes in levels and gene expression of CA transporters are good indicators of specific activation of different CA neurons, especially in the brain.

F. Stress-Related Central Catecholaminergic Pathways

Stressful stimuli may reach the central nervous system through somato- and viscerosensory fibers of spinal or cranial nerves. Somatosensory signals are detected by noxious, mechanical, thermal, or specific (photic, acoustic, gustatory, equilibral) peripheral receptors. Viscerosensory signals arise from the body and may reach spinal and supraspinal neurons through neuronal or humoral (through the circulation) pathways. From here, sensory signals may participate either in short-loop reflexes (spinal and supraspinal) or long-loop neuronal circuits. The spinal short loop is based on spinal reflexes: afferent signals to the spinal sympathetic preganglionic neurons in the IML cell column arise in primary sensory fibers of dorsal root ganglion cells and are relayed by dorsal horn interneurons (65). In contrast to supraspinal neuronal circuits, CA may not be involved directly in this reflex mechanism. The long-loop system consists of ascending (afferent) pathways to forebrain integrative centers, like the hypothalamus, the limbic system, and cerebral cortical areas. The descending (efferent) neuronal pathways carry stress response signals from the integrative forebrain centers to the periphery. The efferent (or output) system involves two major routes: humoral (activation of the HPA axis) and neuronal, exerting their effects through brain stem or spinal autonomic (autonomic preganglionic) and/or somatomotor neurons (Fig. 8). Descending fibers from the hypothalamus may reach sympathetic preganglionic neurons in the thoracic spinal cord directly (monosynaptically), or they may be relayed in lower brain stem catecholamine (A5, C1) cell groups (Figs. 8 and 11). In addition, further brain stem CA neurons in the A6 and A7 cell groups, and noncatecholaminergic neurons in the rostral ventromedial medulla, and serotoninergic medullary raphe nuclei project to the sympathetic preganglionic neurons. All of these neurons are referred to as sympathetic premotor neurons (433). The sympathetic preganglionic neurons in the IML project to the para- and prevertebral sympathetic ganglia (sympatho-neural system), or to chromaffin cells in the adrenal medulla (sympatho-adrenomedullary system) (Fig. 11). NA is released mainly from the postganglionic nerve endings, while the adrenal medulla is the major source of ADR in the circulation. The majority of the sympathetic preganglionic neurons that project to the adrenal medulla are found in thoracic segments T5–T9 (22, 142, 434, 618, 619).

Distinct populations of sympathetic preganglionic neurons innervate ADR- or NA-synthesizing chromaffin cells (142, 433, 434). One group of adrenal projecting IML

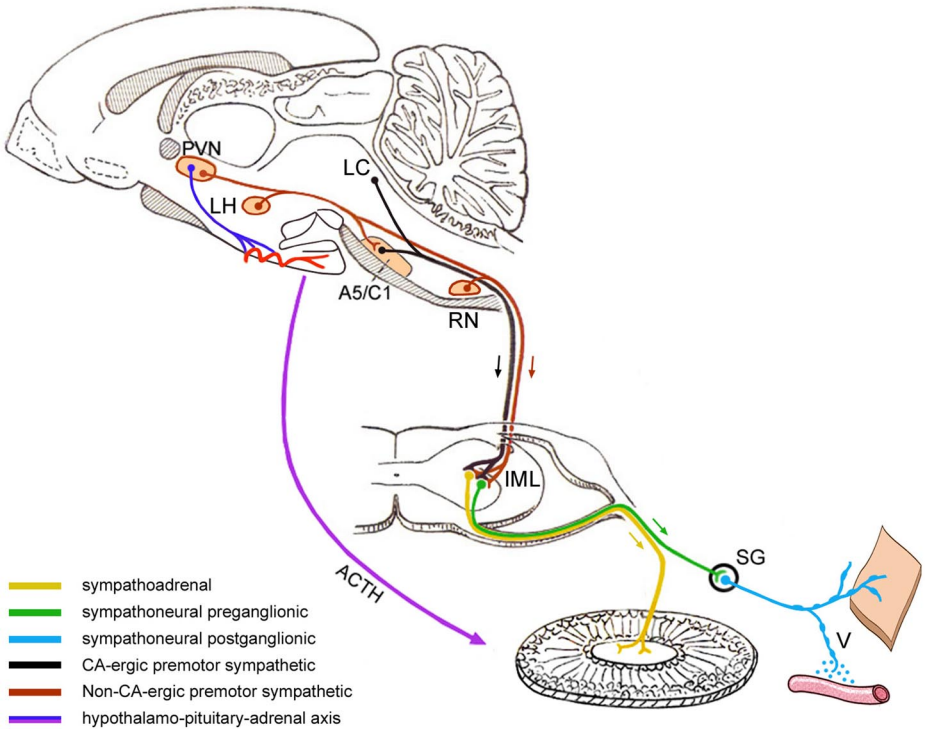

FIG. 11. Humoral (hypothalamo-pituitary-adrenal axis) and neuronal (hypothalamus-brain stem-sympathetic preganglionic) projections from the hypothalamus to the adrenal medulla and sympathetic ganglia. A5, A5 noradrenergic cell group; C1, C1 adrenaline cell group; IML, intermediolateral cell column; LH, lateral hypothalamic area; LC, locus coeruleus; PVN, paraventricular nucleus; RN, medullary raphe nuclei; SG, sympathetic ganglion; V, varicosities of the postganglionic neuron.

neurons has little sensitivity to baroreceptor reflex stimulation from the RVLM, but they are strongly stimulated by 2-deoxyglucose. They have a role in regulation of ADR secretion from adrenal chromaffin cells. The other group of IML neurons is sensitive to baroreceptor reflex activation but unaffected by administration of 2-deoxyglucose. These neurons innervate NA-producing chromaffin cells in the adrenal medulla (434).

Catecholamines may participate in both ascending and descending signal routes. While the ascending catecholamine system, which includes A1, A2, A6, caudal C1 and C2 neurons, influences the activity of neurons in the forebrain integrative centers during stress, the descending catecholamine system (A5, A7, some A6, and rostral C1 neurons) serves as the major connection between the brain and the peripheral catecholaminergic system in the realization of stress responses (Fig. 8).

The sympathetic preganglionic neurons form a longitudinal cell line in the thoracic and the first lumbar spinal cord, at the lateral portion of the central grey matter (Fig. 10, *C* and *D*). In addition, scattered sympathetic preganglionic neurons are found in the nucleus intermediomedialis (also called "central autonomic area") just medial to the IML, and in the lateral funiculus, lateral to the IML (Fig. 10*D*). In the IML, preganglionic sympathetic neurons are topographically organized with respect to their tissue/organ targets (107, 523). Sympathetic innervation to structures in the head and the neck is supplied by ganglionic neurons primarily in the superior cervical ganglion, which is innervated almost exclusively by sympathetic preganglionic neurons situated in segments T1–T4 (107, 526). They are located mainly in the medial and intermediate aspects of the IML cell column (524). Sympathetic preganglionic neurons that innervate ganglion cells in the celiac, superior, and inferior mesenteric ganglia are located in segments T8–T12 (107, 523).

Signals to preganglionic sympathetic neurons arise from spinal and cranial sensory fibers via interneurons, noncatecholaminergic descending inputs from the hypothalamus and the limbic system (Fig. 11). The major input arises from the sympathetic premotor neurons in the rostral ventrolateral medulla, including A5 noradrenergic and rostral C1 adrenergic neurons. In addition, some noradrenergic fibers to the IML arise in the caudal portion of the locus coeruleus (see Ref. 482). The IML cell column also receives dopaminergic innervations, mainly from the A11 cell group (see sect. III*D*).

The preganglionic efferent fibers leave the spinal cord through the ventral roots and terminate on sympathetic ganglion cells and/or chromaffin cells in the adrenal medulla. According to their termination and functional role, the postganglionic sympathetic system (also called sympatho-adrenal system) can be divided into two categories: *1*) chromaffin cells in the adrenal medulla constitute the peripheral portion of the sympatho-adrenomedullary system, which is mainly responsible for the synthesis of the circulating ADR and ~30% of the NA in the plasma, and *2*) sympathetic ganglion cells in the paravertebral (superior cervical, stellate ganglia) and prevertebral sympathetic ganglia that synthesize and release the

vast majority of the circulating NA, called sympatho-neural system (Fig. 11). These two systems constitute the final compartments of the effector loop in the stress response (Fig. 8). The sympatho-adrenomedullary system (ADR release) responds to immobilization, hypoglycemia, hypotensive hemorrhage, emotional distress, shock, and fear, while the sympatho-neural system (NA release) would play key roles in response to cold exposure, exercise, immobilization, active escape, nonhypotensive hemorrhage, pain, glucoprivation, avoidance, altered salt intake, and water immersion (201).

1. Brain stem neuronal circuits

The brain stem neuronal circuits represent the link between sensory and autonomic neurons in the medulla oblongata that finally influence the sympathetic and parasympathetic outflows. Noradrenergic and adrenergic neurons play essential functional roles in both neuronal circuits.

A) VAGAL NEURONAL CIRCUIT. Viscerosensory signals, through the vagus nerve, and partly through the glossopharyngeal and facial nerves, reach interneurons and projection neurons in the NTS (route 1 in Fig. 12, and 1a in Fig. 9). In addition, NTS neurons receive afferent input from spinal dorsal horn neurons, (spino-reticular tract, route 1a in Fig. 9) (163, 412). NTS projection neurons provide ascending signals to forebrain integrative centers, while the interneurons transfer information to efferent preganglionic neurons in the dorsal motor vagal nucleus (route 4 in Fig. 12). These parasympathetic preganglionic fibers terminate in intramural parasympathetic ganglia that innervate almost all of the visceral organs in the body. Collaterals of sensory vagal fibers also innervate noradrenergic (A2) and adrenergic (C2) neurons located within or around the NTS (Fig. 12). Having a high number of collaterals and nerve terminals, these local catecholamine cells may innervate both projection and interneurons in the NTS, as well as dorsal motor vagal neurons (fiber 3 in Fig. 12), influencing their sensitivity to incoming viscero-sensory signals, including stressful stimuli.

Some of the NTS neurons project downward and their axons terminate in the spinal cord (solitario-spinal tract). These neurons are not catecholaminergic. They represent the shortest route other than the intraspinal reflex between primary visceral afferents and viscero- and somatomotor neurons (439).

B) DORSOMEDIAL-VENTROLATERAL NEURONAL CIRCUIT. This neuronal circuit constitutes the morphological basis of the supraspinal viscerosensory-visceromotor regulatory mechanism. Catecholamines participate in this neuronal circuit that represents one of the most important controls of the sympathetic outflow from the spinal cord. The RVLM contains spinal cord-projecting noradrenergic (A5), adrenergic (rostral C1), and noncatecholaminergic neurons (see Refs. 538, 624). These neurons receive mono- and multisynaptic neuronal inputs from the dorsolateral medulla, mainly from the NTS (route 3 in Fig. 9) (85, 283, 709), from neurons located in the caudal ventrolateral

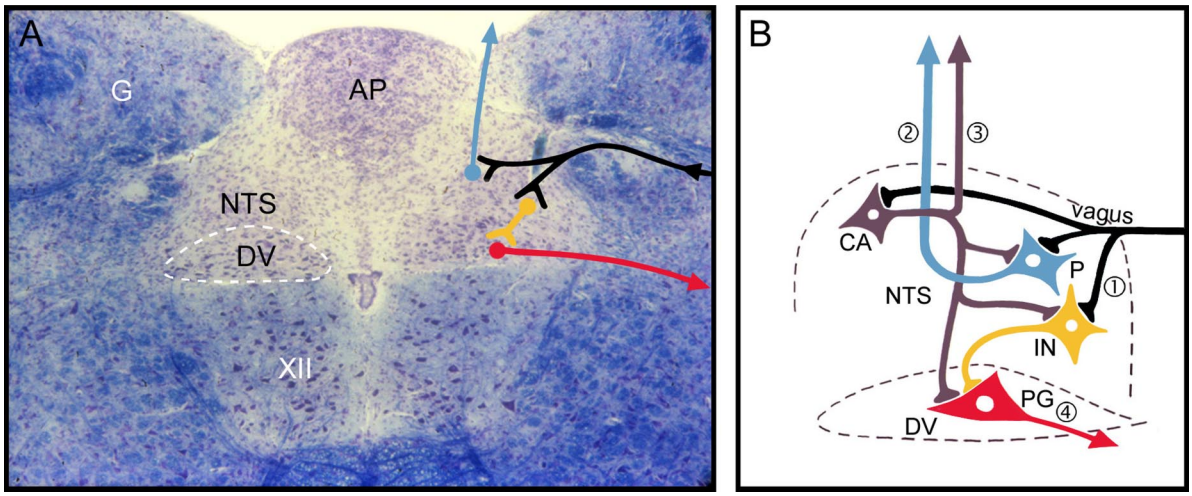

FIG. 12. Inputs of viscerosensory signals to the primary autonomic center, the nucleus of the solitary tract (NTS). Signals reach the NTS mainly through the vagus nerve, and partly via the glossopharyngeal, facial, and trigeminal nerves. *A*: coronal section of the medulla oblongata at the level of vagal nuclei and the area postrema. Luxol fast blue-cresyl violet staining. *B*: terminal pattern of vagal afferents: *1*) Within the NTS, terminals of axon collaterals from the vagus nerve synapse on interneurons (IN) where signals are relayed to parasympathetic preganglionic neurons (PG) in the dorsal motor vagal nucleus (DV), representing a three-neuronal viscerosensory-visceromotor reflex arc. *2*) Vagal fibers terminate on projection neurons of the NTS. Fibers of projection neurons may terminate in brain stem nuclei (the parabrachial nuclei are the major targets) and in the forebrain (hypothalamus, limbic areas, cerebral cortex). *3*) Vagal presynaptic terminals on catecholaminergic (A2 noradrenergic and C2 adrenergic) neurons (CA) located in the territory, or in the close vicinity of the vagal nuclei. CA axon collaterals may innervate neurons in the NTS and the DV, and join the ascending catecholaminergic bundles and terminate in forebrain areas (*3*). *4*) Efferent fibers of dorsal vagal preganglionic neurons to the visceral organs ("periphery"). AP, area postrema; DV, dorsal motor vagal nucleus; G, gracile nucleus; XII, motor hypoglossal nucleus.

medulla (CVLM), from cells in the spinal cord relayed by CVLM neurons (271), and descending fibers participating in this neuronal circuit are not catecholamine but GABAergic neurons (354, 396, 622). These cells may receive signals from the NTS (Fig. 9) (85, 490) and from marginal cells in the spinal cord dorsal horn (spinoreticular tract) conveying noxious and thermal messages to the caudal part of the medulla (367, 413, 642). Besides GABAergic CVLM projections, local GABA interneurons in the RVLM synapse adrenergic and nonadrenergic neurons (420). These GABAergic interneurons in the RVLM are innervated by CVLM neurons (route 4 in Fig. 9).

A small group of cells in the commissural part of the NTS project to the RVLM (Fig. 9) and innervate rostral C1 adrenergic neurons (538). This input appears excitatory and glutamatergic (624), which is inhibited locally by GABAergic interneurons. C1 adrenergic neurons in the RVLM, but none of those located in the CVLM, send descending efferent projections to spinal preganglionic sympathetic neurons (537, 563, 660).

In addition to the NTS-CVLM-RVLM neuronal circuit, C3 adrenergic neurons in the territory of the paragigantocellular reticular nucleus receive neuronal inputs from the NTS (route 5 in Fig. 9) and project to the LC (15, 510). Locus coeruleus neurons receive cardiovascular information, most likely involving NTS projections relayed through the RVLM (Fig. 9) (249, 510).

2. Afferent signal routes to integrative centers

Brain regions that participate in the integration of stress responses can be classified into two major categories: the neuroendocrine hypothalamus and the limbic system. Both of them are bilaterally connected with the lower brain stem and the spinal cord, with brain stem catecholamine neurons, and are also interconnected with each other.

A) ASCENDING NEURONAL PATHWAYS. Visceral and somatic sensory signals are carried to the central nervous system by spinal and cranial afferents. Spinal viscerosensory afferents converge onto dorsal horn neurons and in lamina X around the central canal. Axons from neurons in the lamina VII and X ascend and provide direct and relayed inputs to the nucleus of the solitary tract, lower brain stem, and the hypothalamus.

Three major types of fibers ascend from the spinal cord and the lower brain stem to the forebrain integrative centers: *1*) noncatecholaminergic spinal, *2*) noncatecholaminergic brain stem, and *3*) catecholaminergic brain stem neurons (Fig. 8). In certain conditions, all of these three cell types may relay stressful signals to forebrain neurons. Furthermore, neurons in these three systems may interact, anatomically and functionally, both at spinal/medullary and forebrain levels.

1) Direct (monosynaptic) pathway from spinal sensory neurons terminates in the hypothalamus (spino-hypothalamic tract), and multisynaptic pathways reach limbic and neocortical areas (spino-thalamic and spino-reticulothalamic tracts). The majority of fibers in the spino-hypothalamic tract synapse in the lateral hypothalamic area and relayed to various hypothalamic nuclei, mainly around the paraventricular nucleus.

2) The ventrolateral medulla (peritrigeminal and lateral reticular nuclei) contains stress-sensitive, TH-negative neurons. Some of these neurons constitute the medullary thermoregulatory area and respond to cold stress by rapid c-Fos activation (56). Viscerosensory information enters the medulla via trigeminal, facial, glossopharyngeal, and vagus nerves that terminate in a topographically oriented pattern throughout the NTS (560). In addition, spinal somatosensory fibers also terminate on peptidergic neurons of the NTS (spino-solitary tract). These non-CA NTS neurons, in turn, project to A1, A5, and C1 CA neurons in the ventrolateral medulla (Fig. 9), to the hypothalamus and different compartments of the limbic system (see Refs. 86, 233, 484). Some of the afferent signals reach the NTS and neurons in the ventrolateral medulla through the area postrema (route 1c in Fig. 9) (115, 586).

3) The ascending catecholamine system includes A1, A2, A6 (locus coeruleus) noradrenergic, and C1, C2 adrenergic neurons (Fig. 8). These neurons project to the forebrain and terminate in the hypothalamus and various compartments of the limbic system (484, 491, 503, 530). Axons from caudal medullary neurons travel in the ventral noradrenergic bundle, while fibers from the LC ascend in the dorsal tegmental bundle (Table 1). At the hypothalamic levels, axons from both of these pathways follow their way in the dorsal periventricular system to their hypothalamic, limbic, or cortical destinations. Lesions of the ventral noradrenergic bundle block or attenuate responses to various physical, viscerosensory, and somatosensory (161, 187, 476, 486) but not psychological or emotional stressful stimuli (365).

B) STRESS-RELATED NEURONAL AFFERENTS TO THE HYPOTHALAMUS. Several hypothalamic nuclei participate in the organization of responses to different stressors (see Refs. 129, 231, 233, 474, 477, 564, 565). A great variety of stressors activate neurons in the parvocellular subdivisions of the PVN. These neurons synthesize CRH and vasopressin and release them into the median eminence and represent the neuronal compartment of the so-called final common pathway in the neurohormonal regulation of the ACTH-corticosterone and ACTH-ADR systems in the anterior pituitary and the adrenal gland (Fig. 12). Glucocorticoids inhibit the HPA axis activity by a negative-feedback mechanism (227, 231, 254, 262, 388).

CRH mRNA expression is tonically stimulated by NA and ADR pathways (302). The central CA system stimu-

lates the HPA axis both at perikaryonal (synthesis in the PVN) and also at terminal (release from the median eminence) levels. One of the most extensive excitatory inputs to the PVN arises in medullary NA neurons. NA nerve terminals of lower brain stem origin are also present in the median eminence (488). Here, NA facilitates CRH (632) and vasopressin release into the portal vessels of the median eminence, from where they are transported into the anterior pituitary. Acute stress activates the NA system, which is accompanied by transient activation of the HPA axis.

Neurons in the magnocellular paraventricular and supraoptic nuclei, in the preoptic periventricular nucleus are sensitive to stressors influencing body water and electrolyte homeostasis (hypovolemic stress). Neurons in the preoptic area are very sensitive to thermal stressors. The pain- and restraint-sensitive supramamillary neurons constitute one of the major links between the hypothalamus and the limbic (septo-hippocampal) system.

Selected caudal ventrolateral (A1) and dorsomedial (A2) medullary NA neurons project to the hypothalamus (see Refs. 116, 233, 474, 482, 484, 567). These neurons express TH and α_2-adrenergic receptor, an inhibitory autoreceptor located presynaptically in NA terminals in the hypothalamus. Adrenergic axons with hypothalamic destinations arise in neurons located in the caudal portion of the C1 cell group and in some of the C2 neurons (114). CA nerve terminals establish synaptic contacts on parvocellular neurons of the PVN (368).

Neurons in the rostral subdivision of the LC neurons also participate in NA projections to the hypothalamus (116, 491, 567, 630). There are a fairly high number of stressful stimuli that increase the firing rate of NA neurons in the LC (see Refs. 543, 608).

There is a great deal of evidence for the stimulatory role of brain stem CA on the activity of the HPA axis promoting CRH and ACTH release and CRH gene transcription through activation of adrenergic receptors in response to stressful stimuli (121, 231, 233, 261, 474). Interruption of brain stem projections to the PVN blocks the responses of the HPA axis to physical but not emotional or psychological stressors (365). Unilateral brain stem transections demonstrate that NA fibers travel without any further decussations from the lower brain stem to the hypothalamus. The concentrations and the release of NA as well as the expression of CRH and CRH receptor mRNAs are reduced significantly on the lesioned side of the PVN, but remained unchanged contralateral to the knife cut (388, 476, 478, 486, 562). Glutamatergic interneurons in the PVN may serve as excitatory relays in the afferent NA fibers on CRH secretion (167). At high concentrations, NA can be inhibitory on CRH neurons by acting via β-adrenergic receptors (see Ref. 176).

CRH neurons are directly innervated by GABAergic neurons around the PVN (so-called peri-PVN region). The majority of CRF neurons express multiple $GABA_A$ receptor subunits (110). GABA inhibits the HPA axis, and an inhibitory GABAergic tone exists in the PVN. Excitatory (mainly glutamatergic) inputs to the peri-PVN region from limbic areas, like ventral subiculum, prefrontal (infralimbic) cortex, or amygdala can block the GABA inhibition on the HPA axis (232–234, 251).

PVN neurons receive input from other hypothalamic cell groups, such as the medial preoptic area, the dorsomedial, arcuate, ventromedial or suprachiasmatic nuclei (111, 112). They may act through interneurons in the peri-PVN zone or act inside the PVN. Stressors crucial for immediate survival may act directly on PVN neurons (actions more likely through CA). The hypothalamus, especially the PVN, receives additional neuronal inputs from limbic and cortical regions (see below).

c) STRESS-RELATED SIGNALS TO THE LIMBIC SYSTEM. Both cortical and subcortical limbic structures are sensitive to stressful stimuli, and they are involved in the integration of stress response. A great variety of behavioral responses to stressful stimuli are organized by the limbic system. The subcortical limbic areas include the amygdala, the bed nucleus of the stria terminalis, the septum, one part of the nucleus accumbens, and the substantia innominata, while the medial prefrontal (pre- and infralimbic cortex), anterior cingulate and piriform cortex, as well as the hippocampus (including the ventral subiculum) are classified as limbic cortical areas. Several components of the limbic system are connected to the hypothalamus (207). The septum constitutes an interface between the hypothalamus and the hippocampus: the supramamillary-lateral septal-hippocampus pathway is frequently activated by different stressors.

The amygdala is a key structure within the limbic neuronal circuits. It receives inputs from the hippocampus and the prefrontal cortex that are crucial for acquisition, consolidation, and modulation of memories relative to stressful stimuli (33) and is also widely regarded as being involved in the integration of responses to stressful stimuli. The different amygdaloid nuclei operate in a stress-specific manner. It is more likely that the central nucleus of the amygdala is involved in the neuronal circuitry necessary for associative learning, organizing emotional, anxiety- or fear-related behavioral responses, but not in mediating of responses to physical stressors. Psychological stress elicits an increase in the expression of CRH mRNA in the central nucleus of the amygdala but not in the PVN (389). It is more likely that the central nucleus plays a stressor-specific modulatory role in regulation of HPA axis promoting both CRH synthesis and release in the PVN (see Ref. 235). Recent studies, however, suggest that the central nucleus of the amygdala may modulate the activity of the HPA axis, but is not essential to the stress response. Lesions of the central amygdala nucleus do not affect corticosterone or ACTH secretion induced

by acute stress. The basal CRH mRNA expression is increased in the PVN of central amygdala lesioned rats, but acute restraint does not elicit increased CRH mRNA expression. (520). Adaptation to repeated stress is only modestly dependent on the amygdala (78).

In contrast to the central nucleus, the medial nucleus of the amygdala activates the HPA axis responses to emotional stressors (122, 157, 506).

The noncatecholaminergic inputs from the lower brain stem to the amygdala arise in the NTS. Viscerosensory information from the NTS is relayed in the lateral parabrachial nucleus to the central nucleus of the amygdala (see Refs. 560, 561). Furthermore, the amygdala has access to multimodal sensory inputs from the thalamus and the neocortex.

The locus coeruleus provides the major NA innervation to the amygdala (166). In addition, caudal ventrolateral (A1) and dorsomedial (A2) medullary noradrenergic neurons also project to the amygdala (274, 503, 534). The relatively dense network of ADR fibers arises in both C1 and C2 neurons (489). Acute or repeated immobilization (471), as well as foot-shock (223), increases NA release in the central nucleus of the amygdala. Lesions of the amygdala result in higher c-*fos* expressions in A1 and A2 neurons in response to restraint (123).

The bed nucleus of the stria terminalis (NIST) integrates inputs from the amygdala and ventral subiculum, and it projects to the PVN and the peri-PVN zone. This connection is one of the major links between the limbic system and the HPA axis. Psychological stressors, like foot-shock, that activate CRH neurons in the central nucleus of the amygdala, act also in the NIST. Foot-shock increases CRH mRNA expression in the dorsolateral subdivision of the NIST, in a cell group that projects directly to the PVN (389).

Several reports imply that the lateral septum has an inhibitory role on HPA activity through the peri-PVN zone and other, PVN-projecting hypothalamic GABAergic neurons (234). Lateral septal neurons show strong c-Fos activations in response to psychological stress (see Ref. 235).

The hippocampus is very important in response to stress, especially in adjustment to repeated stressful experience (275). It has an inhibitory action on the HPA axis (234). Excitatory (mainly glutamatergic) inputs to the peri-PVN region from ventral subiculum are relayed in the NIST. Stimulation of hippocampal neurons leads to a depletion of basal and stress-induced glucocorticoid levels in the plasma. In contrast, lesions of the hippocampus or the ventral subiculum, or transections of the fimbria-fornix pathway, result in increased CRH and vasopressin mRNA expressions in the PVN (232, 233). The hippocampus represents one of the critical target sites for the negative-feedback effects of glucocorticoids. The high concentrations of circulating glucocorticoids downregulate the activity of the HPA axis by acting at the level of the hippocampus (275).

D) LIMBIC CORTICAL AREAS. The medial prefrontal cortex, the anterior cingulate cortex, the piriform, and the entorhinal cortex are considered to be members of the so-called limbic cortex. The medial prefrontal cortex can be further divided into prelimbic and infralimbic cortex. The medial prefrontal cortex modulates stress and behavioral responses as well as emotion regulation, and it is important in conditioned stress responses through the interaction with the amygdala (33). Afferent CA pathways, mostly from the mesocortical DA system originating in the A10 DA cell group (ventral tegmental area), modulate prefrontal activity during stress. The medial prefrontal cortex contains high levels of glucocorticoid receptors (see Ref. 620) and acts as a negative-feedback site that suppresses the continued activation of the HPA axis (127). Damage of the medial prefrontal cortex activates stress response, while activation of this area has an inhibitory effect on the HPA axis in response to some of the physical (restraint, pain), but not psychological stressors (127, 235). Neurons in the medial prefrontal cortex, like neurons in the other limbic cortex, manifest strong c-Fos expression following different stressful stimuli (112, 506, 574). Reportedly, foot-shock increases the DA metabolism in the medial prefrontal cortex of rats (256). Lesions of the cingulate cortex are associated with increased plasma levels of both ACTH and corticosterone following restraint stress (127). This finding is consistent with the hypothesis that the limbic cortex mediates inhibitory effect of glucocorticoids on stress-induced HPA activity.

The hippocampus and limbic cortical areas receive NA inputs from the LC (243, 377, 378, 384). The functional significance of NA in these regions is well indicated by observations in TH transgenic mice. In these animals, memory deficits could be restored by NA applications (305).

3. Efferent signal routes in stress response

Neurons in the forebrain integrative centers do not send direct neuronal inputs to the body and the visceral organs (Fig. 8), but they may exert their effects through action on cranial (bulbar) and spinal motoneurons (somatomotor output), or on brain stem and spinal sympathetic or parasympathetic preganglionic neurons (visceromotor output). Preganglionic neuronal cell groups constitute one of the major outputs in the effector loop of stress responses (Figs. 8 and 11). The cholinergic preganglionic neurons in the medulla (dorsal motor vagal nucleus) and the spinal cord (IML) are activated by almost all of the types of stressful stimuli that influence the parasympathetic or sympathetic outflows, respectively. Depending on the stressors and their actions on forebrain integrative centers, the efferent routes of stress response

may arise in the hypothalamus, the amygdala, the cortical areas, or two or three of them combined. The sympathetic nervous system influences stress response through two different pathways, the sympatho-adrenomedullary and the sympatho-neural system (Figs. 8 and 11).

A) HYPOTHALAMIC EFFERENT ROUTES. Besides catecholaminergic and noncatecholaminergic neuronal inputs, the hypothalamus is innervated by limbic and cortical fibers (Fig. 8). The hippocampus lacks significant direct projections to the PVN. The inhibitory action of the hippocampus on PVN neurons is conveyed from the ventral subiculum through the fimbria/fornix system. The amygdala does not have substantial direct (monosynaptic) neuronal input to the PVN (207). Projections from these limbic and cortical areas to the hypothalamus mainly terminate in the peri-PVN zone, an area heavily populated by GABAergic interneurons (see Ref. 235).

The efferent neuronal pathways from the hypothalamus can be classified into two groups (Figs. 8 and 11): *1*) direct, monosynaptic projections from hypothalamic neurons to preganglionic neurons in the lower brain stem and the spinal cord (156, 245, 381, 517, 585, 629, 656, 660); and *2*) hypothalamic projections to brain stem catecholaminergic neurons in the rostral ventrolateral medulla that innervate IML neurons in the thoracic spinal cord (64, 244, 585) (Figs. 8 and 11). One-third of the PVN neurons innervate both RVLM and IML neurons (585). Descending fibers from the PVN to autonomic preganglionic neurons contain oxytocin, vasopressin, CRH, enkephalin, and dynorphins (82, 566, 630). CRH, in addition to governing the HPA axis, may mediate transmission through central neuronal pathways that regulate autonomic outflow and visceromotor activity.

In addition to neuronal efferents, the hypothalamus establishes a special neuroendocrine outflow route, the neurohumoral HPA axis (Figs. 8 and 11). Both the neuronal and neurohumoral efferent routes are crucial in the realization of stress responses.

In addition to the neurohormonal (HPA) and neuronal outputs from the PVN, neurons in other hypothalamic nuclei also participate in several other hypothalamic regulatory circuits, such as control of body temperature (preoptic area), energy homeostasis (dorsolateral hypothalamus), body fluid and mineral balance (anterior hypothalamus), which are in certain conditions, also involved in the central organization of responses to stressful stimuli (82, 156, 646, 667).

B) LIMBIC EFFERENT ROUTES. The limbic stress circuit operates in a stress-specific manner. Stress responses to predicted homeostasis challenges are called "anticipatory responses" (233). These responses are under the control of the limbic system but activate the HPA axis (Fig. 8). The significant sources of limbic output signals arise in central amygdala neurons. These neurons could alter the neuroendocrine (through the HPA axis) and the autonomic outflow (through brain stem/spinal neuronal projections). They may initiate autonomic responses to emotional challenges such as fear or anxiety. Among the projection fibers, only a small percentage consists of monosynaptic projections to the lower brain stem and spinal cord. The majority of the amygdaloid efferents to preganglionic neurons are largely confined to the NIST and the parabrachial nuclei (208). Although neurons in the descending catecholaminergic system receive innervations from the amygdala (676), their influence on the activity of the amygdala brain stem pathway in response to stressful stimuli has not yet been elucidated.

The stress-sensitive cortical areas are anatomically and functionally interconnected to certain limbic and hypothalamic nuclei (175). The ventral prefrontal cortex (ventrolateral orbital and insular cortex) receives sensory and affective signals and projects to the pre- and infralimbic cortex, known as visceromotor cortex (83). The visceromotor cortical areas also receive afferent inputs from the amygdala and the hippocampus and provide outputs to the hypothalamus and brain stem areas to activate the behavioral, neuroendocrine, and sympathetic autonomic systems in response to stressful stimuli (see Ref. 620). They do not establish direct, monosynaptic connections with sympathetic preganglionic neurons, because virtually all limbic cortical projections to the autonomic nervous system require at least one interneuron. Projections from the dorsal part of the visceromotor prefrontal cortex are relayed in the RVLM, while axons from the ventromedial part are relayed in the PVN (251, 584, 670). Psychological stress or repeated stressful stimuli may gain access to limbic or cortical circuits, and they may act on the PVN indirectly (Fig. 8). Most of these interneurons are GABAergic.

4. Neuronal feedback routes

Hypothalamic (mainly paraventricular) and some of the limbic neurons that receive noradrenergic innervations from A1 and A2 TH-positive neurons send descending back to these cells (Fig. 13). These NA cells do not project to the body or the organs, and they are not involved in the organization of the efferent loop of stress-responding pathways. Thus their innervations by hypothalamic or limbic peptidergic (mainly CRH-immunoreactive) neurons (Fig. 13) can be considered feedback signals to cells in the ascending catecholaminergic system. The hypothalamic efferents arise mainly in the PVN (656), some others from the arcuate, perifornical, and dorsomedial nuclei, as well as from neurons in the retrochiasmatic and lateral hypothalamic areas. The limbic "feedback" signals arise mainly from the amygdala relayed by neurons in the bed nucleus of the stria terminalis (see Ref. 482). Neurons in the infralimbic, prelimbic, and the anterior cingulate cortex also project to the NTS (233, 669).

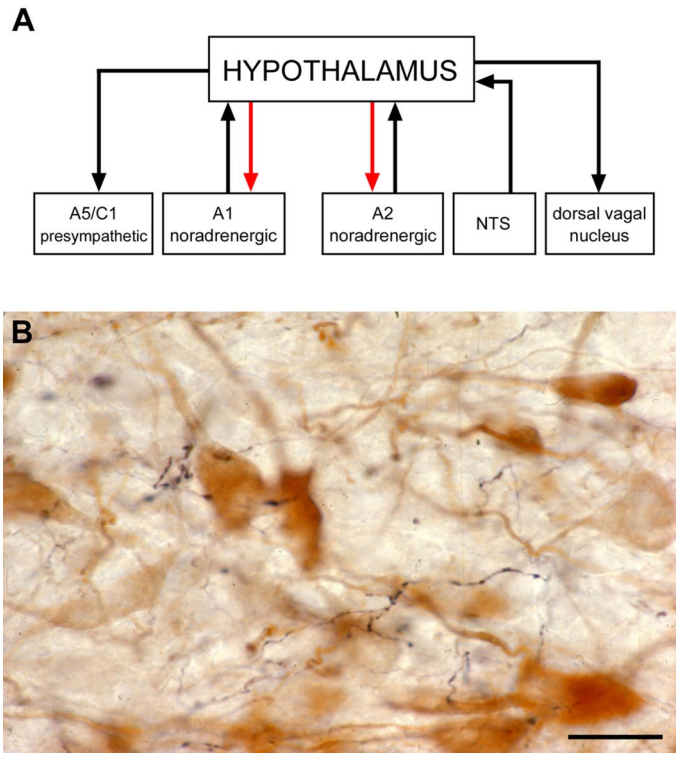

FIG. 13. Possible feedback signals from the hypothalamus to brain stem during stress response. *A*: block diagram indicating projections and bilateral neuronal interconnections between the hypothalamic paraventricular nucleus (PVN) and brain stem CA cell groups and vagal nuclei (NTS and dorsal motor vagal nucleus). *B*: injection of anterograde tracer, *Phaseolus vulgaris* leucoagglutinin (PHA-L) into the paraventricular nucleus. Labeled varicose PHA-L fibers in the NTS, on and around TH-positive (A2 cells) neurons. Scale bar: 25 μm.

5. *Central pathways of selective stressors*

The organization of stress responses appears to be circuit dependent: different stressors may act on different brain areas, and stressful stimuli may reach these areas through diverse neuronal pathways (107, 187, 472, 474, 477). Various stressors utilize different neuronal pathways to elicit stress responses (Fig. 8). Brain stem NA neurons are involved in the central feed-forward drive to the HPA axis. Some of the psychological stressors, like immobilization/restraint, disturbing noise, foot-shock, as well as physical stressors, like immune challenge, pain, nonhypotensive hemorrhage, electrical stimulation, exercise (forced swimming), heat or cold exposure strongly activate brain stem NA neurons, which is followed by the activation of the HPA axis. Both NA turnover and release increase with stress in the PVN, as well as in limbic areas (see Refs. 129, 474). Upon exposure to metabolic stressors (hypoglycemia, hypotensive hemorrhage), PVN NA is only slightly elevated, and it plays a minor role in the activation of the HPA axis.

Many stressors activate the limbic system. Psychological stressors have been found to activate neurons in the medial amygdala, ventral subdivisions of the bed nucleus of the stria terminalis, and the caudal neurons in the A1 and A2 NA cell groups. In contrast, physical stressors activate the central amygdala, dorsomedial NIST, and the rostral neurons in the A1 and A2 cell groups (107).

A) ACUTE AND REPEATED IMMOBILIZATION. Acute (1–3 h) immobilization, considered one of the strongest stressors, is a combination of physical and psychological stimuli. It activates the synthesis and the acute release of both central and peripheral CA, as well as the HPA axis (329, 336, 342, 545). Immobilization stress leads to significant increases in PVN NA and plasma ACTH levels (479), indicating that activation of HPA axis depends on both PVN noradrenergic and PVN or extra-PVN non-CA mechanisms.

Immobilization induces anxiety and conditioned fear through upregulation of CRF_2 receptors in the hippocampus. In response to IMO, neurons in the hypothalamus, the limbic system, the thalamus, the cortical areas, and in the NA and ADR cell groups express c-Fos in very high levels (81, 94, 485, 487, 543, 574). Even a 5-min IMO was found effective in eliciting c-Fos activation in the LC (225). IMO elicited large elevation of TH mRNA and GTPCH mRNA levels in the rostral, medial, and caudal parts of NTS (580). Immobilization stress also activates non-CA neurons in the dorsomedial medulla (see Ref. 474).

Immobilization produces a rapid and marked increase in NA concentrations in the extracellular fluid of the PVN and the central nucleus of the amygdala (471). Immobilization increases CRH mRNA expression in the PVN, but not in the central nucleus of the amygdala (476). During IMO, strong c-Fos activation can be observed in hypothalamic and limbic nuclei and cortical areas (see Ref. 474).

The efferent routes in response to IMO stress could be neurohumoral (routes 3 and 4 in Fig. 8) and neuronal involving hypothalamic, limbic, and cortical neurons that activate motor and autonomic output systems (routes 5 and 6 in Fig. 8). The components of the descending loop are interconnected with each other (limbic-hypothalamic, limbic-brain stem, hypothalamic-brain stem fibers) to coordinate responses to stressful stimuli. During IMO, compartments of the descending CA system are strongly activated. Separate lesions of one of the efferent routes may not block the immobilization-induced activation of the sympatho-adrenomedullary and the sympatho-neural systems. Electrolytic lesions of the A5 or A7 cell groups, or transections of selected neuronal pathways in the lower brain stem (321) did not reduce immobilization-elicited high plasma ADR and NA levels. It was hypothesized that more than one brain area and efferent pathway are involved in the organization of responses to immobilization stress, and the elimination of just one of them might be compensated by others (321).

Immobilization also activates the somatomotor system in the brain as indicated by the strong c-Fos activation in neurons of the cortico-pontine-cerebellar circuit, brain stem, and spinal cord motoneurons (485).

B) RESTRAINT. Although the restraint is a milder version of the IMO with minor and gender-specific nociceptive responses (188), exposure of rats to a 1-h restraint results in the activation of the HPA axis (78). Restraint triggers c-Fos activation in the amygdala nuclei, the thalamus, and the PVN (39, 78, 90, 94). Some of the other hypothalamic nuclei are also sensitive to restraint stress. Monosodium glutamate-induced selective damage of the hypothalamic arcuate nucleus potentiates corticosterone secretion into the plasma in response to restraint. Immediately after 30-min restraint, levels of NA, dopamine, and serotonin increased significantly in the plasma (379) and DA and serotonin levels in the hypothalamic dorsomedial nucleus (379).

Although previous studies indicated a role of the central amygdala on HPA axis in stress response, it is more likely that this nucleus may play a stressor-specific modulatory role in regulation of HPA function (520). Neurons in the subdivisions of the bed nucleus of the stria terminalis that innervate the PVN differently act on the HPA axis in response to restraint stress (93). Acute restraint increases CRH and vasopressin mRNA expression in the PVN, which is not affected by lesions of the central amygdala nucleus (520). Neurons in the medial prefrontal cortex are sensitive to restraint. Lesions of this cortical area, which removes a negative-feedback effect, result in elevated plasma corticosterone levels following restraint (127).

Restraint stress increases TH mRNA expression in the locus coeruleus (559). A1 and A2 NA, few C1 but not C2 ADR neurons respond to restraint stress by increased c-Fos expression (107). In all of the segments of the thoracic spinal cord, restraint activates the sympathetic preganglionic neurons (107). Repeated restraint stress markedly increases Fos expression in the PVN, medial, but not in the central amygdala nucleus, the lateral septum, the lateral preoptic and lateral hypothalamic areas, in the LC and the Barrington nucleus (90, 107).

C) NOCICEPTIVE STIMULI (PAIN). Like other nociceptive stimuli (81, 492), subcutaneous injections of formalin elicit an acute NA response and activate the HPA axis (see Refs. 437, 474, 484). Pain-related signals activate neurons in the ascending CA system leading to the hypothalamus where they evoke marked c-Fos expression and increases in the extracellular levels of NA in the PVN (475, 486). formalin has a depressive effect on plasma ADR both in intact and immobilized rats. This decrease is present in the first phase (extensive acute pain) of the formalin test (436).

Central CA neurons react sensitively to formalin-induced pain. Sixty minutes after unilateral subcutaneous injection, strong bilateral c-Fos labeling is seen in all of the CA and ADR cell groups in the brain stem (485, 487), indicating that the ascending catecholaminergic system is strongly involved in the pain-evoked stress signal routes (Fig. 8) (see Ref. 474). Painful stimuli resulted in marked c-Fos expression in the hypothalamus, particularly in the PVN, as well as in the preoptic and supramamillary nuclei (see Ref. 474). Brain stem hemisection strongly attenuates formalin-induced elevation in the ipsilateral PVN (486).

Other painful stimuli (mechanical pain, mustard oil or capsaicin injections) are also used to investigate pain-induced activation of neurons in the central nervous system. Each of them elicits dose-, intensity-, and time-dependent c-Fos activations.

The stress response is manifested through all of the three possible routes of hypothalamic efferents (Fig. 8): activation of the HPA axis (route 3 in Fig. 8), activation of the descending CA system (route 5), and direct inputs to the preganglionic sympathetic neurons in the spinal cord (route 6). The role of PVN neurons is significant in all these routes. Thus formalin elicits large elevations of corticosterone, ACTH, NA and ADR levels in plasma, and increased TH and CRH mRNA levels in the PVN (see Refs. 474, 484). As a painful stimulus, formalin evokes c-Fos activation in the thalamus (mainly in the midline thalamic nuclei), in the limbic and the somatosensory cortex. Consequently, the cortical stress efferent routes (Fig. 8) are also activated, mainly through the limbic cortex and the hypothalamus.

D) COLD STRESS. Maintaining homeostasis in an organism exposed to cold stress does not require significant activation of the HPA but rather the hypothalamo-pituitary-thyroid axis (184, 341, 479). Cold stress affects metabolic, endocrine, autonomic, and limbic systems. Several brain regions and neuronal pathways are involved in response to thermal stimuli. Signals from peripheral thermoreceptors are carried by sensory neurons to the spinal cord and the sensory trigeminal nucleus in the medulla. From here, thermal signals reach medullary and pontine neurons that relay signals to the medial preoptic nucleus (484). Noncatecholaminergic neurons in different brain regions are activated by either cold or heat stress (see Refs. 474, 484). Cold and heat stress increase c-*fos* mRNA expression in the ventrolateral medulla and in the preoptic area (56). Chronic cold stress elevates TH and NA transporter levels in the prefrontal cortex (422).

Cold exposure exerts only a minor effect on c-Fos or TH activation in central CA neurons (479, 485, 487, 500, 543). The high NA levels in the plasma indicate the activity of the sympatho-neural system (184, 341), while little if any increases in plasma ADR levels are detectable (479).

The efferent (descending) loop of the cold stress response has not yet been topographically localized. Neurohumoral and neuronal routes from the hypothalamus (Fig. 8) are suggested (474). The neurohumoral route may

consist of projections from medial preoptic neurons to the PVN where thyrotropin releasing hormone (TRH)-synthesizing neurons (hypothalamo-pituitary-thyroid axis) are activated. The neuronal efferent route consist of descending axons from the medial preoptic neurons to the lower brain stem (see Ref. 474).

E) IMMUNE CHALLENGE. Immune challenge [lipopolysaccharide (LPS), interleukin-1β (IL-1β)], a physical stressor, increases plasma ACTH and CORT levels, CRH mRNA expression in the PVN, and proopiomelanocortin (POMC) mRNA expression in the anterior pituitary (59, 161). Several cell groups are involved in activation by systemic IL-1β, like the NIST, the central amygdaloid nucleus, the lateral parabrachial nucleus, and the A1 and A2 CA cell groups (161). Central catecholamines modulate the response of the HPA axis. Systemic IL-1β stimulates A1 and A2 neurons (60, 161) by first acting on IL-1β type 1 receptors to activate prostaglandin production, which can then excite CA neurons in the NTS through ER3 and EP4 prostaglandin receptors (see Ref. 59). Lesions or interruptions of the afferent CA pathways inhibit the IL-1β-induced activation of CRH and POMC mRNA expressions (60, 161, 498).

F) HEMORRHAGE. Hemorrhage, depending on whether hypo- or normotensive hemorrhage occurs, activates four main systems: sympatho-adrenomedullary, HPA, renin-angiotensin, and hypothalamic atrial natriuretic hormone-vasopressin systems (routes 3 and 4 in Fig. 8) (see Ref. 474). Loss of more than 20% of blood volume elicits hypotension (hypotensive hemorrhage) (206).

Neurons situated in the medulla and the pons represent the first level where cells are involved in the control of cardiovascular and neuroendocrine functions upon exposure to hemorrhage. The NTS is the principal site of termination of cardiovascular mechano- and chemoreceptor afferents (see Ref. 86). These neurons, mainly noncatecholaminergic, relay baroreceptor information to other regions. Their projections to the hypothalamus seem to be monosynaptic while the amygdala and the bed nucleus of the stria terminals receive cardiovascular information via the lateral parabrachial nucleus. Cardiovascular signals from the NTS to neurons in the caudal portion of the LC are relayed in the rostral ventrolateral medulla (474).

Hypotensive hemorrhage provokes c-Fos, and consequently, CRH and vasopressin expression in the PVN (126). Hemorrhage-induced vasopressin secretion can be abolished by muscimol-induced inhibition of neurons in the CVLM (192). Hemorrhage elicits Fos expression in the supraoptic and paraventricular nuclei in the hypothalamus, the amygdala, the NIST, in the circumventricular organs, like organum vasculosum of the lamina terminalis, subfornical organ, area postrema, in neurons located in the dorsomedial (NTS, A2 and C2 neurons) and the ventrolateral (C1 neurons) medulla (23, 86, 107, 652). Ascending CA neurons, mainly in the A1, A2, and C1 cell groups contribute to the hemorrhage-induced activation of PVN neurons, all of which show moderate Fos activation in hypovolemic hemorrhage (61, 86, 135, 231, 366, 485, 487, 652). Rats fail to respond with c-Fos activation to nonhypotensive hemorrhage (86). Dorsomedial medullary lesions eliminate ACTH responses to hypovolemic hemorrhage (120). Transections of ascending medullary and pontine fibers significantly reduce the hemorrhage-induced responses of ACTH, renin, and vasopressin (see Ref. 474).

In the ventrolateral medulla, spinally projecting cells with presumed vasomotor function show strong Fos immunoreactivity in rats with 15% hemorrhage (86).

I) Activation of the HPA axis by hemorrhage. CRH mRNA expression, portal plasma levels of CRH, and plasma ACTH and corticosteroids are all increased after exposure to hemorrhage (see Ref. 474). Reportedly, hemorrhage increased ACTH (26-fold) and corticosterone (9-fold) after a single, but much less after a second, hemorrhage (652). Both the parvo- and the magnocellular subdivisions of the PVN exhibit increases in Fos expression after hemorrhage (see Ref. 474). Dense concentrations of Fos-immunopositive cells are present in the medial subdivision of the parvocellular PVN that project to the brain stem (to A5 neurons) and to thoracolumbar spinal cord sympathetic preganglionic vasomotor neurons (Fig. 11). In this subdivision, the high proportion of Fos-expressing neurons was found in hypotensive hemorrhage, but in rats with nonhemorrhage, the number of Fos-positive neurons was still higher than that in controls (23).

II) Release of vasopressin in hemorrhage. Hypotensive hemorrhage increases vasopressin and oxytocin release in the PVN, and their levels in the plasma (see Ref. 474). One of the possible pathways for regulation of vasopressin release upon exposure to hypovolemic hemorrhage involves stimulation of cardiac volume receptors. From there, vagal fibers carry signals to the medulla oblongata, stimulate NA neurons which project to the magnocellular subdivision of the PVN to facilitate release of vasopressin (86).

III) Activation of the renin-angiotensin system by hemorrhage. Angiotensin II augments the NA system in the brain and activates presynaptic excitatory receptors on sympathetic postganglionic nerve endings with release of NA. In the brain, the hemorrhage-induced increase in the circulating angiotensin II levels acts on angiotensin II receptors in the subfornical organ and the area postrema. These two circumventricular organs express angiotensin II receptors at the highest level in the central nervous system. Angiotensin II as well as hypotensive hemorrhage elicit Fos expression in their neurons (474). Signals from these areas reach vasopressin synthesizing neurons in the magnocellular PVN and the supraoptic nucleus through mono- or multisynaptic pathways, such as relay by atrial

natriuretic peptide (ANP)-synthesizing neurons in the preoptic area.

Each component of this hypothalamic neuronal circuit receives ascending inputs from brainstem NA (A1) and ADR (C1) neurons. Signals through angiotensin II receptor-containing neurons in the area postrema are relayed by noncatecholaminergic NTS and medullary CA neurons to the hypothalamus (474, 484).

G) INSULIN-INDUCED HYPOGLYCEMIA. Experimental hypoglycemia is achieved by injections of insulin. The fall in blood glucose stimulates autonomic responses to restore levels of circulating glucose. Insulin may not affect TH, NA transporter mRNA levels (543), or Fos activation (485, 487) in NA cell groups in the medulla oblongata. The very high ADR levels in the plasma in hypoglycemia (341, 472) clearly indicate the activation of the sympatho-adrenomedullary system, most probably through central routes (Fig. 8). Intracarotid injections of glucose attenuate and ganglionic blockade or spinal transections abolish the adrenomedullary response to hypoglycemia (51, 186).

Insulin administrations stimulate c-*fos* expression in the PVN, both in neurons that project to the median eminence and the lower brain stem (77, 485), and in other hypothalamic neurons that project to the sympathetic preganglionic neurons in the spinal cord (see Ref. 474). Axons from PVN neurons also project to the dorsal motor vagal nucleus and terminate on parasympathetic preganglionic neurons that innervate the pancreas (374, 517).

Hypoglycemia increases CRH mRNA expression in the PVN, CRH turnover in the median eminence, CRH and vasopressin levels in the hypophysial portal and peripheral blood (see Ref. 474).

The insulin-induced CA secretion into the blood is biphasic and proportional to the insulin dose used. In the first phase, only plasma ADR levels are elevated. In the second phase, both plasma ADR and NA levels are elevated, dramatically (291). Thus the first phase could be elicited by HPA action on the adrenal medulla (route 4 in Fig. 8), while the second phase is neuronal, activation of the sympatho-neural system (routes 6 and 9 in Fig. 8). The central CA neurons (members of either ascending or descending CA systems) may not be involved in this circuit, since lesions of the ventral NA bundle fail to influence ACTH responses to insulin (187). Insulin-induced hypoglycemia does not activate LC neurons (542). In contrast, 2-deoxyglucose (2DG; an antimetabolic glucose analog)-induced glucoprivation affects DBH gene expression in medullary CA cell groups (A1, A2, C1) (364) and increases TH mRNA expression in the LC (542). Local injections of 2DG into the hypothalamic ventromedial nucleus evoke rapid and marked increases in plasma levels of glucose, NA, and ADR (52).

Ritter and co-workers (532, 533) identified the specifc subgroups of hindbrain CA neurons that are selectively activated by 2DG-induced metabolic challenge. TH and PNMT were used as the markers for NA and ADR neurons. In the ventrolateral medulla, doubly labeled neurons were concentrated in the area of A1/C1 and were predominantly adrenergic in phenotype. In the dorsal medulla, doubly labeled neurons were limited to C2 and C3 cell groups. In the pons, some A6 neurons were Fos-positive. Neurons in rostral C1, ventral C3, A2, A5, and A7 areas did not express Fos-ir in response to 2DG (533). These data identify specific subpopulations of CA neurons that are selectively activated by 2DG. Demonstrated connections of these subpopulations are consistent with their participation in hyperglycemic response to glucoprivation. Preferential activation of ADR neurons suggests that they may play a unique role in the brain's response to glucose deficit (533). Selective destruction of hindbrain CA neurons using retrogradely transported immunotoxin (anti-DBH conjugated to saporin) revealed that spinally projecting ADR or NA neurons are required for the adrenal medullary response to glucoprivation, while ADR/NA neurons with hypothalamic projections are required for feeding, corticosterone release, and reproductive responses (532). ADR/NA neurons coexpressing NPY may be the neuronal phenotype required for glucoprivic feeding, since they increase NPY mRNA expression in response to glucoprivation and are nearly eliminated by the immunotoxin administration. In contrast, lesion of arcuate nucleus NPY neurons does not impair glucoprivic feeding or hyperglycemic responses (532). Thus hindbrain ADR/NA neurons orchestrate multiple concurrent glucoregulatory responses. Glucoreceptive control of these neurons resides within the hindbrain (532, 643). CA neurons couple potent orexigenic neural circuitry within the hypothalamus with hindbrain glucose sensors that monitor brain glucose supply (181). Receptor cells responsive to glucose deficit and capable of increasing corticosterone and glucagon levels exist within the hindbrain, thus further delineating central glucoregulatory neural circuitry (8). Hindbrain neurons contribute to the control of energy expenditure seen with food deprivation and increases in expenditure after cold exposure or starvation (209).

H) ETHER STRESS. Ether is a strong acute stressor that elicits high levels of corticosterone, ACTH, NA, and ADR in the plasma. In the brain, ether induces c-*fos* mRNA expression in the hypothalamus and the cerebral cortex. Ether stress recruits more cortical neurons than other stressors, but it does not elicit c-Fos expression in limbic areas, including the amygdala or in the bed nucleus of the stria terminalis (157). Limbic cortical (prefrontal) lesions did not influence plasma ACTH and corticosterone concentrations in response to ether (127).

The ether-induced activation of the HPA axis can be blocked by transections of the ventral NA bundle (187).

I) FOOT-SHOCK. Although c-Fos activation in the PVN has been reported after foot-shock (507), it is more likely that this stressor may not play an essential role in the

activation of the HPA axis: neither plasma corticosterone and ACTH levels, nor CRH mRNA expression in the PVN are elevated after foot-shock (389). In contrast, foot-shock elicits increased NA release (223) and increased CRH mRNA expression in the central nucleus of the amygdala (389).

The hippocampus and the prefrontal cortex are both involved in the inhibition of emotional stress responses. They represent the high level of integration of defensive responses to the kind of stressors from which descending input reaches the medulla where they influence the activity of CA sympathetic premotor neurons (365). It should be noted that both single and repeated foot-shock enhanced CRH mRNA expression in the Barrington nucleus, a cell group in the pontine tegmentum from where neurons project to sacral parasympathetic preganglionic neurons (254).

J) NOVELTY (OPEN FIELD). Exposure to novel environment (open field), as a processive stressor that does not represent an immediate physiological threat, seems to be a strong stimulus concerning c-Fos activation in brain regions like PVN, dorsomedial and suprachiasmatic nuclei in the hypothalamus, in the lateral septum, the medial amygdala, and the fronto-parietal cortex (157). Chronic social stress significantly elevates TH mRNA and TH protein levels in the LC (680). The possible role of CA in novelty-induced changes has to be investigated.

K) AUDIOGENIC STRESS. Acute audiogenic stimuli activate the HPA axis. Intense noise produces strong Fos activation in several brain areas, including limbic cortical areas, auditory (temporal) cortex, PVN, amygdala, parabrachial nuclei, some of the hypothalamic nuclei, A1 noradrenergic and C2 adrenergic neurons (54, 66, 67, 226). In the PVN, CRH-immunostained neurons show Fos activation 30 min after a short-term audiogenic stimulus (226). The efferent route (output) in the audiogenic stress response seems to be the activation of the HPA axis (Fig. 8): strong intensity (decibel)-dependent elevated corticosterone and ACTH levels can be measured in the plasma immediately after audiogenic stimuli (66, 67). The emotional responses to loud noise are processed in the amygdala. Inputs may arise from the posterior intralaminar (subparafascicular) nuclei, and probably from the auditory association cortex, as is indicated by increased c-*fos* expression in the basolateral and central amygdaloid nuclei in response to audiogenic stimuli (226).

G. Molecular Mechanisms in Central Catecholaminergic Systems in Stress

Among central CA systems, the molecular mechanism of the stress response has been studied most extensively in the LC. There are several reasons for this. One is that the LC neurons comprise a broad network of projections that extend throughout the neuroaxis and account for ~70% of all brain NA in primates (179). Moreover, the LC is of great interest since it is involved in controlling attention, vigilance, and activity of the autonomic nervous system. The LC is also implicated in opiate physical dependence and withdrawal. It is likely to play a key role in stress-associated neuropsychiatric disorders such as depression (reviewed in Refs. 16, 34, 306, 455, 663a). In this review, we will mainly focus on molecular mechanisms in the LC.

1. Catecholamines and their biosynthetic enzymes

Acute stress releases NA in the terminal fields of neurons localized in the LC, which leads to depletion of NA and an increase of its metabolites (179, 226). However, during repeated stress, brain NA levels are replenished, as a consequence of increased NA biosynthesis (342, 639). In fact, animals previously exposed to repeated stress are resistant to the depletion of NA in terminal fields upon exposure to acute stress, a phenomena also called immunity to stress (reviewed in Ref. 609).

Early work showed that repeated electrical foot-shock stress increased TH activity in various brain regions, especially the LC, but also in NA-terminal regions of hypothalamus, cortex, and pons-medulla (613). Subsequently, many types of stressors were found to trigger elevated TH activity and protein levels in the LC. These include isolation, foot-shock, restraint or IMO, chronic social stress, forced walking, and chronic cold (reviewed in Refs. 344, 551). In addition, several studies also examined changes in DBH activity or protein levels in LC and found them elevated by stress. For example, while DBH activity was reduced in LC following acute cold (5–10 min at 5°C), probably due to its release with NA, it was elevated in LC following chronic repeated cold stress (4 h daily) for 1–3 wk (119). In addition, repeated daily IMO stress for 3 or 7 consecutive days elicited a large rise in DBH immunoreactive protein in rat LC (582).

The stress-triggered increase in activity of NA-biosynthetic enzymes is accompanied (and in some cases preceded) by changes in their respective mRNAs (387, 438, 544, 582, 602, 678, 680). Most types of stressors increased TH mRNA levels in LC (reviewed in Ref. 551). However, insulin-induced hypoglycemia was an exception; conditions which induced TH gene expression in the adrenal medulla had no effect on the LC (542). The mRNA levels of GTPCH, the rate-limiting enzyme in BH4, the essential cofactor for TH, is also significantly elevated in LC following even single as well as repeated IMO stress (582).

Immobilization stress-induced elevation in TH gene expression and TH protein was also found in the hypothalamic nuclei, which represent important centers regulating neuroendocrine and autonomic systems during

stress, i.e., PVN, periventricular (PeV), and dorsomedial (DMN) nuclei (301, 438). Interruption of ascending CA pathways from the brain stem areas abolished TH gene expression in the PVN and DMN in response to stress. Thus the regulation of the majority of TH-positive cells in these nuclei during stress is under a modulatory control of brain stem and/or spinal cord (301, 438).

2. Role of transcription and posttranscriptional pathways

Several methods have been used to study the role of transcriptional and posttranscriptional mechanisms in the elevation of gene expression of enzymes related to NA biosynthesis in the LC and other brain areas.

Run-on assays of transcription with nuclei of cells from the LC of controls or animals exposed to stress can determine the relative rate of initiation of transcription of different genes. Results from our group revealed that TH transcription initiation rate increased with IMO. About a two- to threefold increase was observed following exposure of rats to 30 or 120 min of a single IMO or following twice repeated IMO for 120 min daily. A similar elevation was observed in transcription rate for DBH and GTPCH genes at these times of IMO (582).

Experiments with mice, which have a transgene encoding a reporter gene, alkaline phosphatase under control of the TH promoter have also been used to evaluate the role of transcription in the response to stress. There was an increase in reporter activity in LC immediately following exposure of mice to single, but not repeated IMO stress (465, 467). The same stimuli increased reporter activity in the adrenal medulla of these animals. A significant, but small induction of reporter activity was also observed by these investigators in LC in response to 3 days of cold stress (467).

Since introns are only present transiently following transcription and are absent in the mature mRNA, use of intron-specific probes can be used to determine if there are stress-triggered changes in transcription. By the method of in situ hybridization with intron-specific probes, intermittent foot-shock was shown to increase TH transcription in LC (87). The increase in levels of intron containing transcripts was rapid and returned to basal levels, while the elevation of TH mRNA was much more sustained. The in situ hybridization showed that there is heterogeneity within the LC in the transcriptional response to the foot-shock stress, with expression below the level of detection in some of the cells. The nature of the variation of the response within the LC remains to be determined. Semi-quantitative RT-PCR has also been used to measure changes in expression of different intron-specific sequences in TH transcripts in the LC. This technique revealed that immediately following repeated IMO stress (2 h daily for 7 days), there is a similar elevation of about fourfold in both mRNA levels and in primary transcripts containing intron 2 of the TH gene (622).

While there is activation of transcription in the LC with stress, it appears that posttranscriptional mechanisms, such as increased stability of TH mRNA, may also play a role in mediating the stress-triggered induction of TH mRNA in the LC (reviewed in Ref. 696). Following cessation of repeated IMO, TH mRNA levels remained at least twice above control levels even 2 days later, while primary transcripts containing intron 2 had declined and were no longer significantly different from levels in control animals (622). Similarly, TH mRNA remains elevated 1 day after intermittent foot-shock stress, although levels of primary transcripts detected by intron-specific probes had declined to basal levels (87). The mechanisms mediating these posttranslational effects remain obscure, and many involve stabilization of the mRNA by proteins binding to the 3′ -UTR and/or possibly coding sequence (5, 117, 535). It is possible that the stress enhances the transcription of these proteins, which can enhance mRNA stability.

3. Transcription factors responsive to stressors

While the relative contribution of direct transcriptional actions compared with posttranscriptional mechanisms remains to be determined, stress has been found to activate or induce a number of transcription factors in brain areas, especially in LC. Figure 14 depicts the changes in expression and/or activation of several transcription factors in the LC, which can regulate TH and DBH gene transcription in response to single or repeated stress as well as signaling pathways that may be involved.

Many studies have determined that exposure to a stressor activates gene expression of c-*fos*, which has been used to map stress responsive brain locations (reviewed in Ref. 484). The induction of c-Fos expression in a number of brain regions has been shown to depend on the intensity and duration of exposure to an acute stress. In the LC, c-Fos was increased after 30 min of mild (restraint) or severe (IMO) stress and remained elevated during an entire 4 h stress, while it had already declined in other brain areas (94). Our results revealed that even 5 min of exposure to IMO stress elicits a significant increase in c-Fos (225).

Upregulation of c-Fos was observed following repeated IMO or restraint stress for 6–14 consecutive days (90, 94, 408). The c-Fos was newly induced in response to the most recent exposure to the stress, as evidenced by the fact that levels of c-Fos declined to control values 24 h after the removal of stress and were reinduced following subsequent exposure to another IMO (225). However, following several weeks of prolonged exposure to the same homotypic stressor, the induction of c-Fos mRNA in

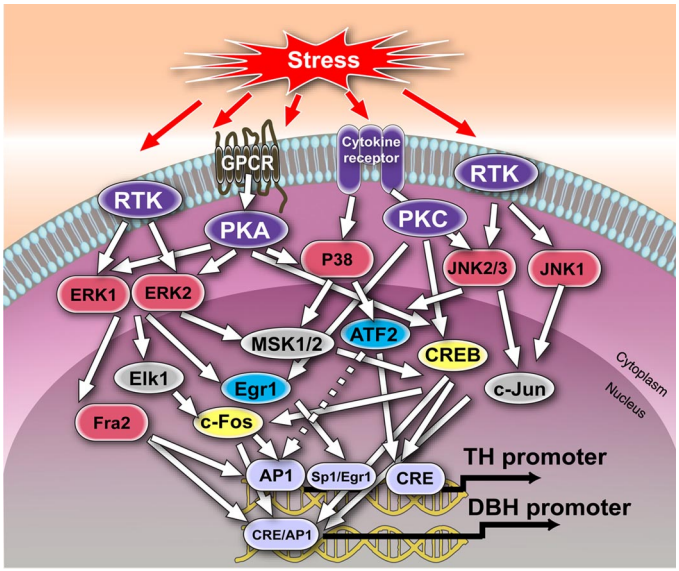

FIG. 14. Diagram of stress-induced signaling events leading to the induction of TH and DBH gene transcription in LC. Membrane receptors, such as receptor tyrosine kinase (RTK), G protein-coupled receptors (GPCR) and cytokine receptors, receive cues from external stress events. These cues are transferred to the nucleus by way of several kinases signaling pathways, such as extracellular signal-regulated protein kinases 1 and 2 (ERK1 and ERK2), protein kinase A (PKA), protein kinase C (PKC), mitogen-activated protein kinase molecular mass 38 kDa (p38), c-Jun NH_2-terminal kinase (JNK1 and JNK2/3), and mitogen-stimulated kinase 1 (MSK1). Within the nucleus, transcription factors, including Elk-1, Fos-related antigen 2 (Fra2), c-Fos, activating transcription factor 2 (ATF2), cAMP response element binding protein (CREB), and c-Jun mediate gene transcription by binding to specific elements within TH and DBH gene promoters. Changes with both single and repeated stress are shown in yellow, and those with repeated stress only in red. Factors not changed or induced in LC are shown in blue.

the LC is reported to be reduced compared with naive not previously stressed animals (664).

Transgenic mice with disruption of the c-*fos* gene were used to examine whether the induction of c-Fos is essential for the stress-triggered elevation of transcription of CA biosynthetic enzymes. Following repeated IMO stress (2 h daily for 3 consecutive days), there was no induction of DBH mRNA in the brain stem in the c-*fos* null animals, despite a normal response in the adrenal glands (583). TH and PNMT mRNA levels in the adrenal medulla of c-*fos* knockout and wild-type mice were elevated to the same extent by a single IMO exposure (311). These results suggest that c-Fos is involved either directly or indirectly in regulation of DBH gene expression in the LC. However, the precise cellular localization, the effect of shorter-term stress, and whether a similar effect is observed on the response of TH have not yet been examined in c-*fos*-deficient animals.

In addition to c-Fos, another Fos-related family member, Fra-2, was found to be elevated in the LC especially in response to repeated IMO stress (225). Overexpression of Fra-2 is capable of inducing TH and DBH promoter-driven reporter activity in cell culture (452). Interestingly, the levels of Fra-2 mRNA are reportedly elevated in postmortem tissue from the LC of individuals with major depressive syndrome, suggesting that elevated *fra-2* gene expression may be linked to the sustained upregulation of TH gene expression in both stress situations and in depression.

Changes in the expression of c-Fos and Fos-related antigens (Fras) in rat LC during space flight and following return to earth were observed. The largest elevations of both c-Fos and Fras were observed in the LC 1 day after landing. In this study, the antiserum used was for the entire Fra family (cross-reacted with Fos, Fra-1, Fra-2 and FraB), and since the changes were based on immunocytochemistry, they were unable to determine changes in specific Fras (514).

CREB also may mediate the transcriptional response to stress: CREB is one of the transcription factors of the CREB/ATF family that then binds as dimers to the cAMP response element (CRE) on a variety of genes, including TH (see sect. II*E*). CREB is well characterized in learning and memory, since it is a crucial mediator of experience-based neuroadaptations (for reviews, see Refs. 74, 397, 398, 591, 704).

CREB phosphorylation (on serine-133, necessary for transcriptional activation) was elevated in the LC during exposure to single and repeated IMO stress (225). This was accompanied by elevated CREB protein levels. The stress-related induction of phospho-CREB was localized primarily to TH-immunoreactive neurons as evidenced by coimmunofluorescence (225). Increased phosphorylation of CREB as well as levels of CBP were also observed in the LC with exposure to single restraint stress (596). In contrast to CREB, another family member ATF-2 was not changed in the LC with single or repeated immobilization stress (225).

Noteworthy is the absence of induction of Egr1, a factor that can enhance TH transcription (493). Immobilization stress-triggered changes in Egr1 in an area adjacent to the LC, but it did not overlap with TH-expressing cells (225). This contrasts with the large stress-triggered induction of Egr1 in the adrenal medulla as described in section IV*E*.

4. *Signaling pathways*

Since AP-1 factors and CREB, but not Egr1 and ATF2, are implicated in mediating the response of the LC to IMO stress, the effects on several upstream kinases that may be involved in mediating these changes were examined (Fig. 14). The phosphorylation of CREB and induction of c-Fos can be mediated by several of these upstream kinases including members of the MAPK family: ERK1/2, JNK1/2/3, and p38 (125, 398). A single IMO led to a modest change in phosphorylation of JNK. However, repeated IMO triggered a robust increase in phosphorylation of all isoforms of JNK. The phosphorylation state of MAPK p38

was not significantly altered by single IMO, but was activated in response to repeated IMO stress (225).

The phosphorylation of CREB can be mediated by several other kinases including cAMP-dependent protein kinase (PKA), protein kinase C (PKC), calmodulin (CaM) kinase II/IV, or mitogen-activated protein (MAP) kinases by way of MSK-1. Activation of the cAMP system in the LC, which is characterized by increased levels of adenylyl cyclase and PKA activities, was demonstrated in response to chronic cold stress (410).

Regarding MAP kinases, upregulation of phospho ERK in the LC was reported following restraint and IMO stress (225, 255, 596). ERK1 is the predominant isoform of ERK in the LC; however, ERK2 was the major phosphorylated form following IMO. A single episode of IMO did not noticeably alter ERK signaling in the LC. In contrast, repeated IMO stress produced significant changes in the levels of phosphorylation of ERK1 and especially ERK2. Following 30 min of twice daily repeated IMO, levels of both phosphorylated ERK isoforms were induced by 50–100%. Within 5 min of stress on a sixth consecutive day, phospho-ERK1 and phospho-ERK2 rose to over 250% of control levels. At 30 min of the sixth daily IMO, the phosphorylation of ERK1 and ERK2 was over fourfold that measured in unstressed animals and remained significantly high throughout the sixth daily exposure to stress (225).

The increase in phospho-ERK1/2 following IMO stress was specifically localized to noradrenergic neurons of the LC using TH coimmunofluorescence. The pERK1/2-immunoreactive neurons within the LC of repeatedly stressed (IMO, for 2 or 6 consecutive days) rats were increased in terms of the number of labeled neurons detected and in the intensity of labeling compared with the LC from control animals.

Hence, acute and repeated IMO differentially regulate the activation of several transcription factors and kinases in the rat LC (Table 2). Multiple MAP kinase pathways are activated, including ERKs, JNKs, and p38. This was especially evident with repeated IMO stress. Among the transcription factors that can regulate TH, a single exposure to IMO elicits induction of c-Fos and activation of CREB, without changing expression of Egr1, Fra-2, or phosphorylation of ATF-2. Repeated IMO continued to trigger activation of CREB, which was accompanied by increased CREB levels. Transient inductions in c-Fos were also observed with repeated stress exposures. Furthermore, repeated IMO led to a significant induction of Fra-2 (Table 2).

The observed induction and/or activation of transcription factors and kinases are likely to take part in mechanisms involved in plasticity of the noradrenergic system following exposure to stress. Stress-induced increase in TH mRNA and TH protein in the LC after single or twice repeated stress may reflect an adaptive regulatory response, perhaps serving to increase the synaptic capacity of the noradrenergic system in anticipation of subsequent stress and to induce the ability to invoke appropriate behavioral adaptation. With prolonged stress, the response may reflect a relatively greater impact of stressful stimuli on noradrenergic neurons of LC resulting from long-term adaptation to stress or failure to initiate appropriate behavioral strategies and may contribute to the susceptibility to stress-related pathology. These distinct alterations in transcriptional pathways following repeated, compared with single stress, may be involved in mediating long-lasting neuronal remodeling and are implicated in the mechanisms by which acute beneficial responses to stress are converted into prolonged adaptive or maladaptive responses.

TABLE 2. *Summary of the changes in locus coeruleus of rats exposed to 2 h of immobilization stress once (single IMO) or on 6 consecutive days (repeated IMO)*

Comparison of Changes in Locus Coeruleus With Single and Repeated IMO	
Single IMO	Repeated IMO
Transcription factors	
↑ P-CREB	↑ P-CREB
↑ c-Fos	↑ ↑ c-Fos
(No change in Fra-2)	↑ Fra-2
Activity of MAP kinases	
	↑ Erk1/2
(No Phos. of MAP kinases)	↑ JNK1/2/3
	↑ p38
Gene expression of CA biosynthetic enzymes	
↑ TH, transcription, mRNA	↑ TH, transcription, mRNA, protein
↑ DBH, transcription, mRNA	↑ DBH, transcription, mRNA, protein
↑ GTPCH, transcription, mRNA	↑ GTPCH, transcription, mRNA

MAP, mitogen-activated protein; TH, tyrosine hydroxylase; DBH, dopamine-β-hydroxylase; CA, catecholamine.

IV. PERIPHERAL CATECHOLAMINERGIC SYSTEMS IN STRESS

A. Sympatho-Adrenomedullary System in Stress

1. Responses of adrenal medullary catecholamines and their biosynthetic enzymes to stressors

The effects of various stressors on adrenomedullary CA levels have been determined in early studies. Exposure to a single episode of IMO stress for up to 2 h is accompanied by a significant decrease of 15–20% in the total adrenal medullary content of ADR without significant changes in NA (335). Following repeated exposures to the same stressor, the CA levels in the adrenal medulla

eventually return to normal and even establish new basal levels, significantly higher than in naive animals (351).

The increased adrenal CA content, urinary excretion, and plasma secretion (132, 335, 339, 347, 400), together with increased maximal adrenal TH, DBH, and PNMT activity (326, 339, 351, 551), suggest that the adrenal medulla responds to repeated IMO stress with an increased capacity to synthesize CA. The administration of labeled CA precursors clearly demonstrated a substantial increase of in vivo biosynthesis of adrenal CA in repeatedly immobilized rats (350). Using differently labeled precursors ([^{14}C]tyrosine and [^{3}H]DOPA) revealed that the increased CA biosynthesis is primarily dependent on the step of tyrosine conversion to L-DOPA, catalyzed by TH; however, after repeated IMO, when DA formation is markedly accelerated, the step catalyzed by DBH, conversion of DA to NA, may also become rate limiting (350).

Given that the synthesis of ADR within the adrenal medulla involves participation of several different enzymes, studies have focused on measurement of activity of three key catecholamine-synthesizing enzymes: TH, DBH, and PNMT. Activity of AAAD, in contrast, is unchanged with stress (468, 648). Several reviews deal with various aspects of the regulation of CA biosynthetic enzymes activity, protein levels, gene expression, and molecular genetics in response to stress (304, 312, 331, 344, 345, 548, 550, 551, 696).

Adrenal TH activity under conditions of stress was also characterized by measuring kinetic parameters, such as K_m for the substrate and cofactor (45, 239). Exposure of rats to a single IMO does not change either K_m or V_{max} compared with the unstressed control group. After repeated IMO, the K_m values remain unchanged, whereas V_{max} for both substrate and cofactor are significantly increased. These data confirmed the idea that repeated IMO stress induces an increase in TH protein synthesis, and this in turn results in elevated TH activity, while the affinity of the enzyme for both substrate and cofactor remains unchanged (45, 239).

Early studies confirmed that alterations in TH activity induced by IMO or cold stress resulted from increases in the amount of enzyme protein and its synthetic rate (99, 100, 239, 350, 351, 649). Repeated or chronic exposure to stress was also found to increase activity of DBH and to a lesser extent PNMT (325, 326, 351, 648, 649). There is a very good correlation between elevated adrenal TH and DBH measured in vitro (351) and the rate of CA synthesis measured in vivo in control and immobilized rats (350).

Other stressors also affect catecholamine biosynthesis and enzyme activities. Thus continuous exposure to cold (4°C) increases adrenal TH, DBH, and PNMT activity; however, the response is transient. After several weeks of continual cold exposure, the activity is no longer significantly elevated (29, 30, 324). Intermittent cold exposure (4h, 21 days) elevates TH activity but not PNMT activity (40). The cold-induced increase in adrenal TH activity is reduced after administration of adrenomedullin, a peptide that elicits a long vasorelaxation (706).

Intermittent repeated swimming at a water temperature of 15°C increases TH activity in the adrenal medulla (468). Elevated activity of adrenal TH and other enzymes after swimming or long-term physical load has also been reported by others (38, 497, 536). Psychosocial stimulation (20, 229, 509), isolation of animals (647), hypokinesia (46), restraint (37) and chronic foot-shock (613) also trigger elevations of TH activity.

Glucoprivation after administration of insulin or 2-DG increased TH activity to a very high extent, similar to IMO stress (177, 178, 300, 346, 598). However, while Kuzmin et al. (316) reported a huge increase in CA secretion from the adrenal gland measured by microdialysis in immobilized and 2-DG-treated rats and elevated TH activity in immobilized animals, they did not observe changes in TH activity after administration of 2-DG.

The only situation in which a reduced adrenal TH activity has been reported is chronic exposure to heat at 34°C (504).

The elevations in TH activity are associated with increased expression of CA biosynthetic enzymes (reviewed in Refs. 331, 345, 551). Early work observed that following several days cold at 4°C, adrenal TH mRNA levels are about three to four times basal levels, and TH protein and activity levels nearly doubled (529, 605, 640). Subsequently, it was shown that chronic or repeated exposure to many types of stressors affect gene expression of CA biosynthetic enzymes and lead to elevated RNA levels. These include physical stressors such as IMO stress (317, 406), exercise (662), hypoglycemia (671), and the chronic mild stress model of depression (136). DBH and PNMT gene expression are also markedly induced by chronic or repeated stress (406, 672). While the majority of these studies were performed in rats, an immobilization-induced increase in TH, DBH, and PNMT mRNA levels in the adrenal medulla of mice was also reported (311, 330, 466, 583).

Social stress and housing conditions also affect gene expression of adrenal CA biosynthetic enzymes. Animals that were group housed were found to have lower PNMT mRNA levels (431). Pohorecky et al. (511) reported that in rats housed in triads, adrenal gene expression of CA biosynthetic enzymes is higher in subordinate compared with dominant and subdominant animals. Alcohol consumption raised plasma CA levels and CA biosynthetic enzymes gene expression, and potentiated the IMO-induced increase in adrenomedullary TH, DBH, and PNMT gene expression (501).

In addition to changes in CA biosynthetic enzymes, there is also upregulation of biosynthesis of the cofactor tetrahydrobiopterin (BH4), essential for TH activity. Gene expression of GTPCH, the rate-limiting enzyme for BH4

biosynthesis, is markedly induced by stress. The rat adrenal medulla expresses both the 1.2- and 3.6-kb isoforms of GTPCH mRNA, and the expression of both are increased by exposure to IMO stress (581). Furthermore, cold stress was found to lead to a doubling of biopterin content (30).

Besides the rise in CA biosynthesis, stress also triggers elevated expression of a number of neuropeptides in the adrenal medulla, such as mRNAs for NPY (220, 237). The deprivation of glucose, caused by either insulin- or 2-DG-induced hypoglycemia, increases not only TH, but also NPY and proenkephalin mRNA levels (355, 671). Cold stress also elicited a large rise in NPY mRNA levels (220, 313, 314).

Thus an abundance of studies confirm that increase in expression of CA biosynthetic enzymes in the adrenal medulla is a prevalent and likely key response to many types of stress.

2. Duration of homotypic stress

An important research question has been how the adrenal medullary response to acute stress differs from that of chronic exposure to stressful situations. When does the response becomes maladaptive? An animal or human experiencing sustained exposure to an acute stressor does not know when the stressor will be terminated, and once terminated, if or when the stressor will begin again. In the face of this uncertainty, how does the adrenal medulla meet the immediate needs of replenishing supplies of ADR and NA in adrenal chromaffin cells and what, if any, provisions are made for an uncertain future?

The duration or repetition of the stress has a marked effect on the changes in gene expression of CA biosynthetic enzymes. This has been studied in great detail in response to cold and IMO stress.

A) COLD STRESS. When examining the effect of cold as a stressor, the response differs depending on duration. Exposure to cold stress for several hours triggers elevation of adrenal medullary TH mRNA levels. The immunoreactive protein is maximal only after several days of the cold exposure, while maximal TH activity required several further days of continual cold stress (30). However, with very prolonged cold (a month at 4°C), TH expression (mRNA levels and the enzyme activity) had returned to levels similar to those observed in the adrenal medulla of unstressed animals (328). These results suggest that there is a habituation of this response with chronic cold stress.

B) IMMOBILIZATION STRESS. Even an acute exposure to IMO stress triggers a large rise in mRNA for CA biosynthetic enzymes. Compared with values in unstressed controls, TH mRNA levels increased ~7 times, DBH mRNA levels ~2.5 times, and PNMT mRNA levels ~5 times following a single 2-h IMO stress (334, 406, 635, 672, 691). TH and PNMT mRNA levels were almost as high as observed with repeated IMO.

While TH mRNA levels are substantially increased following a single exposure to IMO, this is not reflected in comparable changes in TH protein or activity levels (450, 699). The reason for this discordance remains unclear. It might reflect the time of sampling, and the elevation of protein levels might occur at later times than those examined. Posttranscriptional mechanisms may also be involved, either in terms of mRNA stability or differences in translational control (641, 694, 696). The possibility of a translational mechanism is supported by the finding that following a single IMO stress a larger percentage of TH mRNA is not associated with polysomes, compared with RNA from controls or rats exposed to repeated IMO (696, 699). Alternatively with single IMO, the elevation of TH mRNA may not be sustained long enough to be reflected in a marked change in protein levels. The rise in mRNA levels for adrenal CA synthesizing enzymes in response to a single 2-h period of IMO stress reach peak levels 3 h after the IMO is completed, but is short-lived. Twenty-four hours following a single 2-h period of IMO stress, they had returned toward baseline levels and were no longer significantly different from unstressed control levels (334, 450, 672, 691) (see Fig. 15).

However, surprisingly, even with only a second exposure to the same stressor, there is "memory" of the first experience such that the increase in TH mRNA is now much more prolonged and is sustained for longer periods of time after termination of the stress. Repeated, rather than single, IMO were also required for maximal elevation of DBH mRNA levels (406). Chronically repeated stress, in contrast to single stress, triggers persistent activation of TH, DBH, and PNMT genes in the adrenal medulla, which should be crucial for the adaptation (or perhaps maladaptation) to the stressful situation. Compared with unstressed controls, rats repeatedly exposed to IMO stress for 2 h per day for 7 consecutive days have significantly elevated mRNA levels of TH, DBH, and PNMT in the adrenal medulla even 24 h after the last stress exposure (334, 406, 450, 672). In addition, TH, DBH, and PNMT enzyme activities and protein levels are already significantly increased, and these changes persist for quite a while afterwards. In fact, the excess TH activity in the adrenals of repeatedly stressed rats declines toward basal levels with first-order kinetics and a half-life of ~4 days (351).

The relationship between the changes in PNMT activity and protein levels with repeated stress are less correlated. Following single IMO, PNMT protein is up compared with unstressed controls, but after repeated IMO for 7 days, PNMT protein levels have declined (635). This may be indicative of desensitization to the stress or posttranscriptional regulatory effects. However, the interpretation is complicated since the mea-

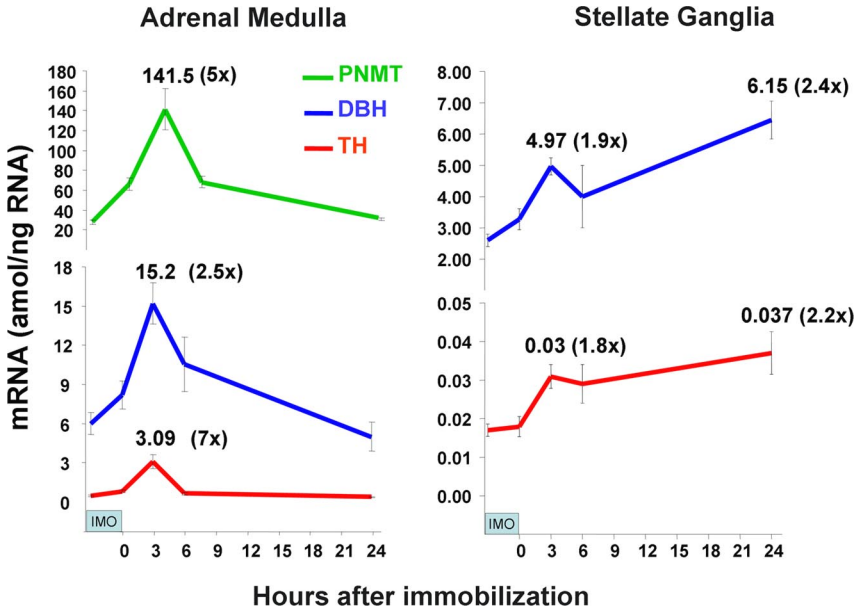

FIG. 15. Kinetics of effect of a single immobilization stress on TH, DBH, and PNMT mRNA concentrations in the adrenal medulla and stellate ganglia. Rats were subjected to immobilization stress (IMO) for 2 h and killed at different intervals after immobilization. Results are expressed as amol/ng of total RNA (means ± SE; n = 7–10). Note the different scales used for the adrenal medulla and ganglia. [Modified from Kvetnansky et al. (334).]

sures of mRNA and protein represent steady-state levels of expression, comprised from both synthesis and degradation and not just synthesis alone. Although the mRNA levels are still high on the day following a repeated IMO stress, there is still a further elevation when animals chronically exposed to IMO for 6 days are subjected to an additional IMO (334) (Fig. 16). TH and PNMT levels of mRNA still responded to even a 42nd (6 wk) daily IMO stress (317, 343), indicating that for induction of TH and PNMT mRNA, in contrast to cold stress, there is no habituation of the adrenal medulla to this stressor.

The increases in adrenal TH protein levels induced by repeated IMO decline after cessation of the stress with a half-life of ~2.5 days and return to basal levels by 14 days (351). On the other hand, after cessation of the stress stimuli, the repeated IMO-induced increases in TH mRNA levels (5- to 10-fold) return back to prestress levels faster, within 5–7 days (406, 450).

Thus, with cold stress, the observed differences in TH and PNMT mRNA levels in the adrenal medulla of rats exposed to short- and long-term cold suggest an adaptation or habituation with respect to changes in gene expression. However, rats exposed to long-term repeated IMO stress do not show such a habituation.

3. Heterotypic or novel stressors

Nowadays, one of the key questions in stress research is how the same stressor can elicit a variant or altered response depending on prior experience with the current or different stressor. It was found that when cold-acclimated or repeatedly immobilized rats were exposed to a new acute stressor, the rise of plasma ADR and NA in response to this novel stressor was substantially higher than the response of naive animals to the same stressor (132, 133, 339). Thus a heterotypic novel stressor triggers an exaggerated elevation in plasma CA in animals previously subjected to long-term cold stress exposure. When animals previously cold stressed for nearly a month are immobilized, the elevation of plasma NA is more than double that observed in naive rats. A similar exaggerated response is obtained whether the novel stressor is IMO stress, insulin-induced hypoglycemia, or glucopenia after 2-DG administration (133). With the novel stressor, plasma ADR reaches a similar level in both groups of animals at the initial phase of stress exposure but remain higher for longer duration of the novel stress in the cold-pretreated animals.

Long-term immobilized rats also show greater activation of the adrenomedullary system when exposed to novel heterotypic stressors than the control previously unstressed group do. Repeatedly immobilized rats exposed to a heterotypic novel stressor have exaggerated plasma CA levels most probably because such animals increasingly utilized their tissue CA stores (132). Repeatedly immobilized rats respond also to acute experimental trauma with substantially higher plasma ADR and NA levels than intact rats that have never before been stressed (339). Rats acclimated to high mountain conditions (1-yr stay outdoors at an altitude of 1,350 m) show significantly higher levels of plasma CA in response to IMO than control rats (25).

An exaggerated response to a novel stressor is also evident in adrenal TH and PNMT gene expression of chronically cold-stressed rats. While exposure to cold stress at 4°C for a number of days elevates adrenal TH

mRNA, protein, and enzyme activity, by 28 days the levels return to baseline (328). PNMT mRNA in adrenal medulla is also elevated by 1 day of cold stress, but returns to control values with chronic continued exposure to cold (317). However, there is "memory" of the stress as indicated by an enhanced or exaggerated response for gene expression to a different type of stressor. The novel stressors (IMO, insulin-induced hypoglycemia, and 2-DG-induced glucopenia) induce an exaggerated elevation of TH mRNA levels, and while a single exposure to these stressors is not sufficient to trigger increased TH protein or activity in naive animals, a significant elevation is observed in the cold-pretreated rats (328). A similar exaggerated response to these novel stressors in cold-preexposed rats is also observed at the level of induction of PNMT mRNA (317).

In addition to sensitization to a heterotypic stressor in cold-prestressed animals, another example of the importance of prior experience is revealed in a study comparing the induction of TH mRNA levels in adrenals of rats with or without prior exercise. In this study, TH mRNA levels were elevated by stress (shaking) only in long-term voluntary exercised rats (361).

Rats acclimated to high-altitude conditions living outdoors for 1 year display a large IMO-induced increase in adrenal TH activity and greater reduction in CA levels than rats that are not adapted to high altitude (25). Similar changes in rat adrenomedullary activity are seen after the

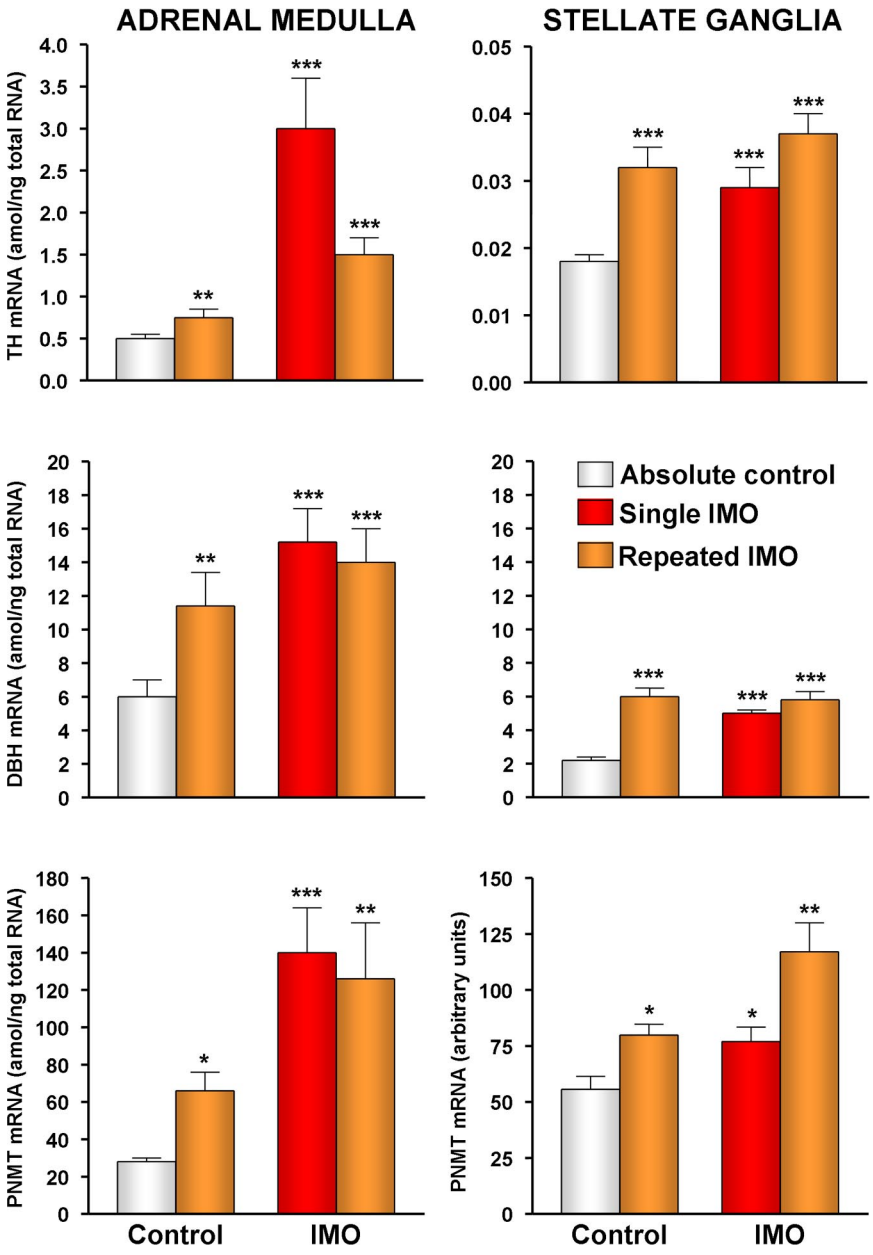

FIG. 16. Effect of repeated exposure to immobilization stress on TH, DBH, and PNMT mRNA concentrations in the adrenal medulla and stellate ganglia of rats. Animals were immobilized 1 or 7 times for 2 h daily and killed 3 h after the immobilization. The repeatedly stressed control group (orange) was immobilized similarly except for the last (7th daily IMO) and killed 24 h after the sixth IMO. Data are expressed as amol/ng of total RNA, except for PNMT mRNA in ganglia, because of the very low mRNA levels (means ± SE; n = 7–10). Statistical significance: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ compared with absolute control group. [Modified from Kvetnansky et al. (334).]

long-term space flight of 18.5 days on board the biosatellite Cosmos 1129 (349). Although adrenal TH activity is unchanged in rats immediately after landing, exposure of such animals to repeated IMO (5 days) induces a much bigger increase in TH activity than observed in rats not subjected to space flight (349). Thus, although weightlessness and other factors associated with space flight do not change adrenal TH activity, they sensitize the adrenal medullary system to a greater response to a novel stressor on Earth. In consequence, it is the readiness of the organism exposed long term to a homotypic stressor to respond to a heterotypic stressor by an exaggerated activation of CA that we consider to be an important adaptive phenomenon of the sympathetic-adrenomedullary system in rats.

In contrast to the above-mentioned findings, TH mRNA and PNMT mRNA levels in the adrenal medulla of repeatedly immobilized rats have elevated levels compared with control naive rats, and no exaggerated response is seen after acute exposure to novel stressors (317, 343). This finding might be explained by the quality and especially intensity of the stressor used. Immobilization is considered one of the most intensive stressors, and therefore, the gene expression is, even in long-term immobilized animals, still significantly activated. An exposure of such animals to a novel weaker stressor is not able to exaggerate gene expression that is already increased compared with control unstressed animals. The system is already prepared to manage the new situation evoked by a novel stressor, and the exaggerated response is not necessary. The results suggest that novel stressors elicit exaggerated responses in prestressed animals when the novel stressor is of equal or greater intensity or duration and/or it is repeated.

The influence of prior experience with homotypic or heterotypic stressors on reactivity of the catecholaminergic system in stress has recently been reviewed (556).

4. Factors and pathways initiating changes in catecholamine enzymes gene expression

Since there is stressor specificity in the mechanism of regulation of gene transcription in the adrenal medulla, for example as described in detail above to the stressors of cold and IMO, it would be expected that there are different upstream inputs eliciting these diverse pathways. The importance of the HPA axis and splanchnic neuronal innervation has been investigated in considerable detail regarding activation of the adrenal medulla by various stressors (reviewed in Ref. 453).

Substantial evidence supports the importance of the activation of the HPA axis for regulation of PNMT gene expression. For example, hypophysectomy prevents the IMO-triggered induction of PNMT gene expression (672). Glucocorticoids have been proposed to regulate PNMT in at least two ways, posttranscriptionally and transcriptionally (634, 635, 692, 696). The PNMT promoter contains strong glucocorticoid response element(s) (373, 634) and also weak one (539) (Fig. 6). The glucocorticoid receptor interacts with the PNMT promoter alone and also synergistically with Egr1 and AP2 transcription factors (693).

Glucocorticoids appear to regulate the availability of *S*-adenosylmethionine, the cofactor for PNMT, by regulating its metabolic enzymes. In turn, *S*-adenosylmethionine binds PNMT and protects it from degradation (697).

CRH-deficient mice were used to clarify the importance of regulation of the HPA axis in the changes in PNMT gene expression observed with stress. In the adrenal medulla of CRH-deficient mice, the induction of PNMT mRNA triggered by both single and repeated IMO is greatly attenuated compared with wild-type animals. There is no response of PNMT protein to IMO in these CRH-deficient mice, compared with a more than doubling of PNMT protein in the wild-type mice. Interestingly, not only is the response of plasma corticosterone to IMO nearly absent in these mice, but the induction of Egr1 protein by IMO is greatly reduced in the adrenal medulla of CRH-deficient mice (330).

The role of the HPA axis is less important for the regulation by stress of TH in the adrenal medulla. In CRH knockout mice, IMO induces a similar increase in TH and DBH mRNA levels as seen in wild-type mice (311). There is a large induction of TH mRNA in response to IMO stress even in hypophysectomized rats (450). However, hypophysectomy prevents the IMO-triggered elevation of mRNA for GTPCH, the rate-limiting enzyme cofactor BH4 (581), and consequently can have an indirect effect on TH activity.

The importance of the splanchnic neuronal input on the stress-evoked regulation of gene expression in the adrenal medulla was extensively investigated. The increase in PNMT mRNA levels by cold stress is diminished, but not abolished, by splanchnic nerve section or by the cholinergic antagonists chlorisondamine and atropine (29). However, adrenal denervation does not significantly alter the elevation of PNMT mRNA in response to a single IMO stress (672).

The induction of TH mRNA triggered by hypoglycemia or cold stress is prevented by splanchnic nerve section or by the nicotinic antagonist chlorisondamine (29, 606, 671). In contrast, splanchnic cholinergic innervation is not essential for the induction of TH or PNMT mRNAs in response to a single IMO stress (337, 345). While the induction of adrenal medulla mRNA coding for NPY is inhibited, neither splanchnic nerve section, nor cholinergic antagonists, chlorisondamine nor atropine, nor their combination are able to prevent the elevation of TH mRNA by a single IMO. In fact, chlorisondamine administration actually increased basal TH mRNA levels in the adrenal (337).

The splanchnic innervation is cholinergic but also includes innervation by other transmitters and multiple neuropeptides, such as PACAP, which can regulate expression of CA biosynthetic enzymes (218, 373, 690). The effects of modulation of the nicotinic cholinergic system on the stress response have been examined. Prior prolonged nicotine infusion to rats, which is expected to lead to desensitization to nicotinic receptors, is one of the few treatments found to reduce the elevation of adrenal TH mRNA in response to a single IMO stress. It also prevents the IMO stress-triggered induction of DBH and PNMT in rat adrenal medulla (576). However, it is not known whether or not this is a direct effect on the adrenal medulla.

Collectively, experiments with many different stressors indicate that regulation of transcription in the adrenal medulla is an important adaptive process. However, there is selectivity based in part on the specific CA biosynthetic enzyme regulated, the type and duration of the stress, and prior experience with the same or different stressors.

B. Sympatho-Neural System in Stress

The main neurotransmitter of the sympatho-neural system is NA, which is released at sympathetic nerve endings and triggers its physiological actions by way of adrenergic receptors on target tissues. The sympatho-neural nervous system is a very important mediator of the response to stressors and is implicated in many of the pathophysiological responses to stress, such as hypertension and other cardiovascular events, gastrointestinal diseases, carcinomas, etc. In spite of its importance, the sympatho-neural system has been much less studied in stress conditions than the adrenal medulla, and little is known about mechanisms of gene expression of CA biosynthetic enzymes in sympathetic neurons following stressful stimulation.

1. Changes in catecholamine biosynthetic enzymes during stress

Cell bodies of peripheral NA neurons are localized in the sympathetic ganglia. Early studies have established that exposure of rats to a variety of stressors triggers increased TH and DBH enzymatic activity in a number of sympathetic ganglia, including the superior cervical (SCG) and stellate (StG) ganglia. This includes cold stress and swimming stress (425, 468, 599, 649, 650). There is no significant change in AADC activity (468, 650). Electric stimulation of preganglionic nerves increases TH and DBH activity but does not affect AAAD activity in the superior cervical ganglia, with maximum increase 3 days after stimulation (714).

TH, but not DBH, activity in these ganglia was found to be elevated in aged rats (499). Other studies also reveal differences in the response of TH in the SCG to cold stress in young and old animals (9).

In vivo hypoxia (10% O_2 for 7 days) increases density of SIF cells immunolabeled for TH and VIP in SCG (505). These data provide evidence that in the sympathetic ganglia not only neuronal cell bodies but also SIF cells are activated under stress. However, sustained and intermittent hypoxia does not signficantly affect TH or DBH expression in SCG (250).

An elegant study by Kiran and Ulus (300) reveals selectivity in the response of TH activity in different sympathetic ganglia to exposure to various stressors. These investigators exposed rats to several stressors: *1*) IMO stress (6 h daily for 4 day), *2*) glucopenia after 2-DG administration, *3*) hypercapnia (20% CO_2, 25% O_2, and 55% N_2), and *4*) cold stress (64 h). In all cases the animals were killed 1 day after termination of the stress, and TH activity was assayed in a variety of sympathetic ganglia and compared with untreated controls. IMO stress significantly increased TH activity mainly in the lumbar and sacral ganglia, and slightly (~30%) in the cervical ganglia, but no changes of TH activity in the thoracic ganglia were seen. In contrast, hypocapnia increased TH activity in celiac ganglia and primarily in thoracic and first two lumbar ganglia, but not in sacral ganglia. Prolonged exposure to cold had a significant effect in the celiac, L2, T9 and 10 and cervical inferior ganglia, but not on other thoracic or lumbar ganglia. Glucopenia, which increases TH activity and gene expression in adrenal medulla and brain catecholaminergic locations, did not significantly alter TH activity in any of the sympathetic ganglia. Swimming stress (15 min daily for 4 days) also showed selectivity and activated TH activity especially in stellate ganglia and coeliac ganglia, but not in SCG (663). These data show selectivity of specific ganglia to particular stressors. It also reveals that stressors can increase TH activity selectively in particular ganglia without triggering changes in all portions of the sympatho-neural system.

The stress-evoked increase in activity of NA biosynthetic enzymes in response to IMO or cold stress is associated with elevated TH immunoreactive protein and elevated levels of TH and DBH mRNAs in StG and SCG (334, 417, 449). Exposure to even a single IMO increases TH mRNA levels two- to threefold in both SCG and StG with more pronounced changes in SCG. Because this change is induced within 2 h of a single IMO, elevation of ganglionic TH mRNA most probably results from transcriptional activation of the gene. Peak levels, however, are reached 24–48 h after the stress exposure (334). Repeated IMO further enhances the expression of TH gene in sympathetic ganglia, eliciting an additional increase approximately twofold over the elevations attained with a single episode of this stressor. Under conditions of repeated

IMO, increased TH immunoreactivity is observed in both StG and SCG (449).

Thus increased CA biosynthetic capacity in the sympathetic nervous system likely helps mediate the stress-triggered elevation of NA in plasma and in target tissues.

Recently, a stress-induced increase in AAAD gene expression was observed in sympathetic ganglia of mice exposed to repeated, but not to single IMO stress (309).

PNMT activity was detected in sympathetic ganglia of newborn rats (159, 372, 480). Culman et al. (113) detected PNMT activity also in the SCG of adult rats, and the activity increased after corticosterone administration. Using an in situ hybridization technique, Schalling et al. (568) observed PNMT mRNA in rat SCG. SIF cells of the paracervical ganglion were shown to contain all enzymes required for ADR biosynthesis, including PNMT (521). These results show that ADR may be a transmitter for SIF cells and that cholinergic neurons can modulate the SIF cell activity via activation of muscarinic receptors (521). Cultured SIF cells from rat SCG are glucocorticoid dependent, displayed PNMT protein, and synthesized and stored ADR in large granular vesicles (100–300 nm) (131). Glucocorticoids increase TH, DBH, and PNMT protein levels in SIF cells of cultured SCG (47, 480).

Nevertheless, convincing data concerning PNMT gene expression in stellate ganglia of adult rats and mice were published only recently (310, 330). PNMT gene expression and PNMT protein levels in the stellate ganglia are elevated after exposure to a single and especially after repeated IMO stress (310).

2. Factors involved in regulation of gene expression

The importance of trans-synaptic nerve stimulation for regulation of CA biosynthetic enzyme gene expression in the sympatho-neural system has been well demonstrated. Preganglionic nerve stimulation elicits increases in TH and DBH activities and elevated TH protein and mRNA levels in rat SCG (41, 257, 714). Denervation of preganglionic inputs prevents the elevation of TH in response to reserpine. However, contribution of trans-synaptic inputs to the stress-triggered changes are less certain. Decentralization of SCG in young rabbits greatly reduced basal TH activity, but nevertheless, exposure to cold stress triggers a large induction in TH activity in SCG even following decentralization (9). This study shows that TH activity is induced in the SCG in the absence of preganglionic input, demonstrating a nonsynaptic regulatory mechanism.

The activation of the HPA axis by stress also appears to be involved in the regulation of the CA biosynthetic enzymes in the sympatho-neural system. Early studies showed that administration of a single dose of the synthetic glucocorticoid dexamethasone increases TH activity (222, 633) and TH mRNA (607) and enhances the response to cold (469) in sympathetic ganglia.

Furthermore, injections of ACTH to rats is as effective as IMO stress in triggering elevation of TH and DBH mRNAs in SCG (449). Further exposure of the ACTH treated animals to a single IMO stress does not lead to an additional increase. The effect of ACTH appears to occur, at least partially, by an adrenal independent mechanism. Adrenalectomy, which as expected, eliminated circulating ADR and increased plasma ACTH, also markedly raised plasma NA levels (340). The adrenalectomized rats display an exaggerated rise of plasma NA and its metabolites in response to stress (323). Moreover, TH and DBH mRNAs in rat SCG are high in adrenalectomized rats (547, 579). ACTH injections still increase TH and DBH mRNA in SCG in adrenalectomized rats in which CORT levels are clamped with CORT pellets to keep basal plasma ACTH near control levels (579).

These findings indicate that ACTH can have a direct effect on gene expression in sympathetic ganglia. Both rat SCG and StG were found to express the MC-2 receptor mRNA (451). Thus the response of the sympathetic ganglia to ACTH may not require the elevation of adrenocortical glucocorticoids. Although the synthetic corticosteroid dexamethasone is effective in increasing TH activity and mRNA in the SCG (222, 607, 633), three other glucocorticoids (corticosterone, hydrocortisone, and triamcinolone) failed to elevate TH activity in the SCG. The effect of dexamethasone was proposed to occur at the preganglionic cholinergic terminals (633). In this regard, infusion of cortisol does not stimulate the expression of TH or DBH gene expression in the SCG (449).

The HPA axis is also important in regulation of PNMT in the sympatho-neural system, and PNMT in stellate ganglia is elevated by IMO stress in wild-type but not in CRH-deficient mice (310, 330).

3. Comparison to the adrenal medulla

A comparison of the regulation of gene expression for CA biosynthetic enzymes in sympathetic ganglia and the adrenal medulla (reviewed in Ref. 555) clearly shows that in contrast to the original designation of the "sympathoadrenal" systems, these are clearly two distinct systems.

Quantitative evaluation of gene expression of CA biosynthetic enzymes was recently determined. In the adrenal medulla, the basal concentration of TH mRNA is ~0.5 amol/ng total RNA. DBH mRNA is ~12 times higher (6.0 amol/ng RNA) and PNMT mRNA ~56 times higher (28.0 amol/ng RNA) than TH mRNA. In stellate ganglia, the basal concentration of TH mRNA (0.02 amol/ng RNA) is ~25 times lower than in the adrenal medulla, but DBH mRNA in ganglia (2.6 amol/ng RNA) is present at similar concentration as in the adrenal medulla (334).

Repeated IMO (2 h daily for 7 days) keeps levels of TH and DBH mRNA elevated both in sympathetic ganglia and the adrenal medulla, but does not produce further increase compared with already elevated levels in adapted control group (immobilized for 6 days, 2 h daily, and killed 22 h later) (334, 417) (Fig. 16). PNMT gene expression is also increased after repeated stress both in StG and the adrenal medulla of rats and mice (310, 330, 334) (Fig. 16).

Thus gene expression of CA biosynthetic enzymes in sympathetic ganglia is, similarly to the adrenal medulla, markedly elevated, which is a part of the adaptation mechanism of the organism to long-term exposure to a homotypic stressor.

Although stress induces CA biosynthetic enzyme gene expression in both the adrenal medulla and sympathetic ganglia, there are important differences. The extent of IMO-induced elevation of TH mRNA and DBH mRNA levels in the two sympathetic ganglia where it was examined, SCG and StG, is about two- to threefold of basal levels (417, 449), which is lower than the maximal induction observed in the adrenal medulla. PNMT mRNA levels in stellate ganglia are also significantly elevated by IMO stress (310, 330).

The time course of the mRNA level changes is different in sympathetic ganglia compared with the adrenal medulla (see Fig. 15). In stellate ganglia, the time course of the elevation of TH and DBH mRNA levels was more gradual and the extent of elevation more modest than in the adrenal medulla. Maximal changes in StG are observed 24 h after cessation of exposure to a 2-h IMO (334, 417). Our unpublished data have shown highly elevated mRNA levels in the StG even 48 h after a single IMO stopped. In contrast, in the adrenal medulla there is a rapid change in TH gene expression with only a single exposure to IMO stress. This rise of about sevenfold above basal levels is maximal 3 h after cessation of 2 h of IMO (450), and returns to near basal levels 1 day later, a time when TH and DBH mRNA in StG are greatly elevated (Fig. 15).

Although stress-induced PNMT gene expression is regulated by the HPA axis in both the adrenal medulla and sympathetic ganglia of rats and mice (310, 318, 330), the involvement of the HPA axis in the IMO-triggered increase in TH and DBH gene expression also differs between sympathetic ganglia and adrenal medulla. While injections of ACTH increase TH and DBH mRNAs as much as IMO stress in SCG, they have no effect on expression of TH or DBH in the adrenal medulla (449), and MC-2 receptor mRNA is not expressed there (451).

In summary, the sympatho-neural system, at least as it relates to TH and DBH, is regulated distinctly from the adrenal medulla. Further work needs to be done to clarify the mechanism of stress-triggered changes in the different components of the sympatho-neural system.

C. Nonneuronal Adrenergic Systems

1. *Extra-adrenal nonneuronal adrenergic systems in stress*

The enzyme PNMT, which catalyzes *N*-methylation of NA to ADR (19), is largely restricted to the adrenal medulla where the majority of body's ADR is synthesized. In small concentrations, ADR is found in almost all organs of the body (73). NA can also be *N*-methylated by a less specific *N*-methyltransferase (NMT) which *N*-methylates many amines including DA (713).

In the past, it was believed that ADR in the heart is taken up mainly from circulation by sympathetic nerve endings (6, 236). However, some investigators predicted that a portion of the heart ADR might be synthesized directly in the cardiac tissue (19, 139–141, 508, 655). Several groups have observed that both rats and humans maintain nearly normal basal levels of blood and urinary ADR following adrenalectomy or medullectomy, which removes the major source of ADR (289, 655, 674). Moreover, Kvetnansky et al. (352) found a slight, but significant elevation of plasma ADR levels in adrenal medullectomized rats exposed to stress, suggesting an extra-adrenal source of ADR. It appears that at least one-third of the ADR in the heart is produced there. The extra-adrenal source of ADR is also proposed in humans, since after adrenalectomy patients maintained nearly normal levels of urinary ADR (674). Patients with heart transplants maintain adequate cardiac function even in the absence of sympathetic reinnervation (203, 540, 590). Abnormal cardiac ADR production in patients with heart failure may be derived in part from sources other than the uptake from plasma or sympathetic nerves, because enhanced cardiac ADR spillover into the coronary circulation is unrelated to stress-induced cardiac sympatho-adrenal activation (284).

PNMT serves as a marker for tissues and cells producing ADR. Besides the adrenal medulla, low PNMT activities have been found in various tissues, e.g., in heart (139–141, 247, 248, 286, 289, 308, 330, 333, 508, 712), spleen (285, 502), kidney (285), lung (286, 502), thymus (679), skeletal muscles (288), skin (522), human red blood cells (285), and different parts of the brain, especially in the brain stem (48, 502, 545) (see sect. III*C*).

In the majority of these tissues, PNMT gene expression has recently been detected as well, which suggests the presence of cells synthesizing PNMT protein. PNMT mRNA has been found in cardiac atria and ventricles (203, 308, 330, 333), spleen (7, 269, 679), thymus (7, 679), and very recently also in the sympathetic ganglia (310, 330) and skin (522). Transient PNMT expression was also described in cardiomyocytes during embryogenesis, prior to establishment of the sinoatrial and atrioventricular nodes and sympathetic innervation (140, 141, 508). ADR might

serve in a pacemaker capacity during early stages of cardiogenesis (140, 141, 508).

In 1996, a new type of cardiac cell capable of adrenergic paracrine signaling was identified in mammalian hearts. These cells, which do not have neuronal or chromaffin cell ultrastructural morphology, were named ICA (intrinsic cardiac adrenergic) cells (247, 248). They contain mRNA and proteins of enzymes involved in CA biosynthesis (mainly PNMT) and may participate in cardiac regulation independently of sympathetic innervation. ICA cells are capable of CA synthesis, release, and uptake, since NET is also localized in these cells (247). They are also able to release ADR (141). Adrenaline released from ICA cells has been shown to play a significant role in the regulation of the rate of beating of cardiac myocytes (247, 248). ICA cells may provide an alternative adrenergic supply to maintain cardiac contractile and pacemaker function at rest and during stress in the absence of sympathetic innervation. ICA cells occur in fetal myocardium in much higher abundance compared with adult heart (141).

Recently two types of catecholamine-containing intrinsic ganglionic neurons have been observed: in addition to the SIF cells, there are also large-diameter neurons (601). SIF cells exhibit TH and also tryptophan hydroxylase-IR immunoreactivity, but they are not positive for DBH. In contrast, large-diameter intrinsic TH-positive neurons also display DBH-IR and PNMT-IR, thus indicating the capacity for the synthesis of NA and also ADR. A majority of these large-diameter intrinsic neurons also show NPY-IR (601). Recent detection of expression of DBH and PNMT genes in cardiac atria supports these findings. The large-diameter neurons are much more numerous within the left atrial plexus than in right one, which is in good agreement with distribution of PNMT mRNA levels (308). Thus SIF cells are most probably dopaminergic, whereas large-diameter intrinsic cells seem to represent a subpopulation of NA and/or ADR-secreting neurons.

Types SIF I and SIF II cells were described in detail (130, 131). They seem to share some similar characteristics with cardiac intrinsic ganglionic neurons. The small ganglionic neurons with the catecholaminergic phenotype seem to be equivalent to SIF cells of sympathetic ganglia (601).

Even if TH-IR has been clearly shown in ICA and SIF cells (247, 248, 601), TH gene expression was detected only in fetal rat hearts before sympathetic innervation (247, 248) and not in the heart of adult animals. TH mRNA levels in the cardiac areas are most probably below the detection limit of the available RT-PCR techniques. It is possible that there exists in the heart a special system synthesizing ADR from uptaken DOPA or NA, which is *N*-methylated by PNMT located in ICA cells and most probably also extra-neuronaly in cardiac muscle cells (289, 308, 333).

These findings have definitely confirmed an extra-adrenal presence of an ADR-producing system including the enzyme PNMT in various tissues mainly in the heart. The findings have also suggested that cardiac PNMT is an extra-neuronal enzyme (287, 308, 333, 654, 713). PNMT was localized immunofluorescently in isolated cardiomyocytes (654).

The extra-adrenal systems producing ADR are also affected by stress. Stress exposure induces PNMT gene expression not only in the adrenal medulla (330, 334, 551, 672, 695), but also in the cardiac atria and ventricles (308, 330, 333) and in spleen (269). The study of Krizanova et al. (308) established for the first time that PNMT gene expression occurs in cardiac atria and to a smaller extent also in ventricles of adult rats and that PNMT mRNA levels in heart tissues are severalfold increased by IMO stress. Both Southern blot and sequencing verified the specificity of PNMT detected by RT-PCR. PNMT gene expression was modulated differently in cardiac atria and ventricles (653). In atria, PNMT mRNA levels are increased by hypoxia but decreased by cold stress. In ventricles, no significant changes are observed (653). In atria, gene expression of PNMT is clearly modulated by glucocorticoids, because adrenalectomy or hypophysectomy prevent the increase in PNMT mRNA levels in response to IMO stress (308). In ventricles, the effect of glucocorticoids on PNMT gene expression is not so clear (330).

Recently, the first data on presence and distribution of the PNMT protein in hearts of adult rats and mice exposed to stress have been reported (330, 333). PNMT mRNA and protein levels are detected in all parts of the heart that were studied (atria, atrial ganglia, ventricles, septum), with the highest levels in the left atrium and its ganglionic part. Single IMO stress exposure increases gene expression of PNMT in right and left atria, but surprisingly, the ganglionic parts of the atria do not respond to stress stimulation (330, 333). Levels of PNMT mRNA remain elevated in cardiac atria of repeatedly immobilized rats (416) and mice (330). This finding suggests that increased ADR synthesis in the heart of mammals is a part of the adaptation process of the organism to chronic or repeated stress exposure.

PNMT mRNA levels were also measured in samples of human myocardium obtained from the right ventricle at regular routine endomyocardial biopsy performed for diagnosis of graft rejection in 18 patients, 0–10 yr after heart transplantation (203), and were significantly higher during the first 3 yr compared with further periods after the transplantation. In addition, a decrease of the average heart rate and an increase of the heart rate variability were documented. Levels of PNMT mRNA do not correlate with blood pressure, left ventricular systolic function at rest, or rejection. The authors speculated that the in-

creased PNMT transcription in human myocardium in early intervals after the heart transplantation reflects "autonomous sympathicotrophy" (203). A decrease in the PNMT gene expression with years after transplantation could be a consequence of the reinnervation process.

Recent data have shown that PNMT gene is also expressed in lymphoid tissues such as spleen and thymus (7, 269). However, the ratio of PNMT mRNA levels (7) and also PNMT activity (502) in adrenal medulla to those in spleen is ~200-fold. PNMT mRNA in spleen was found to be present in the white pulp. Immobilization stress produced a robust rise in spleen PNMT mRNA levels (269). We have recently documented that PNMT gene expression in rat thymus is also significantly activated by exposure to stressors.

PNMT mRNA levels have also been recently detected in the stellate ganglia and were found to be elevated by stress (310, 330). PNMT activity has been detected in sympathetic ganglia especially of newborn rats (159, 372, 429, 480, 481). Culman et al. (113) found PNMT activity in the superior cervical ganglia of adult rats, which increased after a corticosterone administration. Several authors have reported presence of immunoreactive PNMT in sympathetic ganglia, mainly in early postnatal periods of life (159, 481). However, the levels are low, and only one paper showed gene expression of PNMT in superior cervical ganglia measured by in situ hybridization technique after reserpine administration (568). Recently, PNMT mRNA levels were detected in sympathetic ganglia of adult rats or mice by RT-PCR method (310).

These studies clearly suggest that ADR produced by extra-adrenal medullary tissues plays an important role in the handling of stress situations.

2. *Peripheral DOPA-dopamine autocrine/paracrine system (199)*

As indicated earlier, DA plays an important role as a neurotransmitter in the brain (see sect. III*D*). However, understanding of DA functions in the periphery has lagged behind. Even if plasma levels and urinary excretion of DA exceed those of NA, DA seems to function neither as a neurotransmitter released from putative dopaminergic nerves or coreleased with NA from sympathetic nerves, nor as a hormone released from the adrenal medulla along with ADR. Instead, most of DA production in the body occurs in nonneuronal cells, e.g., in kidneys where DA appears to depend mainly on uptake of its precursor L-DOPA from the circulation with conversion to DA by the enzyme L-aromatic-amino acid decarboxylase in proximal tubular, i.e., nonneuronal and nonchromaffin cells (58, 688). Dopamine exiting the nonneuronal cells then appears to act as an autocrine/paracrine substance, promoting natriuresis by local inhibition of Na^+-K^+-ATPase.

More DA production and metabolism takes place in the mesenteric organs than in the brain, sympathetic nerves, or adrenal chromaffin cells (150). Presence of dopamine and its receptors are described also in the stomach, pancreas, and other mesenteric organs (414, 415). It is possible that locally produced DA contributes to regulation of gastrointestinal motility. The function of this system in stress situations is, however, not clear.

D. Catecholamine-Degrading Enzymes in Stress

Surprisingly, unlike the CA biosynthetic enzymes, the activity and especially gene expression of MAO and COMT have not been extensively studied under stress situations. The papers dealing with the stress-induced changes in CA degrading enzymes are discussed in this section.

Activities of CA-degrading enzymes were investigated in liver of rats after stress exposure. Immobilization stress decreased both MAO-A and MAO-B activities (461). Exposure to cold decreases liver MAO-A activity and also the ratio of MAO-A to MAO-B, which suggests that cold, but not IMO, changes the proportion of these forms of MAO activity. Decreased MAO-A activity and unchanged MAO-B activity are also observed in hearts and brains of rats exposed to foot-shock (359).

MAO and COMT activities were measured in separated adrenal cortex and adrenal medulla of rats exposed to a single or repeated IMO stress (348). In controls, the activities of both enzymes are higher in the adrenal cortex than in the adrenal medulla. The MAO activity was unchanged in the adrenal medulla of stressed animals. After a single IMO, activity of COMT is decreased in the adrenal cortex but increased in the adrenal medulla, while animals immobilized repeatedly show lower COMT activity in both parts of the adrenal. Thus the decreased activity of COMT both in adrenal cortex and medulla of repeatedly immobilized rats might reflect participation of this CA-degrading enzyme in the increased levels of CA found in the adrenals of repeatedly stressed rats (335).

MAO and COMT activities were also studied in the adrenals of rats that spent 18–20 days in space on board three COSMOS biosatellites. The data suggest that a prolonged stay in microgravity (weightlessness) does not appear to be a pronounced stressful stimulus for the catecholaminergic systems including CA-degrading enzymes (349).

MAO activity was measured also in superior cervical, stellate, and nodose ganglia of rabbits with different resistance of cardiovascular function to IMO stress exposure. MAO activity declines in sympathetic ganglia of stress-predisposed animals, while in stress-resistant animals it increases (205). In the nodose ganglium, the MAO activity is unaffected by this stressor (204).

Concerning CA degradation in the brain, MAO activity is unchanged or slightly decreased during IMO, while COMT activity is very rapidly (by 5 min) increased in all the hypothalamic nuclei studied (322). In repeatedly IMO rats, there is a further decrease in MAO activity and a reduction of COMT activity in all the hypothalamic nuclei studied, together with highly elevated NA levels. The reduction of CA levels in the hypothalamic nuclei of rats exposed to an acute stressor correlates well with the changes in the activity of CA-degrading enzymes, particularly with the increased COMT activity. In addition to the increased synthesis, a decreased degradation of CA may also participate in the increased NA concentration seen in the hypothalamic nuclei of repeatedly stressed rats (322).

In another study, immobilization stress increases activity of MAO-A and decreases MAO-B activity in the brain of rats (516). Preadministration of actinomycin D completely prevents the changes in activity of both MAO, indicating transcriptionally mediated regulatory pathway of MAO activity during stress. The investigators did not find a regulatory effect of corticosterone in regulation of MAO activities during an acute stress exposure (516). Infusion of MAO inhibitors into the LC region of the brain eliminates both the large depletion of NA in this area and the behavioral depression (measured by swim test) that otherwise results from exposure of animals to uncontrollable shock (600).

Levels of mRNA coding for MAO-A and MAO-B in brain dorsal raphe nucleus of monkeys categorized as high, medium, or low stress resistant were found to be significantly reduced in the stress-sensitive (low-resistant) group (36). Stress-induced reduction in gene expression of these enzymes could participate in increased neurotransmitter levels, which might be a part of the mechanism leading to development of diseases such as anxiety, depression, as well as cardiovascular and immune diseases, etc.

Thus stress-induced changes in CA-degrading enzyme activity are not uniform and certainly are dependent on the strain of the animals, on particular brain regions, model of stress, timing of stressor, and many other factors.

An intriguing question arose concerning a response to stressors in the absence of MAO. A line of transgenic mice has been generated in which the gene that encodes MAO-A is disrupted. MAO-A knockout mice have elevated brain levels of 5-HT, NA, and DA and manifest aggressive behavior (79, 592, 593, 595). Mice deficient in MAO-B by homologous recombination have also been generated (593). Interestingly, MAO-B knockout mice show increased phenylethylamine (PEA) levels only and do not exhibit aggression. MAO A/B double-knockout mice display fear, anxiety, hyperreactivity, and dysregulation of heart rate in novel environment (592, 595). Their levels of NA, DA, serotonin, and PEA are increased much more than in MAO-A or MAO-B single-knockout mice. Both MAO-A-deficient mice (79) and MAO-B-deficient mice (211) show an increased reactivity to stress in the forced-swim test, most probably because of the elevated brain NA and DA levels in MAO-A knockout mice and PEA levels in MAO-B knockout mice.

Does MAO-A knockout affect activity of HPA? The effect of the absence of the gene encoding MAO-A in transgenic mice on corticosterone response to a single or repeated exposure to various stressors, e.g., restraint, cold, water deprivation, and psychosocial stress, has been recently described (515). MAO-A knockout display an attenuated adrenocortical response to these stressors except for the response to psychosocial stressors. The decreased stress response in MAO-A-deficient mice is due to central mechanisms regulating stress-induced ACTH release rather than to the adrenocortical responsiveness to ACTH. Morsink et al. (435) recently reported that gene expression of MAO-A in hippocampus is rapidly downregulated by glucocorticoids via glucocorticoid receptors (435). Pretreatment with the GR antagonist RU 38486 reversed this effect, illustrating the GR specificity of transcriptional regulation by corticosterone. Three glucocorticoid/androgen response elements were found within the first 2 kb of the MAO-A promoter (88, 594); however, the exact mechanism of regulation of MAO gene expression by HPA is not known yet.

There is scant information concerning COMT gene expression under stress conditions. A threefold decrease of COMT mRNA levels is found in the midbrain of the aggressive mice-winners, compared with control mice (170). The absence of changes in COMT mRNA in defeated mice suggests an involvement of COMT in the mechanism of aggressive behavior. COMT-deficient heterozygous null mutant male mice also exhibited increased aggressive behavior (195). COMT activity (319) and also COMT mRNA levels are significantly decreased in liver of adult mice exposed to IMO stress (418). In aged mice, however, the effect of stress on COMT mRNA levels in liver is absent (418).

Genetic differences in COMT have been shown to underlie individual differences in response to psychological and physical stressful challenges (603, 715). A significant association between COMT genotype and history of violent behavior was shown in humans (356). Jabbi et al. (264) examined the effects of the COMT polymorphism on psychological stress. Their observations support a possible role for COMT polymorphism in endocrine and psychological stress responses and may qualify COMT as a possible candidate gene involved in the pathogenesis of major depressive disorders. Thus COMT is considered to be in close correlation with several psychiatric disorders including schizophrenia (264, 356).

Polymorphic variations in genes coding for COMT and MAO-A influence also the regulation of HPA axis

response to acute psychological and endocrine challenges (265).

Therefore, to evaluate a possible function of CA-degrading enzymes in the organism after exposure to stress, we suggest that the activity of MAO and COMT in different organs is preferentially reduced. The reduced degradation process, together with stress-induced increases in CA production, release, and secretion, might be involved in the enhanced availability of CA for adrenergic receptors and for increased activity of metabolic and physiological processes under stress.

E. Molecular Mechanisms of Peripheral Stress Response

1. Role of transcription

It is important to determine the mechanism by which stress triggers long-term changes in gene expression. Transcription plays a major role in the regulation of gene expression of CA biosynthetic enzymes in the adrenal medulla. A number of experimental approaches have been used to establish that the adrenal medulla responds to stress with changes in transcription of CA biosynthetic enzymes.

Early studies tested the requirement for transcription with inhibitors. Administration of the transcriptional inhibitor actinomycin D to rats blocks the stress-evoked increases in activity of adrenal CA biosynthetic enzymes. As early as 1971, it was shown that the increase in adrenal DBH activity is blocked in immobilized rats pretreated by actinomycin D (326). The increase in TH activity induced by 2-h swimming stress in cool water (15°C) is also inhibited by actinomycin D if added during the stress or shortly thereafter, but not a day later (468). Treatment with actinomycin D also prevents the IMO stress-triggered elevation of TH and PNMT mRNA levels as well as their enzymatic activities (450, 672).

While inhibition studies with actinomycin D indicate that transcription is required, they did not determine whether the increase in transcription is occurring specifically in the adrenal medulla. They also did not rule out the possibility of a requirement for transcription of a modulatory factor or coenzyme rather than transcription of the genes for the CA biosynthetic enzymes themselves.

More direct methods were used to determine that the stress-elicited change in transcription involves the genes of the CA biosynthetic enzymes. Run-on assays of transcription were performed with nuclei of cells from adrenal medulla of controls and rats exposed to IMO stress. The results demonstrate that there is an increase in the initiation of both TH and DBH specific transcripts (454). Both single and repeated IMO stress lead to increased initiation of TH and DBH gene transcription to levels about threefold higher than in control animals.

What is especially interesting about the transcriptional pathways is the finding that even brief exposure to a stressor (5-min IMO) is sufficient to increase adrenal TH and DBH gene transcription to levels (3-fold over controls) similar to the ones observed with repeated stress. However, this rise is transient if the stress is not prolonged. With intermediate duration (30 min) of a single immobilization, the transcriptional activation of TH is more stable and still elevated four times above basal levels 90 min later, while DBH transcription is still transient. Following repeated stress (2 h daily for several consecutive days), the activation of TH and DBH transcription is stabilized and significantly elevated even a day later (453). These results reveal that transcriptional mechanisms are also involved in the sustained upregulation of adrenal TH and DBH gene expression in response to repeated stress.

The persistence of elevated TH transcription in the adrenal medulla following repeated IMO stress was also shown with intron-specific probes (623). Following repeated IMO stress (2 h daily for 1 wk), primary transcripts are increased about three times levels in controls and remained high even 2 days after cessation of the stress (623). They correlate with the sustained rise in mRNA levels following repeated IMO.

Transgenic animals in which the reporter activity is under control of the 4.5 kb of the TH 5′-flanking region of the TH promoter confirm that this region of the gene is sufficient to mediate increased transcription in response to stress. Chloramphenicol acetyltransferase or alkaline phosphatase reporter activity under control of the TH promoter increases severalfold in adrenal medulla upon exposure to cold or IMO stress (466, 467). Comparable experiments have not yet been performed for DBH or PNMT promoters.

These experiments clearly show by a variety of independent methods that elevated transcription of CA biosynthetic enzymes plays an important role in adrenal medullary response to stressors.

2. Factors involved in transcriptional activation

A) ADRENAL MEDULLA. Recent work has begun to identify different transcription factors associated with brief or intermediate duration of a single stress or with repeated stress, suggesting that there is a dynamic interplay in converting short-term transient activation of transcription to prolonged potentially maladaptive changes in gene expression (reviewed in Ref. 551). This has been studied in considerable detail for IMO and cold stress.

A diagram of the proximal TH, DBH, and PNMT promoters, and the presence of several characterized regulatory motifs which may be involved in mediating change transcriptional activation are shown in Figure 6.

B) IMMOBILIZATION STRESS. The very rapid activation of transcription of genes for TH and DBH which were already greater than threefold basal levels after only 5 min of IMO is too soon to reflect de novo synthesis of induced transcription factors. Thus a swift increase in transcription may be mediated by rapid phosphorylation of preexisting transcription factors. In this regards, increased phosphorylation of CREB (detected with an antibody specific for the Ser-133 phosphorylated form of CREB, required for activation) is observed in the adrenal medulla of rats immobilized for only 5 min (553) (Fig. 17). As described in section II*E*, the activation of CREB at the CRE motif of the TH promoter is a well-characterized mechanism that is important for activation of TH gene transcription (298, 362). It also is implicated in the regulation of DBH transcription at the CRE/AP1 motif (575). With longer exposure to a single IMO, the amount of phosphorylated CREB decreased gradually, and after 2 h, the levels were similar to those of control animals. The phosphorylation of CREB on Ser-133 can be mediated by a number of kinases, including PKA, MAP kinases, and CaM kinase (426). Activation of the MAP kinases ERK detected by increased phosphor-ERK1/2 in rat adrenal medullary extracts after 5 min of IMO may mediate this rapid activation of CREB (552).

In intermediate duration of a single episode of stress, de novo synthesis of transcription factors can play a role. AP1 factors (e.g., c-Fos) and Egr1 are among the likely candidates for mediating the transcriptional responses of TH to intermediate episodes of stress. Single IMO stress induces immunoreactive c-Fos in the adrenal medulla, which is correlated with its increased binding to the AP1 like sites on the TH and DBH promoters (423, 448, 450).

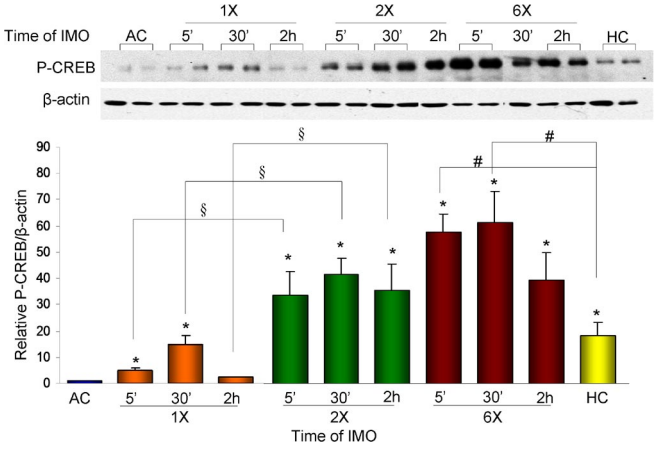

FIG. 17. Changes in phosphorylation of CREB in the adrenal medulla with different durations and repetitions of immobilization stress. Representative immunoblots of IMO-induced changes in levels of P-CREB protein are shown on the upper panel. Blots were stripped and reprobed for β-actin, which was used as a loading control. Summary data of the experiments are shown on the lower panel. *$P < 0.05$ vs. absolute control (AC); §$P < 0.05$ vs. 1xIMO; #$P < 0.05$ vs. handled control (HC). [Modified from Sabban et al. (552), with permission from Springer.]

However, c-Fos does not appear to be essential for the IMO stress-triggered elevation of gene expression of CA biosynthetic enzymes. In fact, even in c-*fos*-deficient mice, IMO stress was able to elevate adrenal TH, DBH and PNMT gene expression to a similar extent as in wild-type animals (311, 583).

The c-Fos is probably not the only AP1 factor manifesting the second wave of gene expression in response to stressors. In this regard, another Fos-family member Fra-2 protein level is also induced even with single IMO and may be compensating for the c-Fos deficiency. The immobilization stress-triggered induction of Fra-2 is slower than the phosphorylation of CREB and is not significantly changed during the first 30 min of IMO (552). After 2-h IMO, however, Fra-2 is significantly elevated, which may lead to elevated TH and DBH gene transcription, since overexpression of Fra-2 in cell cultures of adrenomedullary origin led to elevated TH and DBH promoter activities (452). The increased levels of Fra-2 seen after the first IMO are even potentiated by repeated IMO stress. This might be one of the mechanisms involved in permanently elevating TH mRNA levels in the adrenal medulla of repeatedly immobilized rats. The importance of Fra-2 in the regulation of TH gene expression in the adrenal medulla is also evident in elegant studies with specific angiotensin receptor blockers (13, 272).

The immediate early gene Egr1 is also induced in rat adrenal medulla by a single stress exposure. Increased expression of Sp1 transcription factor and its ability to form protein/DNA complexes is also observed already with single IMO (635). These transcription factors may mediate the changes in TH as well as PNMT gene expression. They require phosphorylation for their binding and transcriptional activation, hence changes in their phosphorylation state is also a crucial determinant of their regulatory function (72, 98, 266). The importance of Egr1 in regulation of PNMT transcription, either alone or in combination with glucocorticoids, and AP2 factors is demonstrated by Wong and coworkers (693, 695). Reduced levels of Egr1 in the adrenal medulla of CRH knockout mice exposed to IMO stress correlate well with reduced PNMT mRNA levels in those animals (330). The role of Egr1 in cholinergic stimulation of PNMT promoter in the adrenal gland was described (430, 432). Egr1 can also regulate transcription of the TH gene. Overexpression of Egr1 in PC12 cells elevates reporter activity under control of the TH promoter and could play an important role in the regulation of TH transcription (493). Egr1 appears to interact with AP-1 factors to regulate TH transcription (447, 493). In contrast, Egr1 was found to reduce DBH promoter-driven transcription (90a).

Many of the transcription factors changed with IMO in the adrenal medulla are also implicated in the response of the LC to stress (see sect. III*G*). This includes CREB, c-Fos, and Fra-2. However, the transcriptional response to stress in the LC is not identical to that of the CA-synthe-

sizing cells of the adrenal medulla. Of particular note, in contrast to the adrenal medulla, Egr1 is not induced by IMO in the TH-positive cells of the LC (225).

To obtain a broader picture of the changes in gene expression in the adrenal medulla in response to a single episode of stress, microarray profiling was used (371). Following a single IMO stress for 2 h, several hundred genes are significantly up- and downregulated (>2-fold). Among the upregulated defined genes, ~20% are transcription factors. Interestingly, an analysis of the direct interactions among the genes affected by stress indicate that Fos, Egr1, and nuclear receptor family member NR4A1 (also known as Nurr 77 and as NGFI-B, which can interact with NBRE motif on TH promoter) are likely to play a central role in interacting with many of the adrenomedullary genes regulated by a single episode of IMO stress (Fig. 18).

A comparison of the time course of changes of several transcription factors during the course of first, second, and sixth daily IMO stress reveal that with the repeated episodes of stress, some of the same transcription factors induced by single stress, such as Egr1, are still induced (695).

Not only is there induction of new transcription factor expression, but also sustained phosphorylation of CREB throughout the entire course of the repeated stress as shown in Figure 7. Adrenal medullae of rats exposed to chronically repeated IMO stress (6× IMO) display an enormous elevation of P-CREB (~60-fold over unstressed controls and ~3-fold over handled controls) even by 5 min of the sixth IMO, and the levels remain very high for an entire 2 h of IMO (552).

Repeated stress also triggers an enormous induction of Fra-2 (370, 452, 552). Fra-2 is also phosphorylated with the repeated stress (370). This elevation in Fra-2 is colocalized with TH in chromaffin cells and displays several forms that differ in the extent of phosphorylation (370).

Microarray analysis of the changes in the adrenal medulla with repeated IMO (6×, 2 h daily) revealed that fewer transcription factors are induced with repeated compared with single IMO (371). About half the genes induced by repeated IMO are also elevated by single IMO. The experiments provide new mechanistic insights and potential new biomarkers for the adrenomedullary response to stress.

The adrenal medulla does not appear to desensitize after repeated episodes of IMO stress, and even with exposure to 2 h of daily IMO stress for 42 days, the final stressor still triggered induction of TH mRNA and Fos related antigens such as Fra-2 (338).

C) COLD STRESS. Cold, like IMO stress, triggers elevation of TH transcription in the adrenal medulla; however, the transcription factors modified were very different. Like IMO, cold exposure for 1–48 h triggers increased binding to AP1 motif of the adrenal TH promoter (423), and a transient elevation of c-Fos observed within the first few hours of cold exposure (370). However, there are no significant changes in phosphorylation of CREB or induction of Egr1 at any of the time points examined from brief (2 h) to prolonged (28 days) exposure to cold (370). Fra-2 is induced by cold stress, but the time course of changes in Fra-2 differed from that observed with c-Fos. Levels of Fra-2 are not significantly changed with 1 or 7 days of cold stress but are about double basal levels after 28 days of

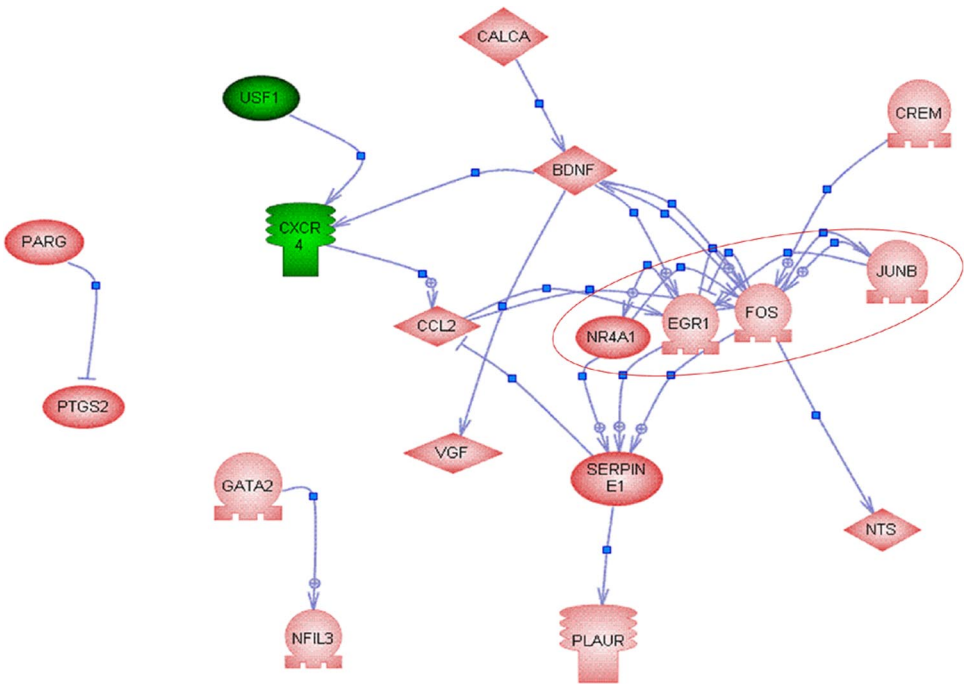

FIG. 18. Results of pathway analysis for direct interactions among top −70 up- and top −50 downregulated genes altered in rat adrenal medulla by 2 h of immobilization stress. The upregulated genes are shown in red and the downregulated genes in green. BDNF, brain-derived neurotrophic factor; CALCA, calcitonin/calcitonin-related polypeptide alpha; CCL2, chemokine (C-C motif) ligand 2; CXCR4, chemokine (C-X-C motif) receptor 4; GATA2, GATA binding protein 2; JUNB, Jun-B oncogene; NFIL3, nuclear factor interleukin-3 regulated; NR4A1, nuclear receptor subfamily 4 group A member 1; NTS, neurotensin; PARG, poly(ADP-ribose) glycohydrolase; PLAUR, plasminogen activator urokinase receptor; PTGS2, prostaglandin-endoperoxide synthase 2; SERPINE1, serine (or cysteine) proteinase inhibitor clade E member 1; USF1, upstream transcription factor 1.

continual cold stress (370). This is still much smaller than the changes in Fra-2 with repeated IMO stress.

Initial microarray analysis reveals that IMO and cold activate a very different repertoire of transcription factors in the adrenal medulla. Cold stress triggers significant changes in expression of fewer genes than IMO stress. There is overlap in only ~5% of the genes up- or down-regulated by IMO stress (Fig. 19).

Thus specificity of response of the organism exposed to various stressors is confirmed even at the molecular genetic level of expression of different genes.

D) HETEROTYPIC NOVEL STRESSOR. To determine whether the exaggerated response of TH to the heterotypic stressor is manifested in terms of transcription factor induction or activation, rats with and without prior exposure to 28 days of cold stress were subjected to IMO (370). A greater response is observed with respect to P-CREB, Egr1, and Fra-2. Rats preexposed to 28 days of cold stress display a significantly higher elevation of P-CREB in response to the IMO, even though cold stress did not alter basal levels of CREB. The increase in phosphorylation of CREB in the cold-preexposed rats following 30 min of IMO was nearly double the response of the naive rats.

The IMO-triggered changes in Egr1 levels are also markedly influenced by cold preexposure. The induction of Egr1 in response to IMO is more pronounced and prolonged in the cold-preexposed rats. The maximal levels attained are about three times higher than in the naive rats. Even 3 h after cessation, the levels of Egr1 in cold-preexposed rats are still significantly elevated.

Fra-2 is dramatically affected by IMO in both groups. Prolonged cold stress triggers about a two- to threefold elevation in Fra-2, and IMO leads to a tremendous (10- to 15-fold) elevation in Fra-2 (370) (Fig. 20). While the extent of the induction of Fra-2 by IMO is similar in both groups of rats, there is substantial phosphorylation of Fra-2 especially with 2 h of IMO of the cold-preexposed animals.

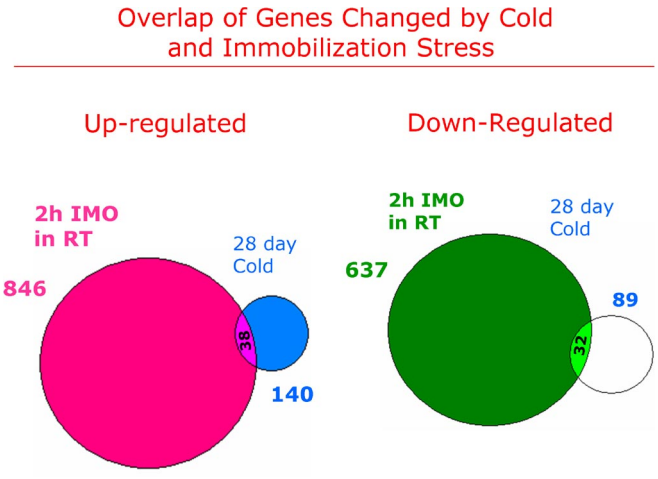

FIG. 19. Comparison of genes in rat adrenal medulla altered by immobilization and cold stress. The overlap is shown in genes whose transcripts were significanly up- or downregulated by 2 h of immobilization stress (IMO) at room temperature (RT) or 28 days of cold stress.

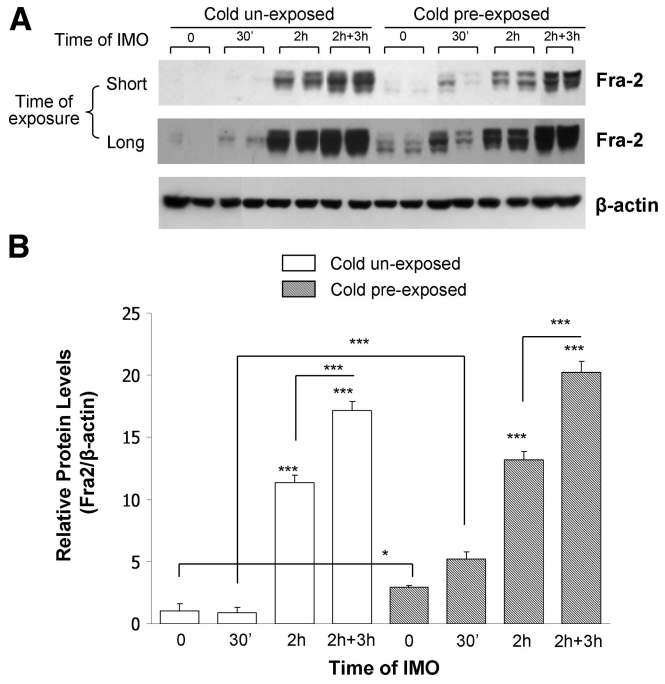

FIG. 20. Immobilization stress elicited induction of Fra-2 protein levels in the adrenal medulla of rats with or without cold preexposure. *A*: rats at room temperature (cold unexposed) or 4°C for 28 days (cold preexposed) were subjected to IMO (30 min or 2 h) and killed immediately, or 3 h after 2 h IMO (2h+3h). Short- and long-term exposure of representative Western blots for Fra-2 levels are shown. *B*: summary data (means ± SE) of Fra-2 protein levels are shown. The area comprising all the phosphorylated forms [multiple bands observed on Western blots (*A*)] of Fra-2 was scanned from each sample and normalized to the values from control untreated rats (0). $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ versus respective unimmobilized controls (0), or by the indicated comparisons. [From Liu et al. (370), with permission from Elsevier.]

Thus, when cold-adapted rats are exposed once to the heterotypic stressor of IMO, there were great differences in terms of the dynamics or magnitude of induction and/or phosphorylation of the transcription factors examined.

E) SYMPATHO-NEURAL SYSTEM. The molecular mechanisms mediating the stress-triggered changes in gene expression of the sympatho-neural system are not as well studied as in the adrenal medulla. It remains to be determined whether the changes observed in mRNA levels in sympathetic ganglia are due to increased transcription of TH, DBH, and PNMT genes or to changes in mRNA stability. Another difference relates to the potential involvement of AP1 transcription factors. The marked induction of AP1 factors, c-Fos, and Fra-2 observed in adrenal medulla (450, 452) do not appear to be important in sympathetic ganglia, as binding to the AP1 motif of the promoter is barely detectable with nuclear extracts from SCG following IMO stress (555) or in response to reserpine (658). The

c-Jun NH_2-terminal kinase (JNK) is also selectively induced in adrenal medulla, but not in SCG (555).

However, IMO stress was found to increase CREB binding at the CRE element on the TH gene promoter in extracts of SCG (555), suggesting increased expression of CREB and related transcriptional mechanisms are likely involved. In contrast in the adrenal medulla, the same stressor did not alter CREB levels, but rather triggered increased phosphorylation of CREB (555).

Thus we can speculate that an induction of CREB or a family member plays a critical role in the response in stress in the SCG, and this might mediate a sustained elevation of transcription of TH, DBH, and other CREB-responsive genes.

V. CONCLUSIONS AND FUTURE DIRECTIONS

A large body of previous research has confirmed that the catecholamine systems, especially the noradrenergic and adrenergic, both in the periphery and in the CNS, play key roles in mediating the physiological responses to stress situations. Within the past decade the anatomical pathways for response to specific stressors have been mapped. Data summarized here indicate that different types of stressors involve discrete regions and pathways, as well as their combinations in the brain. Moreover, responses for many types of stressors involve several brain structures. Knowledge about the existence of stressor-specific pathways and neuronal circuits may help us to understand the mechanism of action of various stressors and to elucidate those specific target areas in the brain that participate in orchestrating stress responses. Whether these same pathways are altered with repeated exposure to the same homotypic stressor or to a novel heterotypic stressor remains to be determined.

The rapid development of brain-imaging techniques will enable researchers to apply this neuromorphological knowledge to clinical practice, to recognize the target sites of chronic stressors in the brain as well as their morphological and functional consequences. It will open new directions and perspectives regarding more specific diagnosis and treatment of stress-related disorders.

It is now evident that there are important differences in the molecular mechanisms of stress responses in various catecholamine locations. This is exemplified in the differences between stress-triggered changes in expression of catecholamine biosynthetic enzymes in the adrenal medulla compared with the sympatho-neural system, or even within different sympathetic ganglia. While this is promising from a selective therapeutic perspective, much work still needs to be done to determine tissue and stressor specific mechanisms.

The repertoire of the stress-triggered alterations in gene expression and the mechanisms mediating these changes is just beginning to be elucidated. Future studies are needed to broaden our understanding of the adaptation and especially maladaptation of the catecholamine systems to stress. Much of the neuroanatomical mapping has concentrated on expression of the immediate early gene c-*fos*, but as studies have shown, so far primarily in the adrenal medulla, this is only one aspect of the transcriptional changes triggered by stress.

An understanding of the mechanisms of various stressors and of the individual response to different stressors can be extraordinarily important in systems biology, physiology, and applied molecular genetics. It is also crucial to understand the mechanism whereby the response to a particular stressor is influenced by prior experience with stress. Reprogramming of gene expression within stressor specific neurons may lead to habituation to a particular stressor. These changes to mild responses in specific neurons could potentially make the target cells resistant to the next, dangerously strong stressor. Such preconditioning for surviving extreme conditions is frequent in nature (hibernation, accommodation to extreme heat or pain), and the potential clinical use of this phenomenon now becomes realistic. However, the mechanism whereby there can be habituation to a particular stressor and nevertheless an exaggerated response or sensitization to a novel heterotypic stressor remains to be better deliniated.

It has become clear that exposure to stress triggers long-term changes in gene expression in catecholamine neurons. It still remains unclear which of these changes are beneficial (protective) adaptive responses mediating resilience to stress and which are detrimental leading to vulnerability to the many stress-related disorders. These changes should be a focus for future research.

Given the essential role for allostatic changes to enable survival of the species throughout human history, it is not surprising that it is so complex. We hope that we have provided a good summary of current knowledge that will help to determine the future directions of the journey towards a comprehensive understanding of stress-triggered regulation of catecholamine physiology.

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REFERENCES

1. **Abel JJ, Crawford AC.** On the blood-pressure-raising constituent of the suprarenal capsule. *Bull Johns Hopkins Hosp* 8: 151–157, 1897.
2. **Abell CW, Kwan SW.** Molecular characterization of monoamine oxidases A and B. *Prog Nucleic Acid Res Mol Biol* 65: 129–156, 2001.
3. **Abercrombie ED, Keefe KA, DiFrischia DS, Zigmond MJ.** Differential effect of stress on in vivo dopamine release in striatum, nucleus accumbens, and medial frontal cortex. *J Neurochem* 52: 1655–1658, 1989.
4. **Ajika K, Hokfelt T.** Ultrastructural identification of catecholamine neurones in the hypothalamic periventricular-arcuate nucleus-median eminence complex with special reference to quantitative aspects. *Brain Res* 57: 97–117, 1973.
5. **Alterio J, Mallet J, Biguet NF.** Multiple complexes involved in tyrosine hydroxylase mRNA stability in rat adrenal medulla, after reserpine stimulation. *Mol Cell Neurosci* 17: 179–189, 2001.
6. **Anden NE.** Uptake and release of dextro- and laevo-adrenaline in noradrenergic stores. *Acta Pharmacol Toxicol* 21: 59–75, 1964.
7. **Andreassi JL 2nd, Eggleston WB, Stewart JK.** Phenylethanolamine *N*-methyltransferase mRNA in rat spleen and thymus. *Neurosci Lett* 241: 75–78, 1998.
8. **Andrew SF, Dinh TT, Ritter S.** Localized glucoprivation of hindbrain sites elicits corticosterone and glucagon secretion. *Am J Physiol Regul Integr Comp Physiol* 292: R1792–R1798, 2007.
9. **Andrews T, Lincoln J, Milner P, Burnstock G, Cowen T.** Differential regulation of tyrosine hydroxylase protein and activity in rabbit sympathetic neurones after long-term cold exposure: altered responses in ageing. *Brain Res* 624: 69–74, 1993.
10. **Anney RJ, Olsson CA, Lotfi-Miri M, Patton GC, Williamson R.** Nicotine dependence in a prospective population-based study of adolescents: the protective role of a functional tyrosine hydroxylase polymorphism. *Pharmacogenetics* 14: 73–81, 2004.
11. **Anstrom KK, Woodward DJ.** Restraint increases dopaminergic burst firing in awake rats. *Neuropsychopharmacology* 30: 1832–1840, 2005.
12. **Apparsundaram S, Moore KR, Malone MD, Hartzell HC, Blakely RD.** Molecular cloning and characterization of an L-epinephrine transporter from sympathetic ganglia of the bullfrog, *Rana catesbiana*. *J Neurosci* 17: 2691–2702, 1997.
13. **Armando I, Jezova M, Bregonzio C, Baiardi G, Saavedra JM.** Angiotensin II AT1 and AT2 receptor types regulate basal and stress-induced adrenomedullary catecholamine production through transcriptional regulation of tyrosine hydroxylase. *Ann NY Acad Sci* 1018: 302–309, 2004.
14. **Armstrong DM, Ross CA, Pickel VM, Joh TH, Reis DJ.** Distribution of dopamine-, noradrenaline-, and adrenaline-containing cell bodies in the rat medulla oblongata: demonstrated by the immunocytochemical localization of catecholamine biosynthetic enzymes. *J Comp Neurol* 212: 173–187, 1982.
15. **Astier B, Kitahama K, Denoroy L, Jouvet M, Renaud B.** Immunohistochemical evidence for the adrenergic medullary longitudinal bundle as a major ascending pathway to the hypothalamus. *Neurosci Lett* 78: 241–246, 1987.
16. **Aston-Jones G, Cohen JD.** An integrative theory of locus coeruleus-norepinephrine function: adaptive gain and optimal performance. *Annu Rev Neurosci* 28: 403–450, 2005.
17. **Aston-Jones G, Shipley MT, Grzanna R.** The locus coeruleus, A5 and A7 noradrenergic cell groups. In: *The Rat Nervous System*, edited by Paxinos G. San Diego, CA: Academic, 1995, p. 182–213.
18. **Axelrod J.** *O*-methylation of (epinephrine and other catecholamines) catecholamines in vitro and in vivo. *Science* 126: 400–401, 1957.
19. **Axelrod J.** Purification and properties of phenylethanolamine-*N*-methyl transferase. *J Biol Chem* 237: 1657–1660, 1962.
20. **Axelrod J, Mueller RA, Henry JP, Stephens PM.** Changes in enzymes involved in the biosynthesis and metabolism of noradrenaline and adrenaline after psychosocial stimulation. *Nature* 225: 1059–1060, 1970.
21. **Axelrod J, Weil-Malherbe H, Tomchick R.** The physiological disposition of H3-epinephrine and its metabolite metanephrine. *J Pharmacol Exp Ther* 127: 251–256, 1959.
22. **Bacon SJ, Smith AD.** Preganglionic sympathetic neurones innervating the rat adrenal medulla: immunocytochemical evidence of synaptic input from nerve terminals containing substance P, GABA or 5-hydroxytryptamine. *J Auton Nerv Syst* 24: 97–122, 1988.
23. **Badoer E, McKinley MJ, Oldfield BJ, McAllen RM.** Distribution of hypothalamic, medullary and lamina terminalis neurons expressing Fos after hemorrhage in conscious rats. *Brain Res* 582: 323–328, 1992.
24. **Baetge EE, Behringer RR, Messing A, Brinster RL, Palmiter RD.** Transgenic mice express the human phenylethanolamine *N*-methyltransferase gene in adrenal medulla and retina. *Proc Natl Acad Sci USA* 85: 3648–3652, 1988.
25. **Balaz V, Balazova E, Blazicek P, Kvetnansky R.** The effect of one-year acclimatization of rats to mountain conditions on plasma catecholamines and dopamine-β-hydroxylase activity. In: *Catecholamines and Stress: Recent Advances*, edited by Usdin E, Kvetnansky R, Kopin IJ. New York: Elsevier North Holland, 1980, p. 259–264.
26. **Banerjee SA, Hoppe P, Brilliant M, Chikaraishi DM.** 5′ Flanking sequences of the rat tyrosine hydroxylase gene target accurate tissue-specific, developmental, transsynaptic expression in transgenic mice. *J Neurosci* 12: 4460–4467, 1992.
27. **Bannon M, Granneman G, Kapatos G.** The dopamine transporter. In: *Psychopharmacology–Fourth Generation of Progress*, edited by Fe B, Kupfer D. New York: Raven, 1995, p. 179–188.
28. **Barbeau P, Litaker MS, Jackson RW, Treiber FA.** A tyrosine hydroxylase microsatellite and hemodynamic response to stress in a multi-ethnic sample of youth. *Ethn Dis* 13: 186–192, 2003.
29. **Baruchin A, Vollmer RR, Miner LL, Sell SL, Stricker EM, Kaplan BB.** Cold-induced increases in phenylethanolamine *N*-methyltransferase (PNMT) mRNA are mediated by non-cholinergic mechanisms in the rat adrenal gland. *Neurochem Res* 18: 759–766, 1993.
30. **Baruchin A, Weisberg EP, Miner LL, Ennis D, Nisenbaum LK, Naylor E, Stricker EM, Zigmond MJ, Kaplan BB.** Effects of cold exposure on rat adrenal tyrosine hydroxylase: an analysis of RNA, protein, enzyme activity, and cofactor levels. *J Neurochem* 54: 1769–1775, 1990.
31. **Bernard C.** Influence du grand sympathique sur la sensibilite et sur la calorification. *C R Soc Biol* 3: 162–164, 1852.
32. **Bernard C.** *Lecons sur les phenomenones de la vie communs aux animaux et aux vegetaux*. *Paris*: Balliere, 1878.
33. **Berretta S.** Cortico-amygdala circuits: role in the conditioned stress response. *Stress* 8: 221–232, 2005.
34. **Berridge CW, Waterhouse BD.** The locus coeruleus-noradrenergic system: modulation of behavioral state and state-dependent cognitive processes. *Brain Res* 42: 33–84, 2003.
35. **Bertocci B, Miggiano V, Da Prada M, Dembic Z, Lahm HW, Malherbe P.** Human catechol-*O*-methyltransferase: cloning and expression of the membrane-associated form. *Proc Natl Acad Sci USA* 88: 1416–1420, 1991.
36. **Bethea CL, Streicher JM, Mirkes SJ, Sanchez RL, Reddy AP, Cameron JL.** Serotonin-related gene expression in female monkeys with individual sensitivity to stress. *Neuroscience* 132: 151–166, 2005.
37. **Betito K, Mitchell JB, Bhatnagar S, Boksa P, Meaney MJ.** Regulation of the adrenomedullary catecholaminergic system after mild, acute stress. *Am J Physiol Regul Integr Comp Physiol* 267: R212–R220, 1994.
38. **Bhagat BD, Horenstein S.** Modulation of adrenal medullary enzymes by stress. In: *Catecholamines and Stress*, edited by Usdin E, Kvetnansky R, Kopin IJ. Oxford: Pergamon, 1976, p. 257–264.
39. **Bhatnagar S, Dallman M.** Neuroanatomical basis for facilitation of hypothalamic-pituitary-adrenal responses to a novel stressor after chronic stress. *Neuroscience* 84: 1025–1039, 1998.
40. **Bhatnagar S, Mitchell JB, Betito K, Boksa P, Meaney MJ.** Effects of chronic intermittent cold stress on pituitary adrenocortical and sympathetic adrenomedullary functioning. *Physiol Behav* 57: 633–639, 1995.
41. **Biguet NF, Rittenhouse AR, Mallet J, Zigmond RE.** Preganglionic nerve stimulation increases mRNA levels for tyrosine hydroxylase in the rat superior cervical ganglion. *Neurosci Lett* 104: 189–194, 1989.

42. **Bjorklund A, Lindvall O.** Dopamine-containing systems in the CNS. In: *Handbook of Chemical Neuroanatomy*, edited by Bjorklund A, Hokfelt T. Amsterdam: Elsevier, 1984, p. 55–122.
43. **Bjorklund A, Lindvall O, Nobin A.** Evidence of an incerto-hypothalamic dopamine neurone system in the rat. *Brain Res* 89: 29–42, 1975.
44. **Bjorklund A, Moore RY, Nobin A, Stenevi U.** The organization of tubero-hypophyseal and reticulo-infundibular catecholamine neuron systems in the rat brain. *Brain Res* 51: 171–191, 1973.
45. **Blazicek P, Kvetnansky R.** Kinetic parameters of rat adrenal TH and PNMT under acute and repeated stress. In: *Stress: Neurochemical and Humoral Mechanisms*, edited by Van Loon GR, Kvetnansky R, McCarty R, Axelrod J. New York: Gordon and Breach, 1989, p. 787–797.
46. **Blazicek P, Kvetnansky R, Tigranian RA, Langos J, Macho L.** Catecholamines and their synthesizing enzymes in rats exposed to prolonged hypokinesia. In: *Catecholamines and Stress: Recent Advances*, edited by Usdin E, Kvetnansky R, Kopin IJ. New York: Elsevier North Holland, 1980, p. 349–354.
47. **Bohn MC, Bloom E, Goldstein M, Black IB.** Glucocorticoid regulation of phenylethanolamine *N*-methyltransferase (PNMT) in organ culture of superior cervical ganglia. *Dev Biol* 105: 130–136, 1984.
48. **Bohn MC, Engele J.** Development of phenylethanolamine *N*-methyltransferase (PNMT) in cultures of dissociated embryonic rat medulla oblongata. *Int J Dev Neurosci* 10: 481–489, 1992.
49. **Bonisch H, Eiden L.** Catecholamine reuptake and storage. In: *Catecholamines Bridging Basic Sience with Clinical Medicine*, edited by Goldstein DS, Eisenhofer G, McCarty R. San Diego, CA: Academic, 1998, p. 149–164.
50. **Bonish H.** Extraneural transport of catecholamines. *Pharmacology* 21: 93–108, 1980.
51. **Borg WP, During MJ, Sherwin RS, Borg MA, Brines ML, Shulman GI.** Ventromedial hypothalamic lesions in rats suppress counterregulatory responses to hypoglycemia. *J Clin Invest* 93: 1677–1682, 1994.
52. **Borg WP, Sherwin RS, During MJ, Borg MA, Shulman GI.** Local ventromedial hypothalamus glucopenia triggers counterregulatory hormone release. *Diabetes* 44: 180–184, 1995.
53. **Borowsky B, Hoffman BJ.** Neurotransmitter transporters: molecular biology, function, and regulation. *Int Rev Neurobiol* 38: 139–199, 1995.
54. **Borrell J, Torrellas A, Guaza C, Borrell S.** Sound stimulation and its effects on the pituitary-adrenocortical function and brain catecholamines in rats. *Neuroendocrinology* 31: 53–59, 1980.
55. **Brady LS.** Stress, antidepressant drugs, and the locus coeruleus. *Brain Res Bull* 35: 545–556, 1994.
56. **Bratincsak A, Palkovits M.** Activation of brain areas in rat following warm and cold ambient exposure. *Neuroscience* 127: 385–397, 2004.
57. **Brown DL, Guyenet PG.** Electrophysiological study of cardiovascular neurons in the rostral ventrolateral medulla in rats. *Circ Res* 56: 359–369, 1985.
58. **Brown MJ, Dollery CT.** A specific radioenzymatic assay for dihydroxyphenylalanine (DOPA). Plasma dopa may be the precursor of urine free dopamine. *Br J Clin Pharmacol* 11: 79–83, 1981.
59. **Brunton PJ, Meddle SL, Ma S, Ochedalski T, Douglas AJ, Russell JA.** Endogenous opioids and attenuated hypothalamic-pituitary-adrenal axis responses to immune challenge in pregnant rats. *J Neurosci* 25: 5117–5126, 2005.
60. **Buller K, Xu Y, Dayas C, Day T.** Dorsal and ventral medullary catecholamine cell groups contribute differentially to systemic interleukin-1beta-induced hypothalamic pituitary adrenal axis responses. *Neuroendocrinology* 73: 129–138, 2001.
61. **Buller KM, Smith DW, Day TA.** Differential recruitment of hypothalamic neuroendocrine and ventrolateral medulla catecholamine cells by non-hypotensive and hypotensive hemorrhages. *Brain Res* 834: 42–54, 1999.
62. **Burman KJ, Ige AO, White JH, Marshall FH, Pangalos MN, Emson PC, Minson JB, Llewellyn-Smith IJ.** $GABA_B$ receptor subunits, R1 and R2, in brainstem catecholamine and serotonin neurons. *Brain Res* 970: 35–46, 2003.
63. **Burman KJ, Sartor DM, Verberne AJ, Llewellyn-Smith IJ.** Cocaine- and amphetamine-regulated transcript in catecholamine and noncatecholamine presympathetic vasomotor neurons of rat rostral ventrolateral medulla. *J Comp Neurol* 476: 19–31, 2004.
64. **Byrum CE, Guyenet PG.** Afferent and efferent connections of the A5 noradrenergic cell group in the rat. *J Comp Neurol* 261: 529–542, 1987.
65. **Cabot JB, Alessi V, Carroll J, Ligorio M.** Spinal cord lamina V and lamina VII interneuronal projections to sympathetic preganglionic neurons. *J Comp Neurol* 347: 515–530, 1994.
66. **Campeau S, Dolan D, Akil H, Watson SJ.** c-*fos* mRNA induction in acute and chronic audiogenic stress: possible role of the orbitofrontal cortex in habituation. *Stress* 5: 121–130, 2002.
67. **Campeau US, Watson SJ.** Neuroendocrine and behavioral responses and brain pattern of c-*fos* induction associated with audiogenic stress. *J Neuroendocrinol* 9: 577–588, 1997.
68. **Cannon WB.** *Bodily Changes in Pain, Hunger, Fear and Rage.* New York: Appleton, 1929.
69. **Cannon WB.** Organization for physiological homeostasis. *Physiol Rev* 9: 399–431, 1929.
70. **Cannon WB.** *The Wisdom of the Body.* New York: Norton, 1939.
71. **Cannon WB, Lissak K.** Evidence for adrenaline in adrenergic neurons. *Am J Physiol* 125: 765–777, 1939.
72. **Cao X, Mahendran R, Guy GR, Tan YH.** Detection and characterization of cellular EGR-1 binding to its recognition site. *J Biol Chem* 268: 16949–16957, 1993.
73. **Caramona MM, Soares da Silva P.** The effects of chemical sympathectomy on dopamine, noradrenaline and adrenaline content in some peripheral tissues. *Br J Pharmacol* 86: 351–356, 1985.
74. **Carlezon WA Jr, Duman RS, Nestler EJ.** The many faces of CREB. *Trends Neurosci* 28: 436–445, 2005.
75. **Carlsson A.** The occurrence, distribution and physiological role of catecholamines in the nervous system. *Pharmacol Rev* 11: 490–493, 1959.
76. **Carlsson A, Lindqvist M, Magnusson T, Waldeck B.** On the presence of 3-hydroxytyramine in brain. *Science* 127: 471, 1958.
77. **Carrasco M, Portillo F, Larsen PJ, Vallo JJ.** Insulin and glucose administration stimulates Fos expression in neurones of the paraventricular nucleus that project to autonomic preganglionic structures. *J Neuroendocrinol* 13: 339–346, 2001.
78. **Carter RN, Pinnock SB, Herbert J.** Does the amygdala modulate adaptation to repeated stress? *Neuroscience* 126: 9–19, 2004.
79. **Cases O, Seif I, Grimsby J, Gaspar P, Chen K, Pournin S, Muller U, Aguet M, Babinet C, Shih JC.** Aggressive behavior and altered amounts of brain serotonin and norepinephrine in mice lacking MAO-A. *Science* 268: 1763–1766, 1995.
80. **Cazorla P, Smidt MP, O'Malley KL, Burbach JP.** A response element for the homeodomain transcription factor Ptx3 in the tyrosine hydroxylase gene promoter. *J Neurochem* 74: 1829–1837, 2000.
81. **Ceccatelli S, Villar MJ, Goldstein M, Hokfelt T.** Expression of c-Fos immunoreactivity in transmitter-characterized neurons after stress. *Proc Natl Acad Sci USA* 86: 9569–9573, 1989.
82. **Cechetto DF, Saper CB.** Neurochemical organization of the hypothalamic projection to the spinal cord in the rat. *J Comp Neurol* 272: 579–604, 1988.
83. **Cechetto DF, Saper CB.** Role of the cerebral cortex in in autonomic function. In: *Central Regulation of Autonomic Functions*, edited by Loewy AD, Spyer KM. Oxford: Oxford Univ. Press, 1990, p. 208–223.
84. **Cederbaum JM, Aghajanian GK.** Afferent projections to the rat locus coeruleus as determined by a retrograde tracing method. *J Comp Neurol* 178: 1–16, 1978.
85. **Chan RK, Peto CA, Sawchenko PE.** A1 catecholamine cell group: fine structure and synaptic input from the nucleus of the solitary tract. *J Comp Neurol* 351: 62–80, 1995.
86. **Chan RK, Sawchenko PE.** Spatially and temporally differentiated patterns of c-*fos* expression in brainstem catecholaminergic cell groups induced by cardiovascular challenges in the rat. *J Comp Neurol* 348: 433–460, 1994.
87. **Chang MS, Sved AF, Zigmond MJ, Austin MC.** Increased transcription of the tyrosine hydroxylase gene in individual locus co-

eruleus neurons following footshock stress. *Neuroscience* 101: 131–139, 2000.
88. **Chen K.** Organization of MAO-A and MAO-B promoters and regulation of gene expression. *Neurotoxicology* 25: 31–36, 2004.
89. **Chen K, Ou XM, Chen G, Choi SH, Shih JC.** R1, a novel repressor of the human monoamine oxidase A. *J Biol Chem* 280: 11552–11559, 2005.
90. **Chen X, Herbert J.** Regional changes in c-*fos* expression in the basal forebrain and brainstem during adaptation to repeated stress: correlations with cardiovascular, hypothermic and endocrine responses. *Neuroscience* 64: 675–685, 1995.
90a. **Cheng SY, Serova LI, Glazkova D, Sabban EL.** Regulation of rat dopamine beta-hydroxylase gene transcription by early growth response gene 1 (Egr1). *Brain Res* 1193: 1–11, 2008.
91. **Cheung S, Ballew JR, Moore KE, Lookingland KJ.** Contribution of dopamine neurons in the medial zona incerta to the innervation of the central nucleus of the amygdala, horizontal diagonal band of Broca and hypothalamic paraventricular nucleus. *Brain Res* 808: 174–181, 1998.
92. **Chiba T, Masuko S.** Direct synaptic contacts of catecholamine axons on the preganglionic sympathetic neurons in the rat thoracic spinal cord. *Brain Res* 380: 405–408, 1986.
93. **Choi DC, Furay AR, Evanson NK, Ostrander MM, Ulrich-Lai YM, Herman JP.** Bed nucleus of the stria terminalis subregions differentially regulate hypothalamic-pituitary-adrenal axis activity: implications for the integration of limbic inputs. *J Neurosci* 27: 2025–2034, 2007.
94. **Chowdhury GM, Fujioka T, Nakamura S.** Induction and adaptation of Fos expression in the rat brain by two types of acute restraint stress. *Brain Res Bull* 52: 171–182, 2000.
95. **Chronwall BM, Zukowska Z.** Neuropeptide Y, ubiquitous and elusive. *Peptides* 25: 359–363, 2004.
96. **Chrousos GP, Gold PW.** The concepts of stress and stress system disorders. Overview of physical and behavioral homeostasis. *JAMA* 267: 1244–1252, 1992.
97. **Chrousos GP, McCarty R, Pacak K, Cizza G, Sternberg E, Gold PW, Kvetnansky R**. *Stress: Basic Mechanisms and Clinical Implications*. New York: NY Acad. Sci., 1995, p. 755.
98. **Chu S, Ferro TJ.** Sp1: regulation of gene expression by phosphorylation. *Gene* 348: 1–11, 2005.
99. **Chuang D, Zsilla G, Costa E.** Turnover rate of tyrosine hydroxylase during trans-synaptic induction. *Mol Pharmacol* 11: 784–794, 1975.
100. **Chuang DM, Costa E.** Biosynthesis of tyrosine hydroxylase in rat adrenal medulla after exposure to cold. *Proc Natl Acad Sci USA* 71: 4570–4574, 1974.
101. **Ciliax BJ, Heilman C, Demchyshyn LL, Pristupa ZB, Ince E, Hersch SM, Niznik HB, Levey AI.** The dopamine transporter: immunochemical characterization and localization in brain. *J Neurosci* 15: 1714–1723, 1995.
102. **Clark FM, Proudfit HK.** The projection of noradrenergic neurons in the A7 catecholamine cell group to the spinal cord in the rat demonstrated by anterograde tracing combined with immunocytochemistry. *Brain Res* 547: 279–288, 1991.
103. **Clark FM, Proudfit HK.** The projections of noradrenergic neurons in the A5 catecholamine cell group to the spinal cord in the rat: anatomical evidence that A5 neurons modulate nociception. *Brain Res* 616: 200–210, 1993.
104. **Comer AM, Qi J, Christie DL, Gibbons HM, Lipski J.** Noradrenaline transporter expression in the pons and medulla oblongata of the rat: localisation to noradrenergic and some C1 adrenergic neurones. *Brain Res* 62: 65–76, 1998.
105. **Craddock N, Owen MJ, O'Donovan MC.** The catechol-*O*-methyl transferase (COMT) gene as a candidate for psychiatric phenotypes: evidence and lessons. *Mol Psychiatry* 11: 446–458, 2006.
106. **Craig SP, Buckle VJ, Lamouroux A, Mallet J, Craig IW.** Localization of the human dopamine-beta-hydroxylase (DBH) gene to chromosome 9q34. *Cytogenet Cell Genet* 48: 48–50, 1988.
107. **Crane JW, French KR, Buller KM.** Patterns of neuronal activation in the rat brain and spinal cord in response to increasing durations of restraint stress. *Stress* 8: 199–211, 2005.
108. **Cubells JF, Zabetian CP.** Human genetics of plasma dopamine beta-hydroxylase activity: applications to research in psychiatry and neurology. *Psychopharmacology* 174: 463–476, 2004.
109. **Cui J, Zhou X, Chazaro I, DeStefano AL, Manolis AJ, Baldwin CT, Gavras H.** Association of polymorphisms in the promoter region of the PNMT gene with essential hypertension in African Americans but not in whites. *Am J Hypertens* 16: 859–863, 2003.
110. **Cullinan WE.** GABA(A) receptor subunit expression within hypophysiotropic CRH neurons: a dual hybridization histochemical study. *J Comp Neurol* 419: 344–351, 2000.
111. **Cullinan WE, Helmreich DL, Watson SJ.** Fos expression in forebrain afferents to the hypothalamic paraventricular nucleus following swim stress. *J Comp Neurol* 368: 88–99, 1996.
112. **Cullinan WE, Herman JP, Battaglia DF, Akil H, Watson SJ.** Pattern and time course of immediate early gene expression in rat brain following acute stress. *Neuroscience* 64: 477–505, 1995.
113. **Culman J, Torda T, Petrikova M, Murgas K.** Effect of corticosterone treatment and adrenalectomy on phenylethanolamine *N*-methyltransferase and catecholamines in brain stem and hypothalamic nuclei and superior cervical ganglion of rats. *Endocrinol Exp* 22: 117–128, 1988.
114. **Cunningham ET Jr, Bohn MC, Sawchenko PE.** Organization of adrenergic inputs to the paraventricular and supraoptic nuclei of the hypothalamus in the rat. *J Comp Neurol* 292: 651–667, 1990.
115. **Cunningham ET Jr, Miselis RR, Sawchenko PE.** The relationship of efferent projections from the area postrema to vagal motor and brain stem catecholamine-containing cell groups: an axonal transport and immunohistochemical study in the rat. *Neuroscience* 58: 635–648, 1994.
116. **Cunningham ET Jr, Sawchenko PE.** Anatomical specificity of noradrenergic inputs to the paraventricular and supraoptic nuclei of the rat hypothalamus. *J Comp Neurol* 274: 60–76, 1988.
117. **Czyzyk-Krzeska MF, Dominski Z, Kole R, Millhorn DE.** Hypoxia stimulates binding of a cytoplasmic protein to a pyrimidine-rich sequence in the 3′-untranslated region of rat tyrosine hydroxylase mRNA. *J Biol Chem* 269: 9940–9945, 1994.
118. **Dahlstrom A, Fuxe K.** Evidence of the existence of monoamine containing neurons in the central nervous system. I. Demonstration of monomaines in cell bodies of brain stem neurons. *Acta Physiol Scand* 62: 1–55, 1964.
119. **Daiguji M, Okada F, Yamashita I.** Dopamine-beta-hydroxylase activity in the locus coeruleus and hypothalamus in cold-stressed rats. *Psychoneuroendocrinology* 7: 223–227, 1982.
120. **Darlington DN, Shinsako J, Dallman MF.** Medullary lesions eliminate ACTH responses to hypotensive hemorrhage. *Am J Physiol Regul Integr Comp Physiol* 251: R106–R115, 1986.
121. **Day HE, Campeau S, Watson SJ Jr, Akil H.** Expression of alpha(1b) adrenoceptor mRNA in corticotropin-releasing hormone-containing cells of the rat hypothalamus and its regulation by corticosterone. *J Neurosci* 19: 10098–10106, 1999.
122. **Dayas CV, Buller KM, Day TA.** Neuroendocrine responses to an emotional stressor: evidence for involvement of the medial but not the central amygdala. *Eur J Neurosci* 11: 2312–2322, 1999.
123. **Dayas CV, Day TA.** Opposing roles for medial and central amygdala in the initiation of noradrenergic cell responses to a psychological stressor. *Eur J Neurosci* 15: 1712–1718, 2002.
124. **De Diego AM, Gandia L, Garcia AG.** A physiological view of the central and peripheral mechanisms that regulate the release of catecholamines at the adrenal medulla. *Acta Physiol* 192: 287–301, 2008.
125. **Deak M, Clifton AD, Lucocq LM, Alessi DR.** Mitogen- and stress-activated protein kinase-1 (MSK1) is directly activated by MAPK and SAPK2/p38, may mediate activation of CREB. *EMBO J* 17: 4426–4441, 1998.
126. **DiMicco JA, Samuels BC, Zaretskaia MV, Zaretsky DV.** The dorsomedial hypothalamus and the response to stress: part renaissance, part revolution. *Pharmacol Biochem Behav* 71: 469–480, 2002.
127. **Diorio D, Viau V, Meaney MJ.** The role of the medial prefrontal cortex (cingulate gyrus) in the regulation of hypothalamic-pituitary-adrenal responses to stress. *J Neurosci* 13: 3839–3847, 1993.
128. **Dobrakovova M, Kvetnansky R, Oprsalova Z, Jezova D.** Specificity of the effect of repeated handling on sympathetic-adreno-

medullary and pituitary-adrenocortical activity in rats. *Psychoneuroendocrinology* 18: 163–174, 1993.
129. **Douglas AJ.** Central noradrenergic mechanisms underlying acute stress responses of the hypothalamo-pituitary-adrenal axis: adaptations through pregnancy and lactation. *Stress* 8: 5–18, 2005.
130. **Doupe AJ, Landis SC, Patterson PH.** Environmental influences in the development of neural crest derivatives: glucocorticoids, growth factors, and chromaffin cell plasticity. *J Neurosci* 5: 2119–2142, 1985.
131. **Doupe AJ, Patterson PH, Landis SC.** Small intensely fluorescent cells in culture: role of glucocorticoids and growth factors in their development and interconversions with other neural crest derivatives. *J Neurosci* 5: 2143–2160, 1985.
132. **Dronjak S, Jezova D, Kvetnansky R.** Different effects of novel stressors on sympathoadrenal system activation in rats exposed to long-term immobilization. *Ann NY Acad Sci* 1018: 113–123, 2004.
133. **Dronjak S, Ondriska M, Svetlovska D, Jezova D, Kvetnansky R.** Effects of novel stressors on plasma catecholamine levels in rats exposed to long-term cold. In: *Stress Neural, Endocrine and Molecular Studies*, edited by McCarty R, Aguilera G, Sabban EL, Kvetnansky R. London: Taylor and Francis, 2002, p. 83–89.
134. **Dumas S, Le Hir H, Bodeau-Pean S, Hirsch E, Thermes C, Mallet J.** New species of human tyrosine hydroxylase mRNA are produced in variable amounts in adrenal medulla and are overexpressed in progressive supranuclear palsy. *J Neurochem* 67: 19–25, 1996.
135. **Dun NJ, Dun SL, Chiaia NL.** Hemorrhage induces Fos immunoreactivity in rat medullary catecholaminergic neurons. *Brain Res* 608: 223–232, 1993.
136. **Duncko R, Brtko J, Kvetnansky R, Jezova D.** Altered function of peripheral organ systems in rats exposed to chronic mild stress model of depression. *Cell Mol Neurobiol* 21: 403–411, 2001.
137. **Dunkley PR, Bobrovskaya L, Graham ME, von Nagy-Felsobuki EI, Dickson PW.** Tyrosine hydroxylase phosphorylation: regulation and consequences. *J Neurochem* 91: 1025–1043, 2004.
138. **Eaton MJ, Wagner CK, Moore KE, Lookingland KJ.** Neurochemical identification of A13 dopaminergic neuronal projections from the medial zona incerta to the horizontal limb of the diagonal band of Broca and the central nucleus of the amygdala. *Brain Res* 659: 201–207, 1994.
139. **Ebert SN, Baden JM, Mathers LH, Siddall BJ, Wong DL.** Expression of phenylethanolamine *N*-methyltransferase in the embryonic rat heart. *J Mol Cell Cardiol* 28: 1653–1658, 1996.
140. **Ebert SN, Rong Q, Boe S, Pfeifer K.** Catecholamine synthesizing cells in the embryonic mouse heart. *Ann NY Acad Sci.* 1148: 317–324, 2008.
141. **Ebert SN, Thompson RP.** Embryonic epinephrine synthesis in the rat heart before innervation: association with pacemaking and conduction tissue development. *Circ Res* 88: 117–124, 2001.
142. **Edwards SL, Anderson CR, Southwell BR, McAllen RM.** Distinct preganglionic neurons innervate noradrenaline and adrenaline cells in the cat adrenal medulla. *Neuroscience* 70: 825–832, 1996.
143. **Eiden LE, Schutz B, Anlauf M, Depboylu C, Schafer MKH, Weihe E.** The vesicular monoamine transporters (VMATs): role in the chemical coding of neuronal transmission and monoamine storage in amine handling immune and inflammatory cells. In: *Catecholamine Research: From Molecular Insights to Clinical Medicine*, edited by Nagatsu T, Nabeshima T, McCarty R, Goldstein DS. New York: Kluwer Academic/Plenum, 2002, p. 23–33.
144. **Eisenhofer G.** The role of neuronal and extraneuronal plasma membrane transporters in the inactivation of peripheral catecholamines. *Pharmacol Ther* 91: 35–62, 2001.
145. **Eisenhofer G, Cox HS, Esler MD.** Parallel increases in noradrenaline reuptake and release into plasma during activation of the sympathetic nervous system in rabbits. *Naunyn-Schmiedeberg's Arch Pharmacol* 342: 328–335, 1990.
146. **Eisenhofer G, Esler MD, Cox HS, Meredith IT, Jennings GL, Brush JE Jr, Goldstein DS.** Differences in the neuronal removal of circulating epinephrine and norepinephrine. *J Clin Endocrinol Metab* 70: 1710–1720, 1990.
147. **Eisenhofer G, Esler MD, Meredith IT, Ferrier C, Lambert G, Jennings G.** Neuronal re-uptake of noradrenaline by sympathetic nerves in humans. *Clin Sci* 80: 257–263, 1991.
148. **Eisenhofer G, Goldstein DS, Kopin IJ.** Plasma dihydroxyphenylglycol for estimation of noradrenaline neuronal re-uptake in the sympathetic nervous system in vivo. *Clin Sci* 76: 171–182, 1989.
149. **Eisenhofer G, Goldstein DS, Ropchak TG, Nguyen HQ, Keiser HR, Kopin IJ.** Source and physiological significance of plasma 3,4-dihydroxyphenylglycol and 3-methoxy-4-hydroxyphenylglycol. *J Auton Nerv Syst* 24: 1–14, 1988.
150. **Eisenhofer G, Keiser H, Friberg P, Mezey E, Huynh TT, Hiremagalur B, Ellingson T, Duddempudi S, Eijsbouts A, Lenders JW.** Plasma metanephrines are markers of pheochromocytoma produced by catechol-*O*-methyltransferase within tumors. *J Clin Endocrinol Metab* 83: 2175–2185, 1998.
151. **Eisenhofer G, Kopin IJ, Goldstein DS.** Catecholamine metabolism: a contemporary view with implications for physiology and medicine. *Pharmacol Rev* 56: 331–349, 2004.
152. **Eisenhofer G, Kopin IJ, Goldstein DS.** Leaky catecholamine stores: undue waste or a stress response coping mechanism? *Ann NY Acad Sci* 1018: 224–230, 2004.
153. **Eisenhofer G, Lenders JW.** Clues to the diagnosis of pheochromocytoma from the differential tissue metabolism of catecholamines. *Adv Pharmacol* 42: 374–377, 1998.
154. **Eisenhofer G, Rundquist B, Aneman A, Friberg P, Dakak N, Kopin IJ, Jacobs MC, Lenders JW.** Regional release and removal of catecholamines and extraneuronal metabolism to metanephrines. *J Clin Endocrinol Metab* 80: 3009–3017, 1995.
155. **Eisenhofer G, Smolich JJ, Cox HS, Esler MD.** Neuronal reuptake of norepinephrine and production of dihydroxyphenylglycol by cardiac sympathetic nerves in the anesthetized dog. *Circulation* 84: 1354–1363, 1991.
156. **Elias CF, Lee C, Kelly J, Aschkenasi C, Ahima RS, Couceyro PR, Kuhar MJ, Saper CB, Elmquist JK.** Leptin activates hypothalamic CART neurons projecting to the spinal cord. *Neuron* 21: 1375–1385, 1998.
157. **Emmert MH, Herman JP.** Differential forebrain c-*fos* mRNA induction by ether inhalation and novelty: evidence for distinctive stress pathways. *Brain Res* 845: 60–67, 1999.
158. **Ennis M, Aston-Jones G.** Two physiologically distinct populations of neurons in the ventrolateral medulla innervate the locus coeruleus. *Brain Res* 425: 275–282, 1987.
159. **Eranko O, Pickel VM, Harkonen M, Eranko L, Joh TH, Reis DJ.** Effect of hydrocortisone on catecholamines and the enzymes synthesizing them in the developing sympathetic ganglion. *Histochem J* 14: 461–478, 1982.
160. **Erickson JD, Schafer MK, Bonner TI, Eiden LE, Weihe E.** Distinct pharmacological properties and distribution in neurons and endocrine cells of two isoforms of the human vesicular monoamine transporter. *Proc Natl Acad Sci USA* 93: 5166–5171, 1996.
161. **Ericsson A, Kovacs KJ, Sawchenko PE.** A functional anatomical analysis of central pathways subserving the effects of interleukin-1 on stress-related neuroendocrine neurons. *J Neurosci* 14: 897–913, 1994.
162. **Esler M, Jackman G, Bobik A, Kelleher D, Jennings G, Leonard P, Skews H, Korner P.** Determination of norepinephrine apparent release rate and clearance in humans. *Life Sci* 25: 1461–1470, 1979.
163. **Esteves F, Lima D, Coimbra A.** Structural types of spinal cord marginal (lamina I) neurons projecting to the nucleus of the tractus solitarius in the rat. *Somatosens Mot Res* 10: 203–216, 1993.
164. **Everitt BJ, Hokfelt T, Terenius L, Tatemoto K, Mutt V, Goldstein M.** Differential co-existence of neuropeptide Y (NPY)-like immunoreactivity with catecholamines in the central nervous system of the rat. *Neuroscience* 11: 443–462, 1984.
165. **Evinger MJ, Mathew E, Cikos S, Powers JF, Lee YS, Sheikh S, Ross RA, Tischler AS.** Nicotine stimulates expression of the PNMT gene through a novel promoter sequence. *J Mol Neurosci* 26: 39–55, 2005.
166. **Fallon JH, Moore RY.** Catecholamine innervation of the basal forebrain. IV. Topography of the dopamine projection to the basal forebrain and neostriatum. *J Comp Neurol* 180: 545–580, 1978.

167. **Feldman S, Weidenfeld J.** Involvement of endogeneous glutamate in the stimulatory effect of norepinephrine and serotonin on the hypothalamo-pituitary-adrenocortical axis. *Neuroendocrinology* 79: 43–53, 2004.
168. **Feng Z, Angeletti RH, Levin BE, Sabban EL.** Glycosylation and membrane insertion of newly synthesized rat dopamine beta-hydroxylase in a cell-free system without signal cleavage. *J Biol Chem* 267: 21808–21815, 1992.
169. **Filipenko ML, Alekseyenko OV, Beilina AG, Kamynina TP, Kudryavtseva NN.** Increase of tyrosine hydroxylase and dopamine transporter mRNA levels in ventral tegmental area of male mice under influence of repeated aggression experience. *Brain Res* 96: 77–81, 2001.
170. **Filipenko ML, Beilina AG, Alexeyenko OV, Timofeeva OA, Avgustinovich DF, Kudryavtseva NN.** Association between brain COMT gene expression and aggressive experience in daily agonistic confrontations in male mice. In: *Stress: Neural, Endocrine and Molecular Studies*, edited by McCarty R, Aguilera G, Sabban E, Kvetnansky R. New York: Taylor & Francis, 2002, p. 157–161.
171. **Finlay JM, Zigmond MJ, Abercrombie ED.** Increased dopamine and norepinephrine release in medial prefrontal cortex induced by acute and chronic stress: effects of diazepam. *Neuroscience* 64: 619–628, 1995.
172. **Fitzpatrick PF.** Tetrahydropterin-dependent amino acid hydroxylases. *Annu Rev Biochem* 68: 355–381, 1999.
173. **Flatmark T.** Catecholamine biosynthesis and physiological regulation in neuroendocrine cells. *Acta Physiol Scand* 168: 1–17, 2000.
174. **Flatmark T, Stevens RC.** Structural insight into the aromatic amino acid hydroxylases and their disease-related mutant forms. *Chem Rev* 99: 2137–2160, 1999.
175. **Floyd NS, Price JL, Ferry AT, Keay KA, Bandler R.** Orbitomedial prefrontal cortical projections to hypothalamus in the rat. *J Comp Neurol* 432: 307–328, 2001.
176. **Flugge G, Van Kampen M, Mijnster MJ.** Perturbations in brain monoamine systems during stress. *Cell Tissue Res* 315: 1–14, 2004.
177. **Fluharty SJ, Rabow LE, Zigmond MJ, Stricker EM.** Tyrosine hydroxylase activity in the sympathoadrenal system under basal and stressful conditions: effect of 6-hydroxydopamine. *J Pharmacol Exp Ther* 235: 354–360, 1985.
178. **Fluharty SJ, Snyder GL, Stricker EM, Zigmond MJ.** Short- and long-term changes in adrenal tyrosine hydroxylase activity during insulin-induced hypoglycemia and cold stress. *Brain Res* 267: 384–387, 1983.
179. **Foote SL, Bloom FE, Aston-Jones G.** Nucleus locus ceruleus: new evidence of anatomical and physiological specificity. *Physiol Rev* 63: 844–914, 1983.
180. **Fossom LH, Sterling CR, Tank AW.** Regulation of tyrosine hydroxylase gene transcription rate and tyrosine hydroxylase mRNA stability by cyclic AMP and glucocorticoid. *Mol Pharmacol* 42: 898–908, 1992.
181. **Fraley GS, Ritter S.** Immunolesion of norepinephrine and epinephrine afferents to medial hypothalamus alters basal and 2-deoxy-D-glucose-induced neuropeptide Y and agouti gene-related protein messenger ribonucleic acid expression in the arcuate nucleus. *Endocrinology* 144: 75–83, 2003.
182. **Fritschy JM, Grzanna R.** Demonstration of two separate descending noradrenergic pathways to the rat spinal cord: evidence for an intragriseal trajectory of locus coeruleus axons in the superficial layers of the dorsal horn. *J Comp Neurol* 291: 553–582, 1990.
183. **Fuke S, Suo S, Takahashi N, Koike H, Sasagawa N, Ishiura S.** The VNTR polymorphism of the human dopamine transporter (DAT1) gene affects gene expression. *Pharmacogenomics J* 1: 152–156, 2001.
184. **Fukuhara K, Kvetnansky R, Weise VK, Ohara H, Yoneda R, Goldstein DS, Kopin IJ.** Effects of continuous and intermittent cold (SART) stress on sympathoadrenal system activity in rats. *J Neuroendocrinol* 8: 65–72, 1996.
185. **Fuxe K, Hokfelt T.** Catecholamines in the hypothalamus and the pituitary gland. In: *Frontiers in Neuroendocrinology*, edited by Ganong W, Martini L. Oxford: Oxford Univ. Press, 1969, p. 47–96.
186. **Gagner JP, Gauthier S, Sourkes TL.** Descending spinal pathways mediating the responses of adrenal tyrosine hydroxylase and catecholamines to insulin and 2-deoxyglucose. *Brain Res* 325: 187–197, 1985.
187. **Gaillet S, Lachuer J, Malaval F, Assenmacher I, Szafarczyk A.** The involvement of noradrenergic ascending pathways in the stress-induced activation of ACTH and corticosterone secretions is dependent on the nature of stressors. *Exp Brain Res* 87: 173–180, 1991.
188. **Gamaro GD, Xavier MH, Denardin JD, Pilger JA, Ely DR, Ferreira MB, Dalmaz C.** The effects of acute and repeated restraint stress on the nociceptive response in rats. *Physiol Behav* 63: 693–697, 1998.
189. **Gasser PJ, Lowry CA, Orchinik M.** Corticosterone-sensitive monoamine transport in the rat dorsomedial hypothalamus: potential role for organic cation transporter 3 in stress-induced modulation of monoaminergic neurotransmission. *J Neurosci* 26: 8758–8766, 2006.
190. **Gelernter J, Kruger S, Pakstis AJ, Pacholczyk T, Sparkes RS, Kidd KK, Amara S.** Assignment of the norepinephrine transporter protein (NET1) locus to chromosome 16. *Genomics* 18: 690–692, 1993.
191. **Gerhard M, Neumayer N, Presecan-Siedel E, Zanner R, Lengyel E, Cramer T, Hocker M, Prinz C.** Gastrin induces expression and promoter activity of the vesicular monoamine transporter subtype 2. *Endocrinology* 142: 3663–3672, 2001.
192. **Gieroba ZJ, Shapoval LN, Blessing WW.** Inhibition of the A1 area prevents hemorrhage-induced secretion of vasopressin in rats. *Brain Res* 657: 330–332, 1994.
193. **Giros B, Jaber M, Jones SR, Wightman RM, Caron MG.** Hyperlocomotion and indifference to cocaine and amphetamine in mice lacking the dopamine transporter. *Nature* 379: 606–612, 1996.
194. **Glatt CE, Wahner AD, White DJ, Ruiz-Linares A, Ritz B.** Gain-of-function haplotypes in the vesicular monoamine transporter promoter are protective for Parkinson disease in women. *Hum Mol Genet* 15: 299–305, 2006.
195. **Gogos JA, Morgan M, Luine V, Santha M, Ogawa S, Pfaff D, Karayiorgou M.** Catechol-*O*-methyltransferase-deficient mice exhibit sexually dimorphic changes in catecholamine levels and behavior. *Proc Natl Acad Sci USA* 95: 9991–9996, 1998.
196. **Goldstein DS.** *Adrenaline and the Inner World. An Introduction to Scientific Integrative Medicine.* Baltimore, MD: Johns Hopkins Univ. Press, 2006, p. 309.
197. **Goldstein DS.** *The Autonomic Nervous System in Health and Disease.* New York: Marcel Bekker, 2001, p. 618.
198. **Goldstein DS.** Catecholamines and stress. *Endocr Regul* 37: 69–80, 2003.
199. **Goldstein DS.** *Stress, Catecholamines, and Cardiovascular Disease.* New York: Oxford Univ. Press, 1995, p. 539.
200. **Goldstein DS, Eisenhofer G, Stull R, Folio CJ, Keiser HR, Kopin IJ.** Plasma dihydroxyphenylglycol and the intraneuronal disposition of norepinephrine in humans. *J Clin Invest* 81: 213–220, 1988.
201. **Goldstein DS, Kopin IJ.** Evolution of concepts of stress. *Stress* 10: 109–120, 2007.
202. **Goldstein DS, McEwen B.** Allostasis, homeostats, and the nature of stress. *Stress* 5: 55–58, 2002.
203. **Goncalvesova E, Micutkova L, Mravec B, Ksinantova L, Krizanova O, Fabian J, Kvetnansky R.** Changes in gene expression of phenylethanolamine *N*-methyltransferase in the transplanted human heart. *Ann NY Acad Sci* 1018: 430–436, 2004.
204. **Gorbunova AV.** Catecholamines in rabbit nodose ganglion following exposure to an acute emotional stressor. *Stress* 2: 231–236, 1998.
205. **Gorbunova AV, Kashtanov SI.** Monoamine oxidase activity in autonomic ganglia of rabbits with differing resistance of cardiovascular functions to emotional stress. *Biull Eksp Biol Med* 96: 111–113, 1983.
206. **Graessler J, Kvetnansky R, Jezova D, Dobrakovova M, Van Loon GR.** Prior immobilization stress alters adrenal hormone responses to hemorrhage in rats. *Am J Physiol Regul Integr Comp Physiol* 257: R661–R667, 1989.
207. **Gray TS, Carney ME, Magnuson DJ.** Direct projections from the central amygdaloid nucleus to the hypothalamic paraventricular

nucleus: possible role in stress-induced adrenocorticotropin release. *Neuroendocrinology* 50: 433–446, 1989.
208. **Gray TS, Magnuson DJ.** Neuropeptide neuronal efferents from the bed nucleus of the stria terminalis and central amygdaloid nucleus to the dorsal vagal complex in the rat. *J Comp Neurol* 262: 365–374, 1987.
209. **Grill HJ.** Distributed neural control of energy balance: contributions from hindbrain and hypothalamus. *Obesity* 14 *Suppl* 5: 216S–221S, 2006.
210. **Grima B, Lamouroux A, Boni C, Julien JF, Javoy-Agid F, Mallet J.** A single human gene encoding multiple tyrosine hydroxylases with different predicted functional characteristics. *Nature* 326: 707–711, 1987.
211. **Grimsby J, Toth M, Chen K, Kumazawa T, Klaidman L, Adams JD, Karoum F, Gal J, Shih JC.** Increased stress response and beta-phenylethylamine in MAOB-deficient mice. *Nat Genet* 17: 206–210, 1997.
212. **Gueorguiev VD, Cheng SY, Sabban EL.** Prolonged activation of element-binding protein and ATF-2 needed for nicotine triggered elevation of tyrosine hydroxylase gene transcription in PC12 cells. *J Biol Chem* 281: 10188–10195, 2006.
213. **Guyenet PG, Young BS.** Projections of nucleus paragigantocellularis lateralis to locus coeruleus and other structures in rat. *Brain Res* 406: 171–184, 1987.
214. **Haag C, Berkels R, Grundemann D, Lazar A, Taubert D, Schomig E.** The localisation of the extraneuronal monoamine transporter (EMT) in rat brain. *J Neurochem* 88: 291–297, 2004.
215. **Hagerty T, Fernandez E, Lynch K, Wang SS, Morgan WW, Strong R.** Interaction of a glucocorticoid-responsive element with regulatory sequences in the promoter region of the mouse tyrosine hydroxylase gene. *J Neurochem* 78: 1379–1388, 2001.
216. **Haller J, Bakos N, Rodriguiz RM, Caron MG, Wetsel WC, Liposits Z.** Behavioral responses to social stress in noradrenaline transporter knockout mice: effects on social behavior and depression. *Brain Res Bull* 58: 279–284, 2002.
217. **Halliday GM, McLachlan EM.** Four groups of tyrosine hydroxylase-immunoreactive neurons in the ventrolateral medulla of rats, guinea-pigs and cats identified on the basis of chemistry, topography and morphology. *Neuroscience* 43: 551–568, 1991.
218. **Hamelink C, Tjurmina O, Damadzic R, Young WS, Weihe E, Lee HW, Eiden LE.** Pituitary adenylate cyclase-activating polypeptide is a sympathoadrenal neurotransmitter involved in catecholamine regulation and glucohomeostasis. *Proc Natl Acad Sci USA* 99: 461–466, 2002.
219. **Hammen C.** Generation of stress in the course of unipolar depression. *J Abnorm Psychol* 100: 555–561, 1991.
220. **Han S, Chen X, Yang CL, Vickery L, Wu Y, Naes L, Macarthur H, Westfall TC.** Influence of cold stress on neuropeptide Y and sympathetic neurotransmission. *Peptides* 26: 2603–2609, 2005.
221. **Hanaoka M, Kanamaru K, Takahashi H, Tanaka K.** Molecular genetic analysis of chloroplast gene promoters dependent on SIG2, a nucleus-encoded sigma factor for the plastid-encoded RNA polymerase, in Arabidopsis thaliana. *Nucleic Acids Res* 31: 7090–7098, 2003.
222. **Hanbauer I, Guidotti A, Costa E.** Dexamethasone induces tyrosine hydroxylase in sympathetic ganglia but not in adrenal medulla. *Brain Res* 85: 527–531, 1975.
223. **Hatfield T, Spanis C, McGaugh JL.** Response of amygdalar norepinephrine to footshock and GABAergic drugs using in vivo microdialysis and HPLC. *Brain Res* 835: 340–345, 1999.
224. **Healy DG, Abou-Sleiman PM, Ozawa T, Lees AJ, Bhatia K, Ahmadi KR, Wullner U, Berciano J, Moller JC, Kamm C, Burk K, Barone P, Tolosa E, Quinn N, Goldstein DB, Wood NW.** A functional polymorphism regulating dopamine beta-hydroxylase influences against Parkinson's disease. *Ann Neurol* 55: 443–446, 2004.
225. **Hebert MA, Serova LI, Sabban EL.** Single and repeated immobilization stress differentially trigger induction and phosphorylation of several transcription factors and MAP kinases in the rat locus coeruleus. *J Neurochem* 95: 483–498, 2005.
226. **Helfferich F, Palkovits M.** Acute audiogenic stress-induced activation of CRH neurons in the hypothalamic paraventricular nucleus and catecholaminergic neurons in the medulla oblongata. *Brain Res* 975: 1–9, 2003.
227. **Helmreich DL, Itoi K, Lopez-Figueroa MO, Akil H, Watson SJ.** Norepinephrine-induced CRH and AVP gene transcription within the hypothalamus: differential regulation by corticosterone. *Brain Res* 88: 62–73, 2001.
228. **Henry JP, Botton D, Sagne C, Isambert MF, Desnos C, Blanchard V, Raisman-Vozari R, Krejci E, Massoulie J, Gasnier B.** Biochemistry and molecular biology of the vesicular monoamine transporter from chromaffin granules. *J Exp Biol* 196: 251–262, 1994.
229. **Henry JP, Kross ME, Stephens PM, Watson FMC.** Evidence that differing psychosocial stimuli lead to adrenal cortical stimulation by autonomic or endocrine pathways. In: *Catecholamines and Stress*, edited by Usdin E, Kvetnansky R, Kopin IJ. Oxford: Pergamon, 1976, p. 457–466.
230. **Her S, Claycomb R, Tai TC, Wong DL.** Regulation of the rat phenylethanolamine *N*-methyltransferase gene by transcription factors Sp1 and MAZ. *Mol Pharmacol* 64: 1180–1188, 2003.
231. **Herman JP, Cullinan WE.** Neurocircuitry of stress: central control of the hypothalamo-pituitary-adrenocortical axis. *Trends Neurosci* 20: 78–84, 1997.
232. **Herman JP, Cullinan WE, Morano MI, Akil H, Watson SJ.** Contribution of the ventral subiculum to inhibitory regulation of the hypothalamo-pituitary-adrenocortical axis. *J Neuroendocrinol* 7: 475–482, 1995.
233. **Herman JP, Figueiredo H, Mueller NK, Ulrich-Lai Y, Ostrander MM, Choi DC, Cullinan WE.** Central mechanisms of stress integration: hierarchical circuitry controlling hypothalamo-pituitary-adrenocortical responsiveness. *Front Neuroendocrinol* 24: 151–180, 2003.
234. **Herman JP, Mueller NK, Figueiredo H.** Role of GABA and glutamate circuitry in hypothalamo-pituitary-adrenocortical stress integration. *Ann NY Acad Sci* 1018: 35–45, 2004.
235. **Herman JP, Tasker JG, Ziegler DR, Cullinan WE.** Local circuit regulation of paraventricular nucleus stress integration: glutamate-GABA connections. *Pharmacol Biochem Behav* 71: 457–468, 2002.
236. **Hertting G, Axelrod J, Kopin IJ, Whitby LG.** Lack of uptake of catecholamines after chronic denervation of sympathetic nerves. *Nature* 189: 66, 1961.
237. **Hiremagalur B, Kvetnansky R, Nankova B, Fleischer J, Geertman R, Fukuhara K, Viskupic E, Sabban EL.** Stress elicits trans-synaptic activation of adrenal neuropeptide Y gene expression. *Brain Res* 27: 138–144, 1994.
238. **Hiremagalur B, Nankova B, Nitahara J, Zeman R, Sabban EL.** Nicotine increases expression of tyrosine hydroxylase gene. Involvement of protein kinase A-mediated pathway. *J Biol Chem* 268: 23704–23711, 1993.
239. **Hoeldtke R, Lloyd T, Kaufman S.** An immunochemical study of the induction of tyrosine hydroxylase in rat adrenal glands. *Biochem Biophys Res Commun* 57: 1045–1053, 1974.
240. **Hoffman BJ, Hansson SR, Mezey E, Palkovits M.** Localization and dynamic regulation of biogenic amine transporters in the mammalian central nervous system. *Front Neuroendocrinol* 19: 187–231, 1998.
241. **Hoffman BJ, Palkovits M, Pacak K, Hansson SR, Mezey E.** Regulation of dopamine transporter mRNA levels in the central nervous system. *Adv Pharmacol* 42: 202–206, 1998.
242. **Hokfelt T, Fuxe K, Goldstein M, Johansson O.** Immunohistochemical evidence for the existence of adrenaline neurons in the rat brain. *Brain Res* 66: 235–251, 1974.
243. **Hokfelt T, Martensson R, Bjorklund A, Kleinau S, Goldstein M.** Distributional maps of tyrosine hydroxylase immunoreactive neurons in the rat brain. In: *Handbook of Chemical Neuroanatomy*, edited by Bjorklund A, Hokfelt T. Amsterdam: Elsevier, 1984, p. 277–379.
244. **Hosoya Y, Sugiura Y, Ito R, Kohno K.** Descending projections from the hypothalamic paraventricular nucleus to the A5 area, including the superior salivatory nucleus, in the rat. *Exp Brain Res* 82: 513–518, 1990.
245. **Hosoya Y, Sugiura Y, Okado N, Loewy AD, Kohno K.** Descending input from the hypothalamic paraventricular nucleus to sympathetic preganglionic neurons in the rat. *Exp Brain Res* 85: 10–20, 1991.

246. **Houhou L, Lamouroux A, Biguet NF, Mallet J.** Expression of human dopamine beta-hydroxylase in mammalian cells infected by recombinant vaccinia virus. Mechanisms for membrane attachment. *J Biol Chem* 270: 12601–12606, 1995.
247. **Huang MH, Bahl JJ, Wu Y, Hu F, Larson DF, Roeske WR, Ewy GA.** Neuroendocrine properties of intrinsic cardiac adrenergic cells in fetal rat heart. *Am J Physiol Heart Circ Physiol* 288: H497–H503, 2005.
248. **Huang MH, Friend DS, Sunday ME, Singh K, Haley K, Austen KF, Kelly RA, Smith TW.** An intrinsic adrenergic system in mammalian heart. *J Clin Invest* 98: 1298–1303, 1996.
249. **Huangfu D, Verberne AJ, Guyenet PG.** Rostral ventrolateral medullary neurons projecting to locus coeruleus have cardiorespiratory inputs. *Brain Res* 598: 67–75, 1992.
250. **Hui AS, Striet JB, Gudelsky G, Soukhova GK, Gozal E, Beitner-Johnson D, Guo SZ, Sachleben LR Jr, Haycock JW, Gozal D, Czyzyk-Krzeska MF.** Regulation of catecholamines by sustained and intermittent hypoxia in neuroendocrine cells and sympathetic neurons. *Hypertension* 42: 1130–1136, 2003.
251. **Hurley KM, Herbert H, Moga MM, Saper CB.** Efferent projections of the infralimbic cortex of the rat. *J Comp Neurol* 308: 249–276, 1991.
252. **Hwang O, Joh TH.** Effects of cAMP, glucocorticoids, and calcium on dopamine beta-hydroxylase gene expression in bovine chromaffin cells. *J Mol Neurosci* 4: 173–183, 1993.
253. **Ichinose H, Ohye T, Fujita K, Yoshida M, Ueda S, Nagatsu T.** Increased heterogeneity of tyrosine hydroxylase in humans. *Biochem Biophys Res Commun* 195: 158–165, 1993.
254. **Imaki T, Nahan JL, Rivier C, Sawchenko PE, Vale W.** Differential regulation of corticotropin-releasing factor mRNA in rat brain regions by glucocorticoids and stress. *J Neurosci* 11: 585–599, 1991.
255. **Imbe H, Murakami S, Okamoto K, Iwai-Liao Y, Senba E.** The effects of acute and chronic restraint stress on activation of ERK in the rostral ventromedial medulla and locus coeruleus. *Pain* 112: 361–371, 2004.
256. **Inoue T, Tsuchiya K, Koyama T.** Regional changes in dopamine and serotonin activation with various intensity of physical and psychological stress in the rat brain. *Pharmacol Biochem Behav* 49: 911–920, 1994.
257. **Ip NY, Perlman RL, Zigmond RE.** Acute transsynaptic regulation of tyrosine 3-monooxygenase activity in the rat superior cervical ganglion: evidence for both cholinergic and noncholinergic mechanisms. *Proc Natl Acad Sci USA* 80: 2081–2085, 1983.
258. **Ishiguro H, Kim KT, Joh TH, Kim KS.** Neuron-specific expression of the human dopamine beta-hydroxylase gene requires both the cAMP-response element and a silencer region. *J Biol Chem* 268: 17987–17994, 1993.
259. **Ishiguro H, Yamada K, Ichino N, Nagatsu T.** Identification and characterization of a novel phorbol ester-responsive DNA sequence in the 5′-flanking region of the human dopamine beta-hydroxylase gene. *J Biol Chem* 273: 21941–21949, 1998.
260. **Isovich E, Mijnster MJ, Flugge G, Fuchs E.** Chronic psychosocial stress reduces the density of dopamine transporters. *Eur J Neurosci* 12: 1071–1078, 2000.
261. **Itoi K, Helmreich DL, Lopez-Figueroa MO, Watson SJ.** Differential regulation of corticotropin-releasing hormone and vasopressin gene transcription in the hypothalamus by norepinephrine. *J Neurosci* 19: 5464–5472, 1999.
262. **Itoi K, Suda T, Tozawa F, Dobashi I, Ohmori N, Sakai Y, Abe K, Demura H.** Microinjection of norepinephrine into the paraventricular nucleus of the hypothalamus stimulates corticotropin-releasing factor gene expression in conscious rats. *Endocrinology* 135: 2177–2182, 1994.
263. **Iversen L.** The uptake of catecholamines at high perfusion concentrations in the rat isolated heart: a novel catecholamine uptake process. *Br J Pharmacol* 25: 18–33, 1965.
264. **Jabbi M, Kema IP, van der Pompe G, te Meerman GJ, Ormel J, den Boer JA.** Catechol-*O*-methyltransferase polymorphism and susceptibility to major depressive disorder modulates psychological stress response. *Psychiatr Genet* 17: 183–193, 2007.
265. **Jabbi M, Korf J, Kema IP, Hartman C, van der Pompe G, Minderaa RB, Ormel J, den Boer JA.** Convergent genetic modulation of the endocrine stress response involves polymorphic variations of 5-HTT, COMT and MAOA. *Mol Psychiatry* 12: 483–490, 2007.
266. **Jackson SP, MacDonald JJ, Lees-Miller S, Tjian R.** GC box binding induces phosphorylation of Sp1 by a DNA-dependent protein kinase. *Cell* 63: 155–165, 1990.
267. **Jaeger CB, Ruggiero DA, Albert VR, Park DH, Joh TH, Reis DJ.** Aromatic L-amino acid decarboxylase in the rat brain: immunocytochemical localization in neurons of the brain stem. *Neuroscience* 11: 691–713, 1984.
268. **Jahn R, Sudhof TC.** Synaptic vesicles and exocytosis. *Annu Rev Neurosci* 17: 219–246, 1994.
269. **Jelokova J, Rusnak M, Kubovcakova L, Buckendahl P, Krizanova O, Sabban EL, Kvetnansky R.** Stress increases gene expression of phenylethanolamine *N*-methyltransferase in spleen of rats via pituitary-adrenocortical mechanism. *Psychoneuroendocrinology* 27: 619–633, 2002.
270. **Jeong H, Kim MS, Kwon J, Kim KS, Seol W.** Regulation of the transcriptional activity of the tyrosine hydroxylase gene by androgen receptor. *Neurosci Lett* 396: 57–61, 2006.
271. **Jeske I, Reis DJ, Milner TA.** Neurons in the barosensory area of the caudal ventrolateral medulla project monosynaptically on to sympathoexcitatory bulbospinal neurons in the rostral ventrolateral medulla. *Neuroscience* 65: 343–353, 1995.
272. **Jezova M, Armando I, Bregonzio C, Yu ZX, Qian S, Ferrans VJ, Imboden H, Saavedra JM.** Angiotensin II AT(1) and AT(2) receptors contribute to maintain basal adrenomedullary norepinephrine synthesis and tyrosine hydroxylase transcription. *Endocrinology* 144: 2092–2101, 2003.
273. **Ji Y, Salavaggione OE, Wang L, Adjei AA, Eckloff B, Wieben ED, Weinshilboum RM.** Human phenylethanolamine *N*-methyltransferase pharmacogenomics: gene re-sequencing and functional genomics. *J Neurochem* 95: 1766–1776, 2005.
274. **Jia HG, Rao ZR, Shi JW.** Projection from the ventrolateral medullary neurons containing tyrosine hydroxylase to the central amygdaloid nucleus in the rat. *Brain Res* 589: 167–170, 1992.
275. **Joels M, Karst H, Krugers HJ, Lucassen PJ.** Chronic stress: implications for neuronal morphology, function and neurogenesis. *Front Neuroendocrinol* 28: 72–96, 2007.
276. **Johnson GRJ.** Accumulation of biological amines into chromaffin granules: a model for hormone and neurotransmitter transport. *Physiol Rev* 68: 232–307, 1988.
277. **Kalia M, Fuxe K, Goldstein M.** Rat medulla oblongata. II. Dopaminergic, noradrenergic (A1 and A2) and adrenergic neurons, nerve fibers, and presumptive terminal processes. *J Comp Neurol* 233: 308–332, 1985.
278. **Kalia M, Fuxe K, Goldstein M.** Rat medulla oblongata. III. Adrenergic (C1 and C2) neurons, nerve fibers and presumptive terminal processes. *J Comp Neurol* 233: 333–349, 1985.
279. **Kaneda N, Ichinose H, Kobayashi K, Oka K, Kishi F, Nakazawa A, Kurosawa Y, Fujita K, Nagatsu T.** Molecular cloning of cDNA and chromosomal assignment of the gene for human phenylethanolamine *N*-methyltransferase, the enzyme for epinephrine biosynthesis. *J Biol Chem* 263: 7672–7677, 1988.
280. **Kaneda N, Kobayashi K, Ichinose H, Kishi F, Nakazawa A, Kurosawa Y, Fujita K, Nagatsu T.** Isolation of a novel cDNA clone for human tyrosine hydroxylase: alternative RNA splicing produces four kinds of mRNA from a single gene. *Biochem Biophys Res Commun* 146: 971–975, 1987.
281. **Kappock TJ, Caradonna JP.** Pterin-dependent amino acid hydroxylases. *Chem Rev* 96: 2659–2756, 1996.
282. **Kaufman S.** Tyrosine hydroxylase. *Adv Enzymol Relat Areas Mol Biol* 70: 103–220, 1995.
283. **Kawano H, Masuko S.** Neurons in the caudal ventrolateral medulla projecting to the paraventricular hypothalamic nucleus receive synaptic inputs from the nucleus of the solitary tract: a light and electron microscopic double-labeling study in the rat. *Neurosci Lett* 218: 33–36, 1996.
284. **Kaye DM, Lefkowits J, Cox HS, Lambert G, Jennings J, Turner A, Esler MD.** Regional epinephrine kinetics in human heart failure: evidence for extra-adrenal, nonneuronal release. *Am J Physiol Heart Circ Physiol* 269: H182–H188, 1995.

285. **Kennedy B, Bigby TD, Ziegler MG.** Nonadrenal epinephrine-forming enzymes in humans. Characteristics, distribution, regulation, and relationship to epinephrine levels. *J Clin Invest* 95: 2896–2902, 1995.
286. **Kennedy B, Elayan H, Ziegler MG.** Glucocorticoid elevation of mRNA encoding epinephrine-forming enzyme in lung. *Am J Physiol Lung Cell Mol Physiol* 265: L117–L120, 1993.
287. **Kennedy B, Elayan H, Ziegler MG.** Glucocorticoid hypertension and nonadrenal phenylethanolamine *N*-methyltransferase. *Hypertension* 21: 415–419, 1993.
288. **Kennedy B, Elayan H, Ziegler MG.** Glucocorticoid induction of epinephrine synthesizing enzyme in rat skeletal muscle and insulin resistance. *J Clin Invest* 92: 303–307, 1993.
289. **Kennedy B, Ziegler MG.** Cardiac epinephrine synthesis. Regulation by a glucocorticoid. *Circulation* 84: 891–895, 1991.
290. **Kennedy BP, Mahata SK, O'Connor DT, Ziegler MG.** Mechanism of cardiovascular actions of the chromogranin A fragment catestatin in vivo. *Peptides* 19: 1241–1248, 1998.
291. **Khalil Z, Marley PD, Livett BG.** Elevation in plasma catecholamines in response to insulin stress is under both neuronal and nonneuronal control. *Endocrinology* 119: 159–167, 1986.
292. **Kim CH, Ardayfio P, Kim KS.** An E-box motif residing in the exon/intron 1 junction regulates both transcriptional activation and splicing of the human norepinephrine transporter gene. *J Biol Chem* 276: 24797–24805, 2001.
293. **Kim CH, Hwang DY, Park JJ, Kim KS.** A proximal promoter domain containing a homeodomain-binding core motif interacts with multiple transcription factors, including HoxA5 and Phox2 proteins, critically regulates cell type-specific transcription of the human norepinephrine transporter gene. *J Neurosci* 22: 2579–2589, 2002.
294. **Kim HS, Hong SJ, LeDoux MS, Kim KS.** Regulation of the tyrosine hydroxylase and dopamine beta-hydroxylase genes by the transcription factor AP-2. *J Neurochem* 76: 280–294, 2001.
295. **Kim HS, Seo H, Yang C, Brunet JF, Kim KS.** Noradrenergic-specific transcription of the dopamine beta-hydroxylase gene requires synergy of multiple *cis*-acting elements including at least two Phox2a-binding sites. *J Neurosci* 18: 8247–8260, 1998.
296. **Kim HS, Yang C, Kim KS.** The cell-specific silencer region of the human dopamine beta-hydroxylase gene contains several negative regulatory elements. *J Neurochem* 71: 41–50, 1998.
297. **Kim KS, Kim CH, Hwang DY, Seo H, Chung S, Hong SJ, Lim JK, Anderson T, Isacson O.** Orphan nuclear receptor Nurr1 directly transactivates the promoter activity of the tyrosine hydroxylase gene in a cell-specific manner. *J Neurochem* 85: 622–634, 2003.
298. **Kim KS, Lee MK, Carroll J, Joh TH.** Both the basal and inducible transcription of the tyrosine hydroxylase gene are dependent upon a cAMP response element. *J Biol Chem* 268: 15689–15695, 1993.
299. **Kim TE, Park MJ, Choi EJ, Lee HS, Lee SH, Yoon SH, Oh CK, Lee BJ, Kim SU, Lee YS, Lee MA.** Cloning and cell type-specific regulation of the human tyrosine hydroxylase gene promoter. *Biochem Biophys Res Commun* 312: 1123–1131, 2003.
300. **Kiran BK, Ulus IH.** Selective response of rat peripheral sympathetic nervous system to various stress situations. In: *Stress: Neuroendocrine and Molecular Approaches*, edited by Kvetnansky R, McCarty R, Axelrod J. New York: Gordon and Breach, 1992, p. 561–568.
301. **Kiss A, Mravec B, Palkovits M, Kvetnansky R.** Stress-induced changes in tyrosine hydroxylase gene expression in rat hypothalamic paraventricular, periventricular, and dorsomedial nuclei. *Ann NY Acad Sci.* 1148: 74–85, 2008.
302. **Kiss A, Palkovits M, Aguilera G.** Neural regulation of corticotropin releasing hormone (CRH) and CRH receptor mRNA in the hypothalamic paraventricular nucleus in the rat. *J Neuroendocrinol* 8: 103–112, 1996.
303. **Kobayashi K, Kurosawa Y, Fujita K, Nagatsu T.** Human dopamine beta-hydroxylase gene: two mRNA types having different 3′-terminal regions are produced through alternative polyadenylation. *Nucleic Acids Res* 17: 1089–1102, 1989.
304. **Kobayashi K, Nagatsu T.** Molecular genetics of tyrosine 3-monooxygenase and inherited diseases. *Biochem Biophys Res Commun* 338: 267–270, 2005.
305. **Kobayashi K, Noda Y, Matsushita N, Nishii K, Sawada H, Nagatsu T, Nakahara D, Fukabori R, Yasoshima Y, Yamamoto T, Miura M, Kano M, Mamiya T, Miyamoto Y, Nabeshima T.** Modest neuropsychological deficits caused by reduced noradrenaline metabolism in mice heterozygous for a mutated tyrosine hydroxylase gene. *J Neurosci* 20: 2418–2426, 2000.
306. **Koob GF, Maldonado R, Stinus L.** Neural substrates of opiate withdrawal. *Trends Neurosci* 15: 186–191, 1992.
307. **Kouzmenko AP, Pereira AM, Singh BS.** Intronic sequences are involved in neural targeting of human dopamine transporter gene expression. *Biochem Biophys Res Commun* 240: 807–811, 1997.
308. **Krizanova O, Micutkova L, Jelokova J, Filipenko M, Sabban E, Kvetnansky R.** Existence of cardiac PNMT mRNA in adult rats: elevation by stress in a glucocorticoid-dependent manner. *Am J Physiol Heart Circ Physiol* 281: H1372–H1379, 2001.
309. **Kubovcakova L, Krizanova O, Kvetnansky R.** Identification of the aromatic L-amino acid decarboxylase gene expression in various mice tissues and its modulation by immobilization stress in stellate ganglia. *Neuroscience* 126: 375–380, 2004.
310. **Kubovcakova L, Micutkova L, Bartosova Z, Sabban EL, Krizanova O, Kvetnansky R.** Identification of phenylethanolamine *N*-methyltransferase gene expression in stellate ganglia and its modulation by stress. *J Neurochem* 97: 1419–1430, 2006.
311. **Kubovcakova L, Tybitanclova K, Sabban EL, Majzoub J, Zorad S, Vietor I, Wagner EF, Krizanova O, Kvetnansky R.** Catecholamine synthesizing enzymes and their modulation by immobilization stress in knockout mice. *Ann NY Acad Sci* 1018: 458–465, 2004.
312. **Kumer SC, Vrana KE.** Intricate regulation of tyrosine hydroxylase activity and gene expression. *J Neurochem* 67: 443–462, 1996.
313. **Kuo LE, Czarnecka M, Kitlinska JB, Tilan JU, Kvetnansky R, Zukowska Z.** Chronic stress, combined with a high-fat/high sugar diet shifts sympathetic signaling towards neuropeptide Y and leads to obesity and the metabolic syndrome. *Ann NY Acad Sci* 1148: 232–237, 2008.
314. **Kuo LE, Kitlinska JB, Tilan JU, Li L, Baker SB, Johnson MD, Lee EW, Burnett MS, Fricke ST, Kvetnansky R, Herzog H, Zukowska Z.** Neuropeptide Y acts directly in the periphery on fat tissue and mediates stress-induced obesity and metabolic syndrome. *Nat Med* 13: 803–811, 2007.
315. **Kurz T, Richardt G, Seyfarth M, Schomig A.** Nonexocytotic noradrenaline release induced by pharmacological agents or anoxia in human cardiac tissue. *Naunyn-Schmiedebergs Arch Pharmacol* 354: 7–16, 1996.
316. **Kuzmin AI, Pogorelov VM, Zaretsky DV, Medvedev OS, Chazov EI.** Comparison of the effects of 2-deoxyglucose and immobilization on secretion and synthesis rate of catecholamines in the adrenal gland: a microdialysis study in conscious rats. *Acta Physiol Scand* 155: 147–155, 1995.
317. **Kvetnansky R.** Stressor specificity and effect of prior experience on catecholamine biosynthetic enzyme phenylethanolamine *N*-methyltransferase. *Ann NY Acad Sci* 1032: 117–129, 2004.
318. **Kvetnansky R.** Transsynaptic and humoral regulation of adrenal catecholamines synthesis in stress. In: *Frontiers in Catecholamine Research*, edited by Usdin E, Snyder S. Oxford, UK: Pergamon, 1973, p. 223–229.
319. **Kvetnansky R, Albrecht I, Torda T, Saleh N, Jahnova E, Mikulaj L.** Effect of stress on catecholamine synthesizing and degrading enzymes in control and spontaneously hypertensive rats. In: *Catecholamines and Stress*, edited by Usdin E, Kvetnansky R, Kopin IJ. Oxford, UK: Pergamon, 1976, p. 237–249.
320. **Kvetnansky R, Armando I, Weise VK, Holmes C, Fukuhara K, Deka-Starosta A, Kopin IJ, Goldstein DS.** Plasma dopa responses during stress: dependence on sympathoneural activity and tyrosine hydroxylation. *J Pharmacol Exp Ther* 261: 899–909, 1992.
321. **Kvetnansky R, Bodnar I, Shahar T, Uhereczky G, Krizanova O, Mravec B.** Effect of lesion of A5 and A7 brainstem noradrenergic areas or transection of brainstem pathways on sympathoadrenal activity in rats during immobilization stress. *Neurochem Res* 31: 267–275, 2006.
322. **Kvetnansky R, Daher F, Culman J, Oprsalova Z, Kiss A.** Degradation of catecholamines in hypothalamic nuclei of rats under stress. In: *Integrative Neurohumoral Mechanisms*, edited by En-

droczi E, de Wied D, Angelucci L, Scapagnini U. Amsterdam: Elsevier Biomedical, 1983, p. 353–366.
323. **Kvetnansky R, Fukuhara K, Pacak K, Cizza G, Goldstein DS, Kopin IJ.** Endogenous glucocorticoids restrain catecholamine synthesis and release at rest and during immobilization stress in rats. *Endocrinology* 133: 1411–1419, 1993.
324. **Kvetnansky R, Gewirtz GP, Weise VK, Kopin IJ.** Catecholamine-synthesizing enzymes in the rat adrenal gland during exposure to cold. *Am J Physiol* 220: 928–931, 1971.
325. **Kvetnansky R, Gewirtz GP, Weise VK, Kopin IJ.** Effect of hypophysectomy on immobilization-induced elevation of tyrosine hydroxylase and phenylethanolamine-*N*-methyl transferase in the rat adrenal. *Endocrinology* 87: 1323–1329, 1970.
326. **Kvetnansky R, Gewirtz GP, Weise VK, Kopin IJ.** Enhanced synthesis of adrenal dopamine beta-hydroxylase induced by repeated immobilization in rats. *Mol Pharmacol* 7: 81–86, 1971.
327. **Kvetnansky R, Goldstein DS, Weise VK, Holmes C, Szemeredi K, Bagdy G, Kopin IJ.** Effects of handling or immobilization on plasma levels of 3,4-dihydroxyphenylalanine, catecholamines, and metabolites in rats. *J Neurochem* 58: 2296–2302, 1992.
328. **Kvetnansky R, Jelokova J, Rusnak M, Dronjak S, Serova L, Nankova B, Sabban EL.** Novel stressors exaggerate tyrosine hydroxylase gene expression in the adrenal medulla of rats exposed to long-term cold stress. In: *Stress Neural, Endocrine and Molecular Studies*, edited by McCarty R, Aguilera G, Sabban EL, Kvetnansky R. New York: Taylor and Francis, 2002, p. 121–128.
329. **Kvetnansky R, Kopin IJ, Saavedra JM.** Changes in epinephrine in individual hypothalamic nuclei after immobilization stress. *Brain Res* 155: 387–390, 1978.
330. **Kvetnansky R, Kubovcakova L, Tillinger A, Micutkova L, Krizanova O, Sabban EL.** Gene expression of phenylethanolamine *N*-methyltransferase in corticotropin-releasing hormone knockout mice during stress exposure. *Cell Mol Neurobiol* 26: 733–752, 2006.
331. **Kvetnansky R, McCarty R.** Adrenal medulla. In: *Encyclopedia of Stress*, edited by Fink G. New York: Academic, 2007, p. 52–59.
332. **Kvetnansky R, McCarty R, Axelrod J.** *Stress: Neuroendocrine and Molecular Approaches*. *New* York: Gordon and Breach Science, 1992, p. 1–1028.
333. **Kvetnansky R, Micutkova L, Kubovcakova L, Sabban EL, Palkovits M, Krizanova O.** Localization and regulation of phenylethanolamine *N*-methyltransferase gene expression in the heart of rats and mice during stress. *Ann NY Acad Sci* 1018: 405–417, 2004.
334. **Kvetnansky R, Micutkova L, Rychkova N, Kubovcakova L, Mravec B, Filipenko M, Sabban EL, Krizanova O.** Quantitative evaluation of catecholamine enzymes gene expression in adrenal medulla and sympathetic ganglia of stressed rats. *Ann NY Acad Sci* 1018: 356–369, 2004.
335. **Kvetnansky R, Mikulaj L.** Adrenal and urinary catecholamines in rats during adaptation to repeated immobilization stress. *Endocrinology* 87: 738–743, 1970.
336. **Kvetnansky R, Mitro A, Palkovits M, Brownstein M, Torda T, Vigas M, Mikulaj L.** Catecholamines in individual hypothalamic nuclei in stressed rats. In: *Catecholamines and Stress*, edited by Usdin E, Kvetnansky R, Kopin IJ. Oxford: Pergamon, 1976, p. 39–50.
337. **Kvetnansky R, Nankova B, Hiremagalur B, Viskupic E, Vietor I, Rusnak M, McMahon A, Kopin IJ, Sabban EL.** Induction of adrenal tyrosine hydroxylase mRNA by single immobilization stress occurs even after splanchnic transection and in the presence of cholinergic antagonists. *J Neurochem* 66: 138–146, 1996.
338. **Kvetnansky R, Nankova B, Rusnak M, Micutkova L, Kubovcakova L, Dronjak S, Krizanova O, Sabban EL.** Differential gene expression of tyrosine hydroxylase in rats exposed long-term to various stressors. In: *Catecholamine Research: From Molecular Insights to Clinical Medicine*, edited by Nagatsu T, Nabeshima T, McCarty R, Goldstein DS. New York: Plenum, 2002, p. 317–320.
339. **Kvetnansky R, Nemeth S, Vigas M, Oprsalova Z, Jurcovicova J.** Plasma catecholamines in rats during adaptation to intermittent exposure to different stressors. In: *Stress: The Role of Catecholamines and Other Neurotransmitters*, edited by Usdin E, Kvetnansky R, Axelrod J. New York: Gordon and Breach Science, 1984, p. 537–562.
340. **Kvetnansky R, Pacak K, Fukuhara K, Viskupic E, Hiremagalur B, Nankova B, Goldstein DS, Sabban EL, Kopin IJ.** Sympathoadrenal system in stress. Interaction with the hypothalamic-pituitary-adrenocortical system. *Ann NY Acad Sci* 771: 131–158, 1995.
341. **Kvetnansky R, Pacak K, Sabban EL, Kopin IJ, Goldstein DS.** Stressor specificity of peripheral catecholaminergic activation. *Adv Pharmacol* 42: 556–560, 1998.
342. **Kvetnansky R, Palkovits M, Mitro A, Torda T, Mikulaj L.** Catecholamines in individual hypothalamic nuclei of acutely and repeatedly stressed rats. *Neuroendocrinology* 23: 257–267, 1977.
343. **Kvetnansky R, Rusnak M, Dronjak S, Krizanova O, Sabban EL.** Effect of novel stressors on tyrosine hydroxylase gene expression in the adrenal medulla of repeatedly immobilized rats. *Neurochem Res* 28: 625–630, 2003.
344. **Kvetnansky R, Sabban EL.** Stress-induced changes in tyrosine hydroxylase and other catecholamine biosynthetic enzymes. In: *Tyrosine Hydroxylase: From Discovery to Cloning*, edited by Naoi M, Parvez SH. Utrecht, The Netherlands: VSP, 1993.
345. **Kvetnansky R, Sabban EL.** Stress and molecular biology of neurotransmitter-related enzymes. *Ann NY Acad Sci* 851: 342–356, 1998.
346. **Kvetnansky R, Silbergeld S, Weise VK, Kopin IJ.** Effects of restraint on rat adrenomedullary response to 2-deoxy-D-glucose. *Psychopharmacologia* 20: 22–31, 1971.
347. **Kvetnansky R, Sun CL, Lake CR, Thoa N, Torda T, Kopin IJ.** Effect of handling and forced immobilization on rat plasma levels of epinephrine, norepinephrine, and dopamine-beta-hydroxylase. *Endocrinology* 103: 1868–1874, 1978.
348. **Kvetnansky R, Torda T, Jahnova E, Saleh N.** Activity of catecholamine degrading enzymes in rat adrenal medulla and cortex after acute and repeated stress. *Endocrinol Exp* 9: 79–86, 1975.
349. **Kvetnansky R, Torda T, Macho L, Tigranian RA, Serova L, Genin AM.** Effect of weightlessness on sympathetic-adrenomedullary activity of rats. *Acta Astronaut* 8: 469–481, 1981.
350. **Kvetnansky R, Weise VK, Gewirtz GP, Kopin IJ.** Synthesis of adrenal catecholamines in rats during and after immobilization stress. *Endocrinology* 89: 46–49, 1971.
351. **Kvetnansky R, Weise VK, Kopin IJ.** Elevation of adrenal tyrosine hydroxylase and phenylethanolamine-*N*-methyl transferase by repeated immobilization of rats. *Endocrinology* 87: 744–749, 1970.
352. **Kvetnansky R, Weise VK, Thoa NB, Kopin IJ.** Effects of chronic guanethidine treatment and adrenal medullectomy on plasma levels of catecholamines and corticosterone in forcibly immobilized rats. *J Pharmacol Exp Ther* 209: 287–291, 1979.
353. **Kwiat GC, Basbaum AI.** Organization of tyrosine hydroxylase- and serotonin-immunoreactive brainstem neurons with axon collaterals to the periaqueductal gray and the spinal cord in the rat. *Brain Res* 528: 83–94, 1990.
354. **Kwiat GC, Liu H, Williamson AM, Basbaum AI.** GABAergic regulation of noradrenergic spinal projection neurons of the A5 cell group in the rat: an electron microscopic analysis. *J Comp Neurol* 330: 557–570, 1993.
355. **La Gamma EF, Agarwal BL, DeCristofaro JD.** Regulation of adrenomedullary preproenkephalin mRNA: effects of hypoglycemia during development. *Brain Res* 13: 189–197, 1992.
356. **Lachman HM, Nolan KA, Mohr P, Saito T, Volavka J.** Association between catechol *O*-methyltransferase genotype and violence in schizophrenia and schizoaffective disorder. *Am J Psychiatry* 155: 835–837, 1998.
357. **Le Bourdelles B, Boularand S, Boni C, Horellou P, Dumas S, Grima B, Mallet J.** Analysis of the 5′ region of the human tyrosine hydroxylase gene: combinatorial patterns of exon splicing generate multiple regulated tyrosine hydroxylase isoforms. *J Neurochem* 50: 988–991, 1988.
358. **Lee KH, Kwak YD, Kim DH, Chang MY, Lee YS.** Human zinc finger protein 161, a novel transcriptional activator of the dopamine transporter. *Biochem Biophys Res Commun* 313: 969–976, 2004.
359. **Lemoine AP, Armando I, Brun JC, Segura ET, Barontini M.** Footshock affects heart and brain MAO and MAO inhibitory activity and open field behavior in rats. *Pharmacol Biochem Behav* 36: 85–88, 1990.
360. **Lenders JW, Keiser HR, Goldstein DS, Willemsen JJ, Friberg P, Jacobs MC, Kloppenborg PW, Thien T, Eisenhofer G.**

Plasma metanephrines in the diagnosis of pheochromocytoma. *Ann Intern Med* 123: 101–109, 1995.
361. **Levenson CW, Moore JB.** Response of rat adrenal neuropeptide Y and tyrosine hydroxylase mRNA to acute stress is enhanced by long-term voluntary exercise. *Neurosci Lett* 242: 177–179, 1998.
362. **Lewis-Tuffin LJ, Quinn PG, Chikaraishi DM.** Tyrosine hydroxylase transcription depends primarily on cAMP response element activity, regardless of the type of inducing stimulus. *Mol Cell Neurosci* 25: 536–547, 2004.
363. **Lewis EJ, Asnani LP.** Soluble and membrane-bound forms of dopamine beta-hydroxylase are encoded by the same mRNA. *J Biol Chem* 267: 494–500, 1992.
364. **Li AJ, Wang Q, Ritter S.** Differential responsiveness of dopamine-beta-hydroxylase gene expression to glucoprivation in different catecholamine cell groups. *Endocrinology* 147: 3428–3434, 2006.
365. **Li HY, Ericsson A, Sawchenko PE.** Distinct mechanisms underlie activation of hypothalamic neurosecretory neurons and their medullary catecholaminergic afferents in categorically different stress paradigms. *Proc Natl Acad Sci USA* 93: 2359–2364, 1996.
366. **Li Q, Goodchild AK, Pilowsky PM.** Effect of haemorrhage on the expression of neurotransmitter-related genes in rat ventrolateral medulla: a quantitative real-time RT-PCR study. *Brain Res* 114: 46–54, 2003.
367. **Lima D, Mendes-Ribeiro JA, Coimbra A.** The spino-latero-reticular system of the rat: projections from the superficial dorsal horn and structural characterization of marginal neurons involved. *Neuroscience* 45: 137–152, 1991.
368. **Liposits Z, Phelix C, Paull WK.** Electron microscopic analysis of tyrosine hydroxylase, dopamine-beta-hydroxylase and phenylethanolamine-*N*-methyltransferase immunoreactive innervation of the hypothalamic paraventricular nucleus in the rat. *Histochemistry* 84: 105–120, 1986.
369. **Liu J, Merlie JP, Todd RD, O'Malley KL.** Identification of cell type-specific promoter elements associated with the rat tyrosine hydroxylase gene using transgenic founder analysis. *Brain Res* 50: 33–42, 1997.
370. **Liu X, Kvetnansky R, Serova L, Sollas A, Sabban EL.** Increased susceptibility to transcriptional changes with novel stressor in adrenal medulla of rats exposed to prolonged cold stress. *Mol Brain Res* 141: 19–29, 2005.
371. **Liu X, Serova L, Kvetnansky R, Sabban EL.** Identifying the stress transcriptome in the adrenal medulla following acute and repeated immobilization. *Ann NY Acad Sci* 1148: 1–28, 2008.
372. **Liuzzi A, Foppen FH, Saavedra JM, Jacobowitz D, Kopin IJ.** Effect of NGF and dexamethasone on phenylethanolamine-*N*-methyl transferase (PNMT) activity in neonatal rat superior cervical ganglia. *J Neurochem* 28: 1215–1220, 1977.
373. **Livett BG, Marley PD, Wan DC, Zhou XF.** Peptide regulation of adrenal medullary function. *J Neural Transm Suppl* 29: 77–89, 1990.
374. **Loewy AD, Haxhiu MA.** CNS cell groups projecting to pancreatic parasympathetic preganglionic neurons. *Brain Res* 620: 323–330, 1993.
375. **Loewy AD, McKellar S, Saper CB.** Direct projections from the A5 catecholamine cell group to the intermediolateral cell column. *Brain Res* 174: 309–314, 1979.
376. **Lorang D, Amara SG, Simerly RB.** Cell-type-specific expression of catecholamine transporters in the rat brain. *J Neurosci* 14: 4903–4914, 1994.
377. **Loughlin SE, Foote SL, Fallon JH.** Locus coeruleus projections to cortex: topography, morphology and collateralization. *Brain Res Bull* 9: 287–294, 1982.
378. **Loughlin SE, Foote SL, Grzanna R.** Efferent projections of nucleus locus coeruleus: morphologic subpopulations have different efferent targets. *Neuroscience* 18: 307–319, 1986.
379. **Lowry CA, Plant A, Shanks N, Ingram CD, Lightman SL.** Anatomical and functional evidence for a stress-responsive, monoamine-accumulating area in the dorsomedial hypothalamus of adult rat brain. *Horm Behav* 43: 254–262, 2003.
380. **Luiten PG, ter Horst GJ, Karst H, Steffens AB.** The course of paraventricular hypothalamic efferents to autonomic structures in medulla and spinal cord. *Brain Res* 329: 374–378, 1985.
381. **Luiten PG, ter Horst GJ, Steffens AB.** The hypothalamus, intrinsic connections and outflow pathways to the endocrine system in relation to the control of feeding and metabolism. *Prog Neurobiol* 28: 1–54, 1987.
382. **Lundberg JM, Hokfelt T.** Multiple co-existence of peptides and classical transmitters in peripheral autonomic and sensory neurons–functional and pharmacological implications. *Prog Brain Res* 68: 241–262, 1986.
383. **Lundstrom K, Tenhunen J, Tilgmann C, Karhunen T, Panula P, Ulmanen I.** Cloning, expression and structure of catechol-*O*-methyltransferase. *Biochim Biophys Acta* 1251: 1–10, 1995.
384. **Luppi PH, Aston-Jones G, Akaoka H, Chouvet G, Jouvet M.** Afferent projections to the rat locus coeruleus demonstrated by retrograde and anterograde tracing with cholera-toxin B subunit and Phaseolus vulgaris leucoagglutinin. *Neuroscience* 65: 119–160, 1995.
385. **Lyons WE, Fritschy JM, Grzanna R.** The noradrenergic neurotoxin DSP-4 eliminates the coeruleospinal projection but spares projections of the A5 and A7 groups to the ventral horn of the rat spinal cord. *J Neurosci* 9: 1481–1489, 1989.
386. **Maharjan S, Serova L, Sabban EL.** Transcriptional regulation of tyrosine hydroxylase by estrogen: opposite effects with estrogen receptors alpha and beta and interactions with cyclic AMP. *J Neurochem* 93: 1502–1514, 2005.
387. **Makino S, Hashimoto K, Gold PW.** Multiple feedback mechanisms activating corticotropin-releasing hormone system in the brain during stress. *Pharmacol Biochem Behav* 73: 147–158, 2002.
388. **Makino S, Schulkin J, Smith MA, Pacak K, Palkovits M, Gold PW.** Regulation of corticotropin-releasing hormone receptor messenger ribonucleic acid in the rat brain and pituitary by glucocorticoids and stress. *Endocrinology* 136: 4517–4525, 1995.
389. **Makino S, Shibasaki T, Yamauchi N, Nishioka T, Mimoto T, Wakabayashi I, Gold PW, Hashimoto K.** Psychological stress increased corticotropin-releasing hormone mRNA and content in the central nucleus of the amygdala but not in the hypothalamic paraventricular nucleus in the rat. *Brain Res* 850: 136–143, 1999.
390. **Mamalaki E, Kvetnansky R, Brady LS, Gold PW, Herkenham M.** Repeated immobilization stress alters tyrosine hydroxylase, corticotrophin-releasing hormone and corticosteroid receptor messenger ribonucleic acid levels in rat brain. *J Neuroendocrinol* 4: 689–699, 1992.
391. **Mann MB, Wu S, Rostamkhani M, Tourtellotte W, MacMurray J, Comings DE.** Phenylethanolamine *N*-methyltransferase (PNMT) gene and early-onset Alzheimer disease. *Am J Med Genet* 105: 312–316, 2001.
392. **Mann MB, Wu S, Rostamkhani M, Tourtellotte W, MacMurray JP, Comings DE.** Association between the phenylethanolamine *N*-methyltransferase gene and multiple sclerosis. *J Neuroimmunol* 124: 101–105, 2002.
393. **Mao W, Iwai C, Qin F, Liang CS.** Norepinephrine induces endoplasmic reticulum stress and downregulation of norepinephrine transporter density in PC12 cells via oxidative stress. *Am J Physiol Heart Circ Physiol* 288: H2381–H2389, 2005.
394. **Mao W, Qin F, Iwai C, Vulapalli R, Keng PC, Liang CS.** Extracellular norepinephrine reduces neuronal uptake of norepinephrine by oxidative stress in PC12 cells. *Am J Physiol Heart Circ Physiol* 287: H29–H39, 2004.
395. **Mason JW.** A re-evaluation of the concept of "non-specificity" in stress theory. *J Psychiatr Res* 8: 323–333, 1971.
396. **Masuda N, Ootsuka Y, Terui N.** Neurons in the caudal ventrolateral medulla mediate the somato-sympathetic inhibitory reflex response via GABA receptors in the rostral ventrolateral medulla. *J Auton Nerv Syst* 40: 91–98, 1992.
397. **Mayford M, Kandel ER.** Genetic approaches to memory storage. *Trends Genet* 15: 463–470, 1999.
398. **Mayr B, Montminy M.** Transcriptional regulation by the phosphorylation-dependent factor CREB. *Nat Rev Mol Cell Biol* 2: 599–609, 2001.
399. **McCarty R, Aguilera G, Sabban E, Kvetnansky R.** *Stress: Neural, Endocrine and Molecular Studies.* New York: Taylor & Francis, 2002, p. 276.
400. **McCarty R, Stone EA.** Chronic stress and regulation of the sympathetic nervous system. In: *Stress: The Role of Catecholamines*

and Other Neurotransmitters, edited by Usdin E, Kvetnansky R, Axelrod J. New York: Gordon and Breach Science, 1984, p. 563–576.
401. **McEwen BS.** Physiology and neurobiology of stress and adaptation: central role of the brain. *Physiol Rev* 87: 873–904, 2007.
402. **McEwen BS.** Protection and damage from acute and chronic stress: allostasis and allostatic overload and relevance to the pathophysiology of psychiatric disorders. *Ann NY Acad Sci* 1032: 1–7, 2004.
403. **McEwen BS.** Stress, adaptation, and disease. Allostasis and allostatic load. *Ann NY Acad Sci* 840: 33–44, 1998.
404. **McEwen BS, Stellar E.** Stress and the individual. Mechanisms leading to disease. *Arch Intern Med* 153: 2093–2101, 1993.
405. **McMahon A, Geertman R, Sabban EL.** Rat dopamine beta-hydroxylase: molecular cloning and characterization of the cDNA and regulation of the mRNA by reserpine. *J Neurosci Res* 25: 395–404, 1990.
406. **McMahon A, Kvetnansky R, Fukuhara K, Weise VK, Kopin IJ, Sabban EL.** Regulation of tyrosine hydroxylase and dopamine beta-hydroxylase mRNA levels in rat adrenals by a single and repeated immobilization stress. *J Neurochem* 58: 2124–2130, 1992.
407. **McMahon A, Sabban EL.** Regulation of expression of dopamine beta-hydroxylase in PC12 cells by glucocorticoids and cyclic AMP analogues. *J Neurochem* 59: 2040–2047, 1992.
408. **Medeiros MA, Canteras NS, Suchecki D, Mello LE.** c-Fos expression induced by electroacupuncture at the Zusanli point in rats submitted to repeated immobilization. *Braz J Med Biol Res* 36: 1673–1684, 2003.
409. **Meister B, Hokfelt T, Steinbusch HW, Skagerberg G, Lindvall O, Geffard M, Joh TH, Cuello AC, Goldstein M.** Do tyrosine hydroxylase-immunoreactive neurons in the ventrolateral arcuate nucleus produce dopamine or only L-dopa? *J Chem Neuroanat* 1: 59–64, 1988.
410. **Melia KR, Rasmussen K, Terwilliger RZ, Haycock JW, Nestler EJ, Duman RS.** Coordinate regulation of the cyclic AMP system with firing rate and expression of tyrosine hydroxylase in the rat locus coeruleus: effects of chronic stress and drug treatments. *J Neurochem* 58: 494–502, 1992.
411. **Meloni R, Albanese V, Ravassard P, Treilhou F, Mallet J.** A tetranucleotide polymorphic microsatellite, located in the first intron of the tyrosine hydroxylase gene, acts as a transcription regulatory element in vitro. *Hum Mol Genet* 7: 423–428, 1998.
412. **Menetrey D, Basbaum AI.** Spinal and trigeminal projections to the nucleus of the solitary tract: a possible substrate for somatovisceral and viscerovisceral reflex activation. *J Comp Neurol* 255: 439–450, 1987.
413. **Menetrey D, Roudier F, Besson JM.** Spinal neurons reaching the lateral reticular nucleus as studied in the rat by retrograde transport of horseradish peroxidase. *J Comp Neurol* 220: 439–452, 1983.
414. **Mezey E, Eisenhofer G, Hansson S, Harta G, Hoffman BJ, Gallatz K, Palkovits M, Hunyady B.** Non-neuronal dopamine in the gastrointestinal system. *Clin Exp Pharmacol Physiol Suppl* 26: S14–S22, 1999.
415. **Mezey E, Eisenhofer G, Harta G, Hansson S, Gould L, Hunyady B, Hoffman BJ.** A novel nonneuronal catecholaminergic system: exocrine pancreas synthesizes and releases dopamine. *Proc Natl Acad Sci USA* 93: 10377–10382, 1996.
416. **Micutkova L, Krepsova K, Sabban E, Krizanova O, Kvetnansky R.** Modulation of catecholamine-synthesizing enzymes in the rat heart by repeated immobilization stress. *Ann NY Acad Sci* 1018: 424–429, 2004.
417. **Micutkova L, Rychkova N, Sabban EL, Krizanova O, Kvetnansky R.** Quantitation of changes in gene expression of norepinephrine biosynthetic enzymes in rat stellate ganglia induced by stress. *Neurochem Int* 43: 235–242, 2003.
418. **Mikhailova ON, Gulyaeva LF, Filipenko ML.** Gene expression of drug metabolizing enzymes in adult and aged mouse liver: a modulation by immobilization stress. *Toxicology* 210: 189–196, 2005.
419. **Milner TA, Morrison SF, Abate C, Reis DJ.** Phenylethanolamine *N*-methyltransferase-containing terminals synapse directly on sympathetic preganglionic neurons in the rat. *Brain Res* 448: 205–222, 1988.
420. **Milner TA, Pickel VM, Chan J, Massari VJ, Oertel WH, Park DH, Joh TH, Reis DJ.** Phenylethanolamine *N*-methyltransferase-containing neurons in the rostral ventrolateral medulla. II. Synaptic relationships with GABAergic terminals. *Brain Res* 411: 46–57, 1987.
421. **Min N, Joh TH, Kim KS, Peng C, Son JH.** 5′ Upstream DNA sequence of the rat tyrosine hydroxylase gene directs high-level and tissue-specific expression to catecholaminergic neurons in the central nervous system of transgenic mice. *Brain Res* 27: 281–289, 1994.
422. **Miner LH, Jedema HP, Moore FW, Blakely RD, Grace AA, Sesack SR.** Chronic stress increases the plasmalemmal distribution of the norepinephrine transporter and the coexpression of tyrosine hydroxylase in norepinephrine axons in the prefrontal cortex. *J Neurosci* 26: 1571–1578, 2006.
423. **Miner LL, Pandalai SP, Weisberg EP, Sell SL, Kovacs DM, Kaplan BB.** Cold-induced alterations in the binding of adrenomedullary nuclear proteins to the promoter region of the tyrosine hydroxylase gene. *J Neurosci Res* 33: 10–18, 1992.
424. **Minson J, Llewellyn-Smith I, Neville A, Somogyi P, Chalmers J.** Quantitative analysis of spinally projecting adrenaline-synthesising neurons of C1, C2 and C3 groups in rat medulla oblongata. *J Auton Nerv Syst* 30: 209–220, 1990.
425. **Molinoff PB, Brimijoin S, Axelrod J.** Induction of dopamine-β-hydroxylase and tyrosine hydroxylase in rat hearts and sympathetic ganglia. *J Pharmacol Exp Ther* 182: 116–129, 1972.
426. **Montminy MR, Gonzalez GA, Yamamoto KK.** Regulation of cAMP-inducible genes by CREB. *Trends Neurosci* 13: 184–188, 1990.
427. **Moore H, Rose HJ, Grace AA.** Chronic cold stress reduces the spontaneous activity of ventral tegmental dopamine neurons. *Neuropsychopharmacology* 24: 410–419, 2001.
428. **Moore KE, Lookingland KJ.** Dopaminergic neuronal systems in the hypothalamus. In: *Psychopharmacology: The Fourth Generation of Progress*, edited by Bloom FE, Kupfer DJ. New York: Raven, 1995, p. 245–256.
429. **Moore KE, Phillipson OT.** Effects of dexamethasone on phenylethanolamine *N*-methyltransferase and adrenaline in the brains and superior cervical ganglia of adult and neonatal rats. *J Neurochem* 25: 289–294, 1975.
430. **Morita K, Bell RA, Siddall BJ, Wong DL.** Neural stimulation of Egr-1 messenger RNA expression in rat adrenal gland: possible relation to phenylethanolamine *N*-methyltransferase gene regulation. *J Pharmacol Exp Ther* 279: 379–385, 1996.
431. **Morita K, Kuwada A, Sano A, Sakata M, Sei H, Morita Y.** Semi-quantitative RT-PCR analysis of environmental influence on P450scc and PNMT mRNA expression in rat adrenal glands. *Life Sci* 70: 73–80, 2001.
432. **Morita K, Wong DL.** Role of Egr-1 in cholinergic stimulation of phenylethanolamine *N*-methyltransferase promoter. *J Neurochem* 67: 1344–1351, 1996.
433. **Morrison SF.** Differential control of sympathetic outflow. *Am J Physiol Regul Integr Comp Physiol* 281: R683–R698, 2001.
434. **Morrison SF, Cao WH.** Different adrenal sympathetic preganglionic neurons regulate epinephrine and norepinephrine secretion. *Am J Physiol Regul Integr Comp Physiol* 279: R1763–R1775, 2000.
435. **Morsink MC, Van Gemert NG, Steenbergen PJ, Joels M, De Kloet ER, Datson NA.** Rapid glucocorticoid effects on the expression of hippocampal neurotransmission-related genes. *Brain Res* 1150: 14–20, 2007.
436. **Mravec B, Bodnar I, Kubovcakova L, Nagy GM, Palkovits M, Kvetnansky R.** Inhibitory effect of formalin administration on immobilization-induced epinephrine release. *Ann NY Acad Sci* 1018: 105–112, 2004.
437. **Mravec B, Bodnar I, Uhereczky G, Nagy GM, Kvetnansky R, Palkovits M.** Formalin attenuates the stress-induced increase in plasma epinephrine levels. *J Neuroendocrinol* 17: 727–732, 2005.
438. **Mravec B, Lukackova R, Bodnar I, Kiss A, Pacak K, Palkovits M, Kvetnansky R.** Stress-induced alterations in catecholamine enzymes gene expression in the hypothalamic dorsomedial nucleus are modulated by caudal brain and not hypothalamic paraventricular nucleus neurons. *Brain Res Bull* 74: 147–154, 2007.

439. **Mtui EP, Anwar M, Gomez R, Reis DJ, Ruggiero DA.** Projections from the nucleus tractus solitarii to the spinal cord. *J Comp Neurol* 337: 231–252, 1993.
440. **Nagamoto-Combs K, Piech KM, Best JA, Sun B, Tank AW.** Tyrosine hydroxylase gene promoter activity is regulated by both cyclic AMP-responsive element and AP1 sites following calcium influx. Evidence for cyclic AMP-responsive element binding protein-independent regulation. *J Biol Chem* 272: 6051–6058, 1997.
441. **Nagatsu T.** Genes for human catecholamine-synthesizing enzymes. *Neurosci Res* 12: 315–345, 1991.
442. **Nagatsu T.** Progress in monoamine oxidase (MAO) research in relation to genetic engineering. *Neurotoxicology* 25: 11–20, 2004.
443. **Nagatsu T.** Tyrosine hydroxylase: human isoforms, structure and regulation in physiology and pathology. *Essays Biochem* 30: 15–35, 1995.
444. **Nagatsu T, Ichinose H.** Regulation of pteridine-requiring enzymes by the cofactor tetrahydrobiopterin. *Mol Neurobiol* 19: 79–96, 1999.
445. **Nagatsu T, Nabeshima T, McCarty R, Goldstein DS.** *Catecholamine Research: From Molecular Insight to Clinical Medicine*. New York: Plenum, 2002, p. 558.
446. **Nagatsu T, Stjarne L.** Catecholamine synthesis and release. Overview. *Adv Pharmacol* 42: 1–14, 1998.
447. **Nakashima A, Ota A, Sabban EL.** Interactions between Egr1 and AP1 factors in regulation of tyrosine hydroxylase transcription. *Brain Res* 112: 61–69, 2003.
448. **Nankova B, Devlin D, Kvetnansky R, Kopin IJ, Sabban EL.** Repeated immobilization stress increases the binding of c-Fos-like proteins to a rat dopamine beta-hydroxylase promoter enhancer sequence. *J Neurochem* 61: 776–779, 1993.
449. **Nankova B, Kvetnansky R, Hiremagalur B, Sabban B, Rusnak M, Sabban EL.** Immobilization stress elevates gene expression for catecholamine biosynthetic enzymes and some neuropeptides in rat sympathetic ganglia: effects of adrenocorticotropin and glucocorticoids. *Endocrinology* 137: 5597–5604, 1996.
450. **Nankova B, Kvetnansky R, McMahon A, Viskupic E, Hiremagalur B, Frankle G, Fukuhara K, Kopin IJ, Sabban EL.** Induction of tyrosine hydroxylase gene expression by a nonneuronal nonpituitary-mediated mechanism in immobilization stress. *Proc Natl Acad Sci USA* 91: 5937–5941, 1994.
451. **Nankova BB, Kvetnansky R, Sabban EL.** Adrenocorticotropic hormone (MC-2) receptor mRNA is expressed in rat sympathetic ganglia and up-regulated by stress. *Neurosci Lett* 344: 149–152, 2003.
452. **Nankova BB, Rivkin M, Kelz M, Nestler EJ, Sabban EL.** Fos-related antigen 2: potential mediator of the transcriptional activation in rat adrenal medulla evoked by repeated immobilization stress. *J Neurosci* 20: 5647–5653, 2000.
453. **Nankova BB, Sabban EL.** Multiple signalling pathways exist in the stress-triggered regulation of gene expression for catecholamine biosynthetic enzymes and several neuropeptides in the rat adrenal medulla. *Acta Physiol Scand* 167: 1–9, 1999.
454. **Nankova BB, Tank AW, Sabban EL.** Transient or sustained transcriptional activation of the genes encoding rat adrenomedullary catecholamine biosynthetic enzymes by different durations of immobilization stress. *Neuroscience* 94: 803–808, 1999.
455. **Nestler EJ, Alreja M, Aghajanian GK.** Molecular control of locus coeruleus neurotransmission. *Biol Psychiatry* 46: 1131–1139, 1999.
456. **Nichol CA, Smith GK, Duch DS.** Biosynthesis and metabolism of tetrahydrobiopterin and molybdopterin. *Annu Rev Biochem* 54: 729–764, 1985.
457. **Nikulina EM, Covington HE, 3rd Ganschow L, Hammer RP Jr, Miczek KA.** Long-term behavioral and neuronal cross-sensitization to amphetamine induced by repeated brief social defeat stress: Fos in the ventral tegmental area and amygdala. *Neuroscience* 123: 857–865, 2004.
458. **Nuseir K, Proudfit HK.** Bidirectional modulation of nociception by GABA neurons in the dorsolateral pontine tegmentum that tonically inhibit spinally projecting noradrenergic A7 neurons. *Neuroscience* 96: 773–783, 2000.
459. **Nygren LG, Olson L.** A new major projection from locus coeruleus: the main source of noradrenergic nerve terminals in the ventral and dorsal columns of the spinal cord. *Brain Res* 132: 85–93, 1977.
460. **O'Malley KL, Anhalt MJ, Martin BM, Kelsoe JR, Winfield SL, Ginns EI.** Isolation and characterization of the human tyrosine hydroxylase gene: identification of 5′ alternative splice sites responsible for multiple mRNAs. *Biochemistry* 26: 6910–6914, 1987.
461. **Obata T, Yamanaka Y.** Changes in monoamine oxidase activity in rat liver during stress. *Jpn J Pharmacol* 66: 149–150, 1994.
462. **Ohye T, Ichinose H, Yoshizawa T, Kanazawa I, Nagatsu T.** A new splicing variant for human tyrosine hydroxylase in the adrenal medulla. *Neurosci Lett* 312: 157–160, 2001.
463. **Oliver G, Schafer EA.** On the physiological action of extract of the suprarenal capsules. *J Physiol* 17: 9–14, 1895.
464. **Oliver G, Schafer EA.** The physiological effects of extracts of the suprarenal capsules. *J Physiol* 18: 230–276, 1895.
465. **Osterhout C, Sun B, Tank AW.** Regulation of tyrosine hydroxylase gene transcription rate by stress: use of transgenic mice. In: *Stress: Neural, Endocrine and Molecular Studies*, edited by McCarty R, Aguilera G, Sabban E, Kvetnansky R. London: Taylor & Francis, 2002, p. 107–113.
466. **Osterhout CA, Chikaraishi DM, Tank AW.** Induction of tyrosine hydroxylase protein and a transgene containing tyrosine hydroxylase 5′ flanking sequences by stress in mouse adrenal gland. *J Neurochem* 68: 1071–1077, 1997.
467. **Osterhout CA, Sterling CR, Chikaraishi DM, Tank AW.** Induction of tyrosine hydroxylase in the locus coeruleus of transgenic mice in response to stress or nicotine treatment: lack of activation of tyrosine hydroxylase promoter activity. *J Neurochem* 94: 731–741, 2005.
468. **Otten U, Paravicini U, Oesch F, Thoenen H.** Time requirement for the single steps of trans-synaptic induction of tyrosine hydroxylase in the peripheral sympathetic nervous system. *Naunyn-Schmiedeberg's Arch Pharmacol* 280: 117–127, 1973.
469. **Otten U, Thoenen H.** Circadian rhythm of tyrosine hydroxylase induction by short-term cold stress: modulatory action of glucocorticoids in newborn and adult rats. *Proc Natl Acad Sci USA* 72: 1415–1419, 1975.
470. **Pacak K, Aguilera G, Sabban EL, Kvetnansky R.** *Stress: Current Neuroendocrine and Genetic Approaches*. New York: NY Acad. Sci., 2004, p. 590.
471. **Pacak K, Armando I, Fukuhara K, Kvetnansky R, Palkovits M, Kopin IJ, Goldstein DS.** Noradrenergic activation in the paraventricular nucleus during acute and chronic immobilization stress in rats: an in vivo microdialysis study. *Brain Res* 589: 91–96, 1992.
472. **Pacak K, Baffi JS, Kvetnansky R, Goldstein DS, Palkovits M.** Stressor-specific activation of catecholaminergic systems: implications for stress-related hypothalamic-pituitary-adrenocortical responses. *Adv Pharmacol* 42: 561–564, 1998.
473. **Pacak K, Keiser HR, Eisenhofer G.** Pheochromocytoma In: *Textbook of Endocrinology*, edited by DeGroot LJ, Jameson LJ. Philadelphia, PA: Elsevier Saunders, 2005, p. 2501–2534.
474. **Pacak K, Palkovits M.** Stressor specificity of central neuroendocrine responses: implications for stress-related disorders. *Endocr Rev* 22: 502–548, 2001.
475. **Pacak K, Palkovits M, Kopin IJ, Goldstein DS.** Stress-induced norepinephrine release in the hypothalamic paraventricular nucleus and pituitary-adrenocortical and sympathoadrenal activity: in vivo microdialysis studies. *Front Neuroendocrinol* 16: 89–150, 1995.
476. **Pacak K, Palkovits M, Kvetnansky R, Kopin IJ, Goldstein DS.** Stress-induced norepinephrine release in the paraventricular nucleus of rats with brainstem hemisections: a microdialysis study. *Neuroendocrinology* 58: 196–201, 1993.
477. **Pacak K, Palkovits M, Kvetnansky R, Yadid G, Kopin IJ, Goldstein DS.** Effects of various stressors on in vivo norepinephrine release in the hypothalamic paraventricular nucleus and on the pituitary-adrenocortical axis. *Ann NY Acad Sci* 771: 115–130, 1995.
478. **Pacak K, Palkovits M, Makino S, Kopin IJ, Goldstein DS.** Brainstem hemisection decreases corticotropin-releasing hormone mRNA in the paraventricular nucleus but not in the central amygdaloid nucleus. *J Neuroendocrinol* 8: 543–551, 1996.
479. **Pacak K, Palkovits M, Yadid G, Kvetnansky R, Kopin IJ, Goldstein DS.** Heterogeneous neurochemical responses to differ-

ent stressors: a test of Selye's doctrine of nonspecificity. *Am J Physiol Regul Integr Comp Physiol* 275: R1247–R1255, 1998.
480. **Paivarinta H, Pickel VM, Eranko L, Joh TH.** Glucocorticoid-induced PNMT-immunoreactive sympathetic cells in the superior cervical ganglion of the rat. *J Electron Microsc Tech* 12: 389–396, 1989.
481. **Paivarinta H, Soinila S, Eranko O, Joh TH.** Effect of hydrocortisone on immunohistochemically demonstrable phenylethanolamine-*N*-methyltransferase in cultures of embryonic and postnatal superior cervical ganglia. *Histochemistry* 81: 247–252, 1984.
482. **Palkovits M.** Interconnections between the neuroendocrine hypothalamus and the central autonomic system. Geoffrey Harris Memorial Lecture, Kitakyushu, Japan, October 1998. *Front Neuroendocrinol* 20: 270–295, 1999.
483. **Palkovits M.** Locus coeruleus. In: *Advances in Cellular Neurobiology*, edited by Fedoroff S, Hertz L. New York: Taylor & Francis, 1983, p. 81–103.
484. **Palkovits M.** Stress-related central neuronal regulatory circuits. In: *Stress: Neural, Endocrine and Molecular Studies*, edited by McCarty R, Aguillera G, Sabban EL, Kvetnansky R. London: Taylor and Francis, 2002, p. 1–11.
485. **Palkovits M, Baffi JS, Dvori S.** Neuronal organization of stress response. Pain-induced c-fos expression in brain stem catecholaminergic cell groups. *Ann NY Acad Sci* 771: 313–326, 1995.
486. **Palkovits M, Baffi JS, Pacak K.** The role of ascending neuronal pathways in stress-induced release of noradrenaline in the hypothalamic paraventricular nucleus of rats. *J Neuroendocrinol* 11: 529–539, 1999.
487. **Palkovits M, Baffi JS, Pacak K.** Stress-induced Fos-like immunoreactivity in the pons and the medulla oblongata of rats. *Stress* 1: 155–168, 1997.
488. **Palkovits M, Leranth C, Zaborszky L, Brownstein MJ.** Electron microscopic evidence of direct neuronal connections from the lower brain stem to the median eminence. *Brain Res* 136: 339–344, 1977.
489. **Palkovits M, Mezey E, Skirboll LR, Hokfelt T.** Adrenergic projections from the lower brainstem to the hypothalamic paraventricular nucleus, the lateral hypothalamic area and the central nucleus of the amygdala in rats. *J Chem Neuroanat* 5: 407–415, 1992.
490. **Palkovits M, Zaborszky L.** Neuroanatomy of central cardiovascular control. Nucleus tractus solitarii: afferent and efferent neuronal connections in relation to the baroreceptor reflex arc. *Prog Brain Res* 47: 9–34, 1977.
491. **Palkovits M, Zaborszky L, Feminger A, Mezey E, Fekete MI, Herman JP, Kanyicska B, Szabo D.** Noradrenergic innervation of the rat hypothalamus: experimental biochemical and electron microscopic studies. *Brain Res* 191: 161–171, 1980.
492. **Pan B, Castro-Lopes JM, Coimbra A.** Central afferent pathways conveying nociceptive input to the hypothalamic paraventricular nucleus as revealed by a combination of retrograde labeling and c-*fos* activation. *J Comp Neurol* 413: 129–145, 1999.
493. **Papanikolaou NA, Sabban EL.** Ability of Egr1 to activate tyrosine hydroxylase transcription in PC12 cells. Cross-talk with AP-1 factors. *J Biol Chem* 275: 26683–26689, 2000.
494. **Papanikolaou NA, Sabban EL.** Sp1/Egr1 motif: a new candidate in the regulation of rat tyrosine hydroxylase gene transcription by immobilization stress. *J Neurochem* 73: 433–436, 1999.
495. **Parareda A, Villaescusa JC, Sanchez de Toledo J, Gallego S.** New splicing variants for human tyrosine hydroxylase gene with possible implications for the detection of minimal residual disease in patients with neuroblastoma. *Neurosci Lett* 336: 29–32, 2003.
496. **Pardon MC, Ma S, Morilak DA.** Chronic cold stress sensitizes brain noradrenergic reactivity and noradrenergic facilitation of the HPA stress response in Wistar Kyoto rats. *Brain Res* 971: 55–65, 2003.
497. **Parizkova J, Kvetnansky R.** Catecholamine metabolism and compositional growth in exercised and hypokinetic male rats. In: *Catecholamines and Stress: Recent Advances*, edited by Usdin E, Kvetnansky R, Kopin IJ. New York: Elsevier, 1980, p. 355–358.
498. **Parsadaniantz SM, Gaillet S, Malaval F, Lenoir V, Batsche E, Barbanel G, Gardier A, Terlain B, Jacquot C, Szafarczyk A.** Lesions of the afferent catecholaminergic pathways inhibit the temporal activation of the CRH and POMC gene expression and ACTH release induced by human interleukin-1beta in the male rat. *Neuroendocrinology* 62: 586–595, 1995.
499. **Partanen M, Waller SB, London ED, Hervonen A.** Indices of neurotransmitter synthesis and release in aging sympathetic nervous system. *Neurobiol Aging* 6: 227–232, 1985.
500. **Patronas P, Horowitz M, Simon E, Gerstberger R.** Differential stimulation of c-*fos* expression in hypothalamic nuclei of the rat brain during short-term heat acclimation and mild dehydration. *Brain Res* 798: 127–139, 1998.
501. **Patterson-Buckendahl P, Kubovcakova L, Krizanova O, Pohorecky LA, Kvetnansky R.** Ethanol consumption increases rat stress hormones and adrenomedullary gene expression. *Alcohol* 37: 157–166, 2005.
502. **Pendleton RG, Gessner G, Sawyer J.** Studies on the distribution of phenylethanolamine *N*-methyltransferase and epinephrine in the rat. *Res Commun Chem Pathol Pharmacol* 21: 315–325, 1978.
503. **Petrov T, Krukoff TL, Jhamandas JH.** Branching projections of catecholaminergic brainstem neurons to the paraventricular hypothalamic nucleus and the central nucleus of the amygdala in the rat. *Brain Res* 609: 81–92, 1993.
504. **Petrovic VM, Janic-Sibalic V.** Catecholamine synthesizing and degrading enzymes in the heat stressed or adapted rats. In: *Catecholamines and Stress: Recent Advances*, edited by Usdin E, Kvetnansky R, Kopin IJ. New York: Elsevier North Holland, 1980, p. 365–370.
505. **Peyronnet J, Poncet L, Denoroy L, Pequignot JM, Lagercrantz H, Dalmaz Y.** Plasticity in the phenotypic expression of catecholamines and vasoactive intestinal peptide in adult rat superior cervical and stellate ganglia after long-term hypoxia in vivo. *Neuroscience* 91: 1183–1194, 1999.
506. **Pezzone MA, Lee WS, Hoffman GE, Pezzone KM, Rabin BS.** Activation of brainstem catecholaminergic neurons by conditioned and unconditioned aversive stimuli as revealed by c-Fos immunoreactivity. *Brain Res* 608: 310–318, 1993.
507. **Pezzone MA, Lee WS, Hoffman GE, Rabin BS.** Induction of c-Fos immunoreactivity in the rat forebrain by conditioned and unconditioned aversive stimuli. *Brain Res* 597: 41–50, 1992.
508. **Pfeifer K, Boe SP, Rong Q, Ebert SN.** Generating mouse models for studying the function and fate of intrinsic cardiac adrenergic cells. *Ann NY Acad Sci* 1018: 418–423, 2004.
509. **Pfeifer WD, Davis LC.** Modification of the adrenal stress response by age and prior experience. *Exp Aging Res* 2: 27–36, 1976.
510. **Pieribone VA, Aston-Jones G, Bohn MC.** Adrenergic and nonadrenergic neurons in the C1 and C3 areas project to locus coeruleus: a fluorescent double labeling study. *Neurosci Lett* 85: 297–303, 1988.
511. **Pohorecky LA, Blakley GG, Kubovcakova L, Krizanova O, Patterson-Buckendahl P, Kvetnansky R.** Social hierarchy affects gene expression for catecholamine biosynthetic enzymes in rat adrenal glands. *Neuroendocrinology* 80: 42–51, 2004.
512. **Pollard HB, Caohuy H, Minton AP, Srivastava M.** Synexin (annexin VII) hypothesis for Ca^{2+}/GTP-regulated exocytosis. *Adv Pharmacol* 42: 81–87, 1998.
513. **Polymeropoulos MH, Xiao H, Rath DS, Merril CR.** Tetranucleotide repeat polymorphism at the human tyrosine hydroxylase gene (TH). *Nucleic Acids Res* 19: 3753, 1991.
514. **Pompeiano M, d'Ascanio P, Centini C, Pompeiano O, Balaban E.** Short-term (Fos) and long-term (FRA) protein expression in rat locus coeruleus neurons during the neurolab mission: contribution of altered gravitational fields, stress, and other factors. *Neuroscience* 115: 111–123, 2002.
515. **Popova NK, Maslova LN, Morosova EA, Bulygina VV, Seif I.** MAO-A knockout attenuates adrenocortical response to various kinds of stress. *Psychoneuroendocrinology* 31: 179–186, 2006.
516. **Popova NK, Voitenko NN, Maslova LN.** The effect of emotional stress and corticosterone on the activity of monoaminoxidase in the rat brain. *Vopr Med Khim* 35: 114–119, 1989.
517. **Portillo F, Carrasco M, Vallo JJ.** Separate populations of neurons within the paraventricular hypothalamic nucleus of the rat project to vagal and thoracic autonomic preganglionic levels and express c-Fos protein induced by lithium chloride. *J Chem Neuroanat* 14: 95–102, 1998.

518. **Porzgen P, Bonisch H, Bruss M.** Molecular cloning and organization of the coding region of the human norepinephrine transporter gene. *Biochem Biophys Res Commun* 215: 1145–1150, 1995.
519. **Powell JF, Boni C, Lamouroux A, Craig IW, Mallet J.** Assignment of the human tyrosine hydroxylase gene to chromosome 11. *FEBS Lett* 175: 37–40, 1984.
520. **Prewitt CM, Herman JP.** Hypothalamo-pituitary-adrenocortical regulation following lesions of the central nucleus of the amygdala. *Stress* 1: 263–280, 1997.
521. **Prud'homme MJ, Houdeau E, Serghini R, Tillet Y, Schemann M, Rousseau JP.** Small intensely fluorescent cells of the rat paracervical ganglion synthesize adrenaline, receive afferent innervation from postganglionic cholinergic neurones, and contain muscarinic receptors. *Brain Res* 821: 141–149, 1999.
522. **Pullar CE, Rizzo A, Isseroff RR.** Beta-adrenergic receptor antagonists accelerate skin wound healing: evidence for a catecholamine synthesis network in the epidermis. *J Biol Chem* 281: 21225–21235, 2006.
523. **Pyner S, Coote JH.** Evidence that sympathetic preganglionic neurones are arranged in target-specific columns in the thoracic spinal cord of the rat. *J Comp Neurol* 342: 15–22, 1994.
524. **Pyner S, Coote JH.** Rostroventrolateral medulla neurons preferentially project to target-specified sympathetic preganglionic neurons. *Neuroscience* 83: 617–631, 1998.
525. **Quillen EW Jr, Cowley AW Jr.** Influence of volume changes on osmolality-vasopressin relationships in conscious dogs. *Am J Physiol Heart Circ Physiol* 244: H73–H79, 1983.
526. **Rando TA, Bowers CW, Zigmond RE.** Localization of neurons in the rat spinal cord which project to the superior cervical ganglion. *J Comp Neurol* 196: 73–83, 1981.
526a. **Rani SC, Elango N, Wang SS, Kobayashi K, Strong R.** Identification of an AP-1-like sequence as the glucocorticoid response element in the rat tyrosine hydroxylase gene. *Mol Pharmacol* 2008.
527. **Rao F, Zhang L, Wessel J, Zhang K, Wen G, Kennedy BP, Rana BK, Das M, Rodriguez-Flores JL, Smith DW, Cadman PE, Salem RM, Mahata SK, Schork NJ, Taupenot L, Ziegler MG, O'Connor DT.** Tyrosine hydroxylase, the rate-limiting enzyme in catecholamine biosynthesis: discovery of common human genetic variants governing transcription, autonomic activity, and blood pressure in vivo. *Circulation* 116: 993–1006, 2007.
528. **Rao F, Zhang L, Wessel J, Zhang K, Wen G, Kennedy BP, Rana BK, Das M, Rodriguez JL, Smith DW, Cadman PE, Salem RM, Mahata SK, Schork NJ, Taupenot L, Ziegler MG, O'Connor DT.** Adrenergic polymorphism and the human stress response. *Ann NY Acad Sci* 1148: 282–296, 2008.
529. **Richard F, Faucon-Biguet N, Labatut R, Rollet D, Mallet J, Buda M.** Modulation of tyrosine hydroxylase gene expression in rat brain and adrenals by exposure to cold. *J Neurosci Res* 20: 32–37, 1988.
530. **Rinaman L.** Visceral sensory inputs to the endocrine hypothalamus. *Front Neuroendocrinol* 28: 50–60, 2007.
531. **Ritter S, Bugarith K, Dinh TT.** Immunotoxic destruction of distinct catecholamine subgroups produces selective impairment of glucoregulatory responses and neuronal activation. *J Comp Neurol* 432: 197–216, 2001.
532. **Ritter S, Dinh TT, Li AJ.** Hindbrain catecholamine neurons control multiple glucoregulatory responses. *Physiol Behav* 89: 490–500, 2006.
533. **Ritter S, Llewellyn-Smith I, Dinh TT.** Subgroups of hindbrain catecholamine neurons are selectively activated by 2-deoxy-D-glucose induced metabolic challenge. *Brain Res* 805: 41–54, 1998.
534. **Roder S, Ciriello J.** Innervation of the amygdaloid complex by catecholaminergic cell groups of the ventrolateral medulla. *J Comp Neurol* 332: 105–122, 1993.
535. **Roe DF, Craviso GL, Waymire JC.** Nicotinic stimulation modulates tyrosine hydroxylase mRNA half-life and protein binding to the 3′UTR in a manner that requires transcription. *Brain Res* 120: 91–102, 2004.
536. **Roffman M, Freedman LS, Goldstein DS.** The effect of acute and chronic swim stress on dopamine-beta-hydroxylase activity. *Life Sci* 12: 369–376, 1973.
537. **Ross CA, Ruggiero DA, Joh TH, Park DH, Reis DJ.** Rostral ventrolateral medulla: selective projections to the thoracic autonomic cell column from the region containing C1 adrenaline neurons. *J Comp Neurol* 228: 168–185, 1984.
538. **Ross CA, Ruggiero DA, Reis DJ.** Projections from the nucleus tractus solitarii to the rostral ventrolateral medulla. *J Comp Neurol* 242: 511–534, 1985.
539. **Ross ME, Evinger MJ, Hyman SE, Carroll JM, Mucke L, Comb M, Reis DJ, Joh TH, Goodman HM.** Identification of a functional glucocorticoid response element in the phenylethanolamine *N*-methyltransferase promoter using fusion genes introduced into chromaffin cells in primary culture. *J Neurosci* 10: 520–530, 1990.
540. **Rowan RA, Billingham ME.** Myocardial innervation in long-term heart transplant survivors: a quantitative ultrastructural survey. *J Heart Transplant* 7: 448–452, 1988.
541. **Ruggiero DA, Ross CA, Anwar M, Park DH, Joh TH, Reis DJ.** Distribution of neurons containing phenylethanolamine *N*-methyltransferase in medulla and hypothalamus of rat. *J Comp Neurol* 239: 127–154, 1985.
542. **Rusnak M, Jelokova J, Vietor I, Sabban EL, Kvetnansky R.** Different effects of insulin and 2-deoxy-D-glucose administration on tyrosine hydroxylase gene expression in the locus coeruleus and the adrenal medulla in rats. *Brain Res Bull* 46: 447–452, 1998.
543. **Rusnak M, Kvetnansky R, Jelokova J, Palkovits M.** Effect of novel stressors on gene expression of tyrosine hydroxylase and monoamine transporters in brainstem noradrenergic neurons of long-term repeatedly immobilized rats. *Brain Res* 899: 20–35, 2001.
544. **Rusnak M, Zorad S, Buckendahl P, Sabban EL, Kvetnansky R.** Tyrosine hydroxylase mRNA levels in locus ceruleus of rats during adaptation to long-term immobilization stress exposure. *Mol Chem Neuropathol* 33: 249–258, 1998.
545. **Saavedra JM, Kvetnansky R, Kopin IJ.** Adrenaline, noradrenaline and dopamine levels in specific brain stem areas of acutely immobilized rats. *Brain Res* 160: 271–280, 1979.
546. **Saavedra JM, Torda T.** Increased brain stem and decreased hypothalamic adrenaline-forming enzyme after acute and repeated immobilization stress in the rat. *Neuroendocrinology* 31: 142–146, 1980.
547. **Sabban E, Serova L, Schilt N, Veerasirikul M, Gueorguiev V.** Role of ACTH in regulation of gene expresion of norepinephrine biosynthetic enzymes in superior cervical ganglia by stress. *Front Neuroendocrinol* 27: 61, 2006.
548. **Sabban EL.** Catecholamines in stress: molecular mechanisms of gene expression. *Endocr Regul* 41: 61–73, 2007.
549. **Sabban EL, Hebert MA, Liu X, Nankova B, Serova L.** Differential effects of stress on gene transcription factors in catecholaminergic systems. *Ann NY Acad Sci* 1032: 130–140, 2004.
550. **Sabban EL, Hiremagalur B, Nankova B, Kvetnansky R.** Molecular biology of stress-elicited induction of catecholamine biosynthetic enzymes. *Ann NY Acad Sci* 771: 327–338, 1995.
551. **Sabban EL, Kvetnansky R.** Stress-triggered activation of gene expression in catecholaminergic systems: dynamics of transcriptional events. *Trends Neurosci* 24: 91–98, 2001.
552. **Sabban EL, Liu X, Serova L, Gueorguiev V, Kvetnansky R.** Stress triggered changes in gene expression in adrenal medulla: transcriptional responses to acute and chronic stress. *Cell Mol Neurobiol* 26: 845–856, 2006.
553. **Sabban EL, Nankova B, Hiremagalur B, Rusnak M, Viskupic E, Kvetnansky R.** Molecular mechanisms of immobilization stress elicited rise in expression of genes for adrenal catecholamine biosynthetic enzymes, neuropeptide Y and proenkephalin. In: *Stress: Molecular, Genetic and Neurobiological Advances*, edited by McCarty R, Aguillera G, Sabban EL, Kvetnansky R. New York: Gordon and Breach, 1996, p. 611–628.
554. **Sabban EL, Nankova BB.** Multiple pathways in regulation of dopamine beta-hydroxylase. *Adv Pharmacol* 42: 53–56, 1998.
555. **Sabban EL, Nankova BB, Serova LI, Kvetnansky R, Liu X.** Molecular regulation of gene expression of catecholamine biosynthetic enzymes by stress: sympathetic ganglia versus adrenal medulla. *Ann NY Acad Sci* 1018: 370–377, 2004.
556. **Sabban EL, Serova LI.** Influence of prior experience with homotypic or heterotypic stressor on stress reactivity in catecholaminergic systems. *Stress* 10: 137–143, 2007.

557. **Sabol SZ, Hu S, Hamer D.** A functional polymorphism in the monoamine oxidase A gene promoter. *Hum Genet* 103: 273–279, 1998.
558. **Sacchetti P, Brownschidle LA, Granneman JG, Bannon MJ.** Characterization of the 5′-flanking region of the human dopamine transporter gene. *Brain Res* 74: 167–174, 1999.
559. **Sands SA, Strong R, Corbitt J, Morilak DA.** Effects of acute restraint stress on tyrosine hydroxylase mRNA expression in locus coeruleus of Wistar and Wistar-Kyoto rats. *Brain Res* 75: 1–7, 2000.
560. **Saper CB.** The central autonomic nervous system: conscious visceral perception and autonomic pattern generation. *Annu Rev Neurosci* 25: 433–469, 2002.
561. **Saper CB.** Central autonomic system. In: *The Rat Nervous System*, edited by Paxinos G. San Diego, CA: Academic, 1995, p. 107–135.
562. **Sawchenko PE.** Effects of catecholamine-depleting medullary knife cuts on corticotropin-releasing factor and vasopressin immunoreactivity in the hypothalamus of normal and steroid-manipulated rats. *Neuroendocrinology* 48: 459–470, 1988.
563. **Sawchenko PE, Bohn MC.** Glucocorticoid receptor-immunoreactivity in C1, C2, and C3 adrenergic neurons that project to the hypothalamus or to the spinal cord in the rat. *J Comp Neurol* 285: 107–116, 1989.
564. **Sawchenko PE, Brown ER, Chan RK, Ericsson A, Li HY, Roland BL, Kovacs KJ.** The paraventricular nucleus of the hypothalamus and the functional neuroanatomy of visceromotor responses to stress. *Prog Brain Res* 107: 201–222, 1996.
565. **Sawchenko PE, Li HY, Ericsson A.** Circuits and mechanisms governing hypothalamic responses to stress: a tale of two paradigms. *Prog Brain Res* 122: 61–78, 2000.
566. **Sawchenko PE, Swanson LW.** Immunohistochemical identification of neurons in the paraventricular nucleus of the hypothalamus that project to the medulla or to the spinal cord in the rat. *J Comp Neurol* 205: 260–272, 1982.
567. **Sawchenko PE, Swanson LW.** The organization of noradrenergic pathways from the brainstem to the paraventricular and supraoptic nuclei in the rat. *Brain Res* 257: 275–325, 1982.
568. **Schalling M, Franco-Cereceda A, Hemsen A, Dagerlind A, Seroogy K, Persson H, Hokfelt T, Lundberg JM.** Neuropeptide Y and catecholamine synthesizing enzymes and their mRNAs in rat sympathetic neurons and adrenal glands: studies on expression, synthesis and axonal transport after pharmacological and experimental manipulations using hybridization techniques and radioimmunoassay. *Neuroscience* 41: 753–766, 1991.
569. **Schomig A, Fischer S, Kurz T, Richardt G, Schomig E.** Non-exocytotic release of endogenous noradrenaline in the ischemic and anoxic rat heart: mechanism and metabolic requirements. *Circ Res* 60: 194–205, 1987.
570. **Schuldiner S.** A molecular glimpse of vesicular monoamine transporters. *J Neurochem* 62: 2067–2078, 1994.
571. **Selye H.** Stress and the general adaptation syndrome. *Br Med J* 4667: 1383–1392, 1950.
572. **Selye H.** *Stress Without Distress*. New York: New America Library, 1974.
573. **Selye H.** A syndrome produced by diverse nocious agents. *Nature* 138: 32, 1936.
574. **Senba E, Matsunaga K, Tohyama M, Noguchi K.** Stress-induced c-*fos* expression in the rat brain: activation mechanism of sympathetic pathway. *Brain Res Bull* 31: 329–344, 1993.
575. **Seo H, Yang C, Kim HS, Kim KS.** Multiple protein factors interact with the *cis*-regulatory elements of the proximal promoter in a cell-specific manner and regulate transcription of the dopamine beta-hydroxylase gene. *J Neurosci* 16: 4102–4112, 1996.
576. **Serova L, Danailov E, Chamas F, Sabban EL.** Nicotine infusion modulates immobilization stress-triggered induction of gene expression of rat catecholamine biosynthetic enzymes. *J Pharmacol Exp Ther* 291: 884–892, 1999.
577. **Serova L, Rivkin M, Nakashima A, Sabban EL.** Estradiol stimulates gene expression of norepinephrine biosynthetic enzymes in rat locus coeruleus. *Neuroendocrinology* 75: 193–200, 2002.
578. **Serova L, Sabban EL, Zangen A, Overstreet DH, Yadid G.** Altered gene expression for catecholamine biosynthetic enzymes and stress response in rat genetic model of depression. *Brain Res* 63: 133–138, 1998.
579. **Serova LI, Gueorguiev V, Cheng SY, Sabban EL.** Adrenocorticotropic hormone elevates gene expression for catecholamine biosynthesis in rat superior cervical ganglia and locus coeruleus by an adrenal independent mechanism. *Neuroscience.* 153: 1380–1389, 2008.
580. **Serova LI, Maharjan S, Sabban EL.** Estrogen modifies stress response of catecholamine biosynthetic enzyme genes and cardiovascular system in ovariectomized female rats. *Neuroscience* 132: 249–259, 2005.
581. **Serova LI, Nankova B, Kvetnansky R, Sabban EL.** Immobilization stress elevates GTP cyclohydrolase I mRNA levels in rat adrenals predominantly by hormonally mediated mechanisms. *Stress* 1: 135–144, 1997.
582. **Serova LI, Nankova BB, Feng Z, Hong JS, Hutt M, Sabban EL.** Heightened transcription for enzymes involved in norepinephrine biosynthesis in the rat locus coeruleus by immobilization stress. *Biol Psychiatry* 45: 853–862, 1999.
583. **Serova LI, Saez E, Spiegelman BM, Sabban EL.** c-Fos deficiency inhibits induction of mRNA for some, but not all, neurotransmitter biosynthetic enzymes by immobilization stress. *J Neurochem* 70: 1935–1940, 1998.
584. **Sesack SR, Deutch AY, Roth RH, Bunney BS.** Topographical organization of the efferent projections of the medial prefrontal cortex in the rat: an anterograde tract-tracing study with Phaseolus vulgaris leucoagglutinin. *J Comp Neurol* 290: 213–242, 1989.
585. **Shafton AD, Ryan A, Badoer E.** Neurons in the hypothalamic paraventricular nucleus send collaterals to the spinal cord and to the rostral ventrolateral medulla in the rat. *Brain Res* 801: 239–243, 1998.
586. **Shapiro RE, Miselis RR.** The central neural connections of the area postrema of the rat. *J Comp Neurol* 234: 344–364, 1985.
587. **Sharma P, Hingorani A, Jia H, Ashby M, Hopper R, Clayton D, Brown MJ.** Positive association of tyrosine hydroxylase microsatellite marker to essential hypertension. *Hypertension* 32: 676–682, 1998.
588. **Shaskus J, Greco D, Asnani LP, Lewis EJ.** A bifunctional genetic regulatory element of the rat dopamine beta-hydroxylase gene influences cell type specificity and second messenger-mediated transcription. *J Biol Chem* 267: 18821–18830, 1992.
589. **Shaskus J, Zellmer E, Lewis EJ.** A negative regulatory element in the rat dopamine beta-hydroxylase gene contributes to the cell type specificity of expression. *J Neurochem* 64: 52–60, 1995.
590. **Shaver JA, Leon DF, Gray S 3rd, Leonard JJ, Bahnson HT.** Hemodynamic observations after cardiac transplantation. *N Engl J Med* 281: 822–827, 1969.
591. **Shaywitz AJ, Greenberg ME.** CREB: a stimulus-induced transcription factor activated by a diverse array of extracellular signals. *Annu Rev Biochem* 68: 821–861, 1999.
592. **Shih JC.** Cloning, after cloning, and knock-out mice, physiological functions of MAO A and B. *Neurotoxicology* 25: 21–30, 2004.
593. **Shih JC, Chen K.** MAO-A and -B gene knock-out mice exhibit distinctly different behavior. *Neurobiology* 7: 235–246, 1999.
594. **Shih JC, Chen K.** Regulation of MAO-A and MAO-B gene expression. *Curr Med Chem* 11: 1995–2005, 2004.
595. **Shih JC, Chen K, Ridd MJ.** Monoamine oxidase: from genes to behavior. *Annu Rev Neurosci* 22: 197–217, 1999.
596. **Shimizu Y, Sugama S, Degiorgio LA, Cho BP, Joh TH.** Cell-type specific signal transduction and gene regulation via mitogen-activated protein kinase pathway in catecholaminergic neurons by restraint stress. *Neuroscience* 129: 831–839, 2004.
597. **Shokunbi MT, Hrycyshyn AW, Flumerfelt BA.** Spinal projections to the lateral reticular nucleus in the rat: a retrograde labelling study using horseradish peroxidase. *J Comp Neurol* 239: 216–226, 1985.
598. **Silbergeld S, Kvetnansky R, Weise VK, Kopin IJ.** Effect of repeated administration of 2-deoxy-D-glucose or insulin on catecholamine-synthesizing enzymes in rat adrenals. *Biochem Pharmacol* 20: 1763–1768, 1971.
599. **Silberstein SD, Brimijoin S, Molinoff PB, Lemberger L.** Induction of dopamine-β-hydroxylase in rat superior cervical ganglia in organ culture. *J Neurochem* 19: 919–921, 1972.
600. **Simson PG, Weiss JM, Ambrose MJ, Webster A.** Infusion of a monoamine oxidase inhibitor into the locus coeruleus can prevent

stress-induced behavioral depression. *Biol Psychiatry* 21: 724–734, 1986.
601. **Slavikova J, Kuncova J, Reischig J, Dvorakova M.** Catecholaminergic neurons in the rat intrinsic cardiac nervous system. *Neurochem Res* 28: 593–598, 2003.
602. **Smith MA, Brady LS, Glowa J, Gold PW, Herkenham M.** Effects of stress and adrenalectomy on tyrosine hydroxylase mRNA levels in the locus ceruleus by in situ hybridization. *Brain Res* 544: 26–32, 1991.
603. **Smolka MN, Schumann G, Wrase J, Grusser SM, Flor H, Mann K, Braus DF, Goldman D, Buchel C, Heinz A.** Catechol-*O*-methyltransferase val158met genotype affects processing of emotional stimuli in the amygdala and prefrontal cortex. *J Neurosci* 25: 836–842, 2005.
604. **Somogyi J, Llewellyn-Smith IJ.** Patterns of colocalization of GABA, glutamate and glycine immunoreactivities in terminals that synapse on dendrites of noradrenergic neurons in rat locus coeruleus. *Eur J Neurosci* 14: 219–228, 2001.
605. **Stachowiak M, Sebbane R, Stricker EM, Zigmond MJ, Kaplan BB.** Effect of chronic cold exposure on tyrosine hydroxylase mRNA in rat adrenal gland. *Brain Res* 359: 356–359, 1985.
606. **Stachowiak M, Stricker EM, Zigmond MJ, Kaplan BB.** A cholinergic antagonist blocks cold stress-induced alterations in rat adrenal tyrosine hydroxylase mRNA. *Brain Res* 427: 193–195, 1988.
607. **Stachowiak MK, Rigual RJ, Lee PH, Viveros OH, Hong JS.** Regulation of tyrosine hydroxylase and phenylethanolamine *N*-methyltransferase mRNA levels in the sympathoadrenal system by the pituitary-adrenocortical axis. *Brain Res* 427: 275–286, 1988.
608. **Stanford C.** The relation of organ noradrenaline concentration and the fluorescence histochemical reaction. *J Physiol* 245: 108P–109P, 1975.
609. **Stanford SC.** Central noradrenergic neurones and stress. *Pharmacol Ther* 68: 297–242, 1995.
610. **Sterling P, Eyer J.** Allostasis: a new paradigm to explain arousal pathology. In: *Handbook of Life Stress, Cognition and Health*, edited by Fischer S, Reason J. New York: Wiley, 1988, p. 629–649.
611. **Stewart LC, Klinman JP.** Dopamine β-hydroxylase of adrenal chromaffin granules: structure and function. *Annu Rev Biochem* 57: 551–592, 1988.
612. **Stjarne L.** Basic mechanisms and local modulation of nerve impulse-induced secretion of neurotransmitters from individual sympathetic nerve varicosities. *Rev Physiol Biochem Pharmacol* 112: 1–137, 1989.
613. **Stone EA, Freedman LS, Morgano LE.** Brain and adrenal tyrosine hydroxylase activity after chronic footshock stress. *Pharmacol Biochem Behav* 9: 551–553, 1978.
614. **Stone EA, McCarty R.** Adaptation to stress: tyrosine hydroxylase activity and catecholamine release. *Neurosci Biobehav Rev* 7: 29–34, 1983.
615. **Stornetta RL, Guyenet PG.** Distribution of glutamic acid decarboxylase mRNA-containing neurons in rat medulla projecting to thoracic spinal cord in relation to monoaminergic brainstem neurons. *J Comp Neurol* 407: 367–380, 1999.
616. **Stornetta RL, Schreihofer AM, Pelaez NM, Sevigny CP, Guyenet PG.** Preproenkephalin mRNA is expressed by C1 and non-C1 barosensitive bulbospinal neurons in the rostral ventrolateral medulla of the rat. *J Comp Neurol* 435: 111–126, 2001.
617. **Stornetta RL, Sevigny CP, Guyenet PG.** Vesicular glutamate transporter DNPI/VGLUT2 mRNA is present in C1 and several other groups of brainstem catecholaminergic neurons. *J Comp Neurol* 444: 191–206, 2002.
618. **Strack AM, Sawyer WB, Marubio LM, Loewy AD.** Spinal origin of sympathetic preganglionic neurons in the rat. *Brain Res* 455: 187–191, 1988.
619. **Strack AM, Sawyer WB, Platt KB, Loewy AD.** CNS cell groups regulating the sympathetic outflow to adrenal gland as revealed by transneuronal cell body labeling with pseudorabies virus. *Brain Res* 491: 274–296, 1989.
620. **Sullivan RM.** Hemispheric asymmetry in stress processing in rat prefrontal cortex and the role of mesocortical dopamine. *Stress* 7: 131–143, 2004.
621. **Sumal KK, Blessing WW, Joh TH, Reis DJ, Pickel VM.** Synaptic interaction of vagal afferents and catecholaminergic neurons in the rat nucleus tractus solitarius. *Brain Res* 277: 31–40, 1983.
622. **Sun B, Chen X, Xu L, Sterling C, Tank AW.** Chronic nicotine treatment leads to induction of tyrosine hydroxylase in locus ceruleus neurons: the role of transcriptional activation. *Mol Pharmacol* 66: 1011–1021, 2004.
623. **Sun B, Sterling CR, Tank AW.** Chronic nicotine treatment leads to sustained stimulation of tyrosine hydroxylase gene transcription rate in rat adrenal medulla. *J Pharmacol Exp Ther* 304: 575–588, 2003.
624. **Sun MK.** Central neural organization and control of sympathetic nervous system in mammals. *Prog Neurobiol* 47: 157–233, 1995.
625. **Suzuki T, Yamakuni T, Hagiwara M, Ichinose H.** Identification of ATF-2 as a transcriptional regulator for the tyrosine hydroxylase gene. *J Biol Chem* 277: 40768–40774, 2002.
626. **Swanson DJ, Adachi M, Lewis EJ.** The homeodomain protein Arix promotes protein kinase A-dependent activation of the dopamine beta-hydroxylase promoter through multiple elements and interaction with the coactivator cAMP-response element-binding protein-binding protein. *J Biol Chem* 275: 2911–2923, 2000.
627. **Swanson DJ, Zellmer E, Lewis EJ.** AP1 proteins mediate the cAMP response of the dopamine beta-hydroxylase gene. *J Biol Chem* 273: 24065–24074, 1998.
628. **Swanson LW.** The projections of the ventral tegmental area and adjacent regions: a combined fluorescent retrograde tracer and immunofluorescence study in the rat. *Brain Res Bull* 9: 321–353, 1982.
629. **Swanson LW, Kuypers HG.** The paraventricular nucleus of the hypothalamus: cytoarchitectonic subdivisions and organization of projections to the pituitary, dorsal vagal complex, spinal cord as demonstrated by retrograde fluorescence double-labeling methods. *J Comp Neurol* 194: 555–570, 1980.
630. **Swanson LW, Sawchenko PE.** Paraventricular nucleus: a site for the integration of neuroendocrine and autonomic mechanisms. *Neuroendocrinology* 31: 410–417, 1980.
631. **Sykova E.** Extrasynaptic volume transmission and diffusion parameters of the extracellular space. *Neuroscience* 129: 861–876, 2004.
632. **Szafarczyk A, Guillaume V, Conte-Devolx B, Alonso G, Malaval F, Pares-Herbute N, Oliver C, Assenmacher I.** Central catecholaminergic system stimulates secretion of CRH at different sites. *Am J Physiol Endocrinol Metab* 255: E463–E468, 1988.
633. **Sze PY, Hedrick BJ.** Effects of dexamethasone and other glucocorticoid steroids on tyrosine hydroxylase activity in the superior cervical ganglion. *Brain Res* 265: 81–86, 1983.
634. **Tai TC, Claycomb R, Her S, Bloom AK, Wong DL.** Glucocorticoid responsiveness of the rat phenylethanolamine N-methyltransferase gene. *Mol Pharmacol* 61: 1385–1392, 2002.
635. **Tai TC, Claycomb R, Siddall BJ, Bell RA, Kvetnansky R, Wong DL.** Stress-induced changes in epinephrine expression in the adrenal medulla in vivo. *J Neurochem* 101: 1108–1118, 2007.
636. **Tai TC, Wong DL.** Protein kinase A and protein kinase C signaling pathway interaction in phenylethanolamine *N*-methyltransferase gene regulation. *J Neurochem* 85: 816–829, 2003.
637. **Takamine J.** The isolation of the active principle of the suprarenal gland. *J Physiol* 27: 29P–30P, 1901.
638. **Taljanidisz J, Stewart L, Smith AJ, Klinman JP.** Structure of bovine adrenal dopamine beta-monooxygenase, as deduced from cDNA and protein sequencing: evidence that the membrane-bound form of the enzyme is anchored by an uncleaved signal peptide. *Biochemistry* 28: 10054–10061, 1989.
639. **Tanaka M, Kohno Y, Nakagawa R, Ida Y, Takeda S, Nagasaki N.** Time-related differences in noradrenaline turnover in rat brain regions by stress. *Pharmacol Biochem Behav* 16: 315–319, 1982.
640. **Tank AW, Lewis EJ, Chikaraishi DM, Weiner N.** Elevation of RNA coding for tyrosine hydroxylase in rat adrenal gland by reserpine treatment and exposure to cold. *J Neurochem* 45: 1030–1033, 1985.
641. **Tank AW, Xu L, Chen X, Radcliffe P, Sterling CR.** Post-transcriptional regulation of tyrosine hydroxylase expression in adrenal medulla and brain. *Ann NY Acad Sci* 1148: 238–248, 2008.

642. **Tavares I, Lima D, Coimbra A.** Neurons in the superficial dorsal horn of the rat spinal cord projecting to the medullary ventrolateral reticular formation express c-fos after noxious stimulation of the skin. *Brain Res* 623: 278–286, 1993.
643. **Taylor K, Lester E, Hudson B, Ritter S.** Hypothalamic and hindbrain NPY, AGRP and NE increase consummatory feeding responses. *Physiol Behav* 90: 744–750, 2007.
644. **Ter Horst GJ, de Boer P, Luiten PG, van Willigen JD.** Ascending projections from the solitary tract nucleus to the hypothalamus. A *Phaseolus vulgaris* lectin tracing study in the rat. *Neuroscience* 31: 785–797, 1989.
645. **Ter Horst GJ, Luiten PG.** The projections of the dorsomedial hypothalamic nucleus in the rat. *Brain Res Bull* 16: 231–248, 1986.
646. **Ter Horst GJ, Luiten PG, Kuipers F.** Descending pathways from hypothalamus to dorsal motor vagus and ambiguus nuclei in the rat. *J Auton Nerv Syst* 11: 59–75, 1984.
647. **Thoa NB, Tizabi Y, Kopin IJ, Maengwyn-Davies GD.** Alterations of mouse adrenal medullary catecholamines and enzymes in response to attack: effect of pre- and post-treatment with phenobarbital. *Psychopharmacology* 51: 53–57, 1976.
648. **Thoenen H.** Comparison between the effect of neuronal activity and nerve growth factor on the enzymes involved in the synthesis of norepinephrine. *Pharmacol Rev* 24: 255–267, 1972.
649. **Thoenen H.** Induction of tyrosine hydroxylase in peripheral and central adrenergic neurones by cold-exposure of rats. *Nature* 228: 861–862, 1970.
650. **Thoenen H, Mueller RA, Axelrod J.** Trans-synaptic induction of adrenal tyrosine hydroxylase. *J Pharmacol Exp Ther* 169: 249–254, 1969.
651. **Thony B, Auerbach G, Blau N.** Tetrahydrobiopterin biosynthesis, regeneration and functions. *Biochem J* 347: 1–16, 2000.
652. **Thrivikraman KV, Su Y, Plotsky PM.** Patterns of Fos-immunoreactivity in the CNS induced by repeated hemorrhage in conscious rats: correlations with pituitary-adrenal axis activity. *Stress* 2: 145–158, 1997.
653. **Tillinger A, Bruderova V, Kubovcakova L, Zeman M, Kopacek J, Novakova M, Kvetnansky R, Krizanova O.** Gene expression of the phenylethanolamine *N*-methyltransferase is differently modulated in cardiac atria and ventricles. *Gen Physiol Biophys* 25: 355–364, 2006.
654. **Tillinger A, Novakova M, Pavlovicova M, Lacinova L, Zatovicova M, Pastorekova S, Krizanova O, Kvetnansky R.** Modulation by 6-hydroxydopamine of expression of the phenylethanolamine *N*-methyltransferase (PNMT) gene in the rat heart during immobilization stress. *Stress* 9: 207–213, 2006.
655. **Torda T, Culman J, Petrikova M.** Distribution of phenylethanolamine-*N*-methyltransferase in the rat heart: effect of 6-hydroxydopamine. *Eur J Pharmacol* 141: 305–308, 1987.
656. **Toth ZE, Gallatz K, Fodor M, Palkovits M.** Decussations of the descending paraventricular pathways to the brainstem and spinal cord autonomic centers. *J Comp Neurol* 414: 255–266, 1999.
657. **Trendelenburg U.** The extraneuronal uptake and metabolism of catecholamines. In: *Handbook of Experimental Pharmacology*, edited by Trendelenburg U. Berlin: Springer-Verlag, 1988, p. 279–319.
658. **Trocme C, Mallet J, Biguet NF.** AP-1 mediates trans-synaptic induction of tyrosine hydroxylase gene expression in adrenal medulla but not in superior cervical ganglia. *J Neurosci Res* 48: 489–498, 1997.
659. **Trocme C, Sarkis C, Hermel JM, Duchateau R, Harrison S, Simonneau M, Al-Shawi R, Mallet J.** CRE and TRE sequences of the rat tyrosine hydroxylase promoter are required for TH basal expression in adult mice but not in the embryo. *Eur J Neurosci* 10: 508–521, 1998.
660. **Tucker DC, Saper CB, Ruggiero DA, Reis DJ.** Organization of central adrenergic pathways. I. Relationships of ventrolateral medullary projections to the hypothalamus and spinal cord. *J Comp Neurol* 259: 591–603, 1987.
661. **Tumer N, Demirel HA, Serova L, Sabban EL, Broxson CS, Powers SK.** Gene expression of catecholamine biosynthetic enzymes following exercise: modulation by age. *Neuroscience* 103: 703–711, 2001.
662. **Tumer N, Hale C, Lawler J, Strong R.** Modulation of tyrosine hydroxylase gene expression in the rat adrenal gland by exercise: effects of age. *Brain Res* 14: 51–56, 1992.
663. **Ulus IH, Wurtman RJ.** Selective response of rat peripheral sympathetic nervous system to various stimuli. *J Physiol* 293: 513–523, 1979.
663a. **Valentino RJ, Van Bockstaele E.** Convergent regulation of locus coeruleus activity as an adaptive response to stress. *Eur J Pharmacol* 2008.
664. **Valles A, Marti O, Armario A.** Long-term effects of a single exposure to immobilization: a c-*fos* mRNA study of the response to the homotypic stressor in the rat brain. *J Neurobiol* 66: 591–602, 2006.
665. **Van Bockstaele EJ, Bajic D, Proudfit H, Valentino RJ.** Topographic architecture of stress-related pathways targeting the noradrenergic locus coeruleus. *Physiol Behav* 73: 273–283, 2001.
666. **Van Bockstaele EJ, Colago EE, Valentino RJ.** Corticotropin-releasing factor-containing axon terminals synapse onto catecholamine dendrites and may presynaptically modulate other afferents in the rostral pole of the nucleus locus coeruleus in the rat brain. *J Comp Neurol* 364: 523–534, 1996.
667. **Van den Pol AN.** Hypothalamic hypocretin (orexin): robust innervation of the spinal cord. *J Neurosci* 19: 3171–3182, 1999.
668. **Van der Gugten J, Palkovits M, Wijnen HL, Versteeg DH.** Regional distribution of adrenaline in rat brain. *Brain Res* 107: 171–175, 1976.
669. **Van der Kooy D, Koda LY, McGinty JF, Gerfen CR, Bloom FE.** The organization of projections from the cortex, amygdala, hypothalamus to the nucleus of the solitary tract in rat. *J Comp Neurol* 224: 1–24, 1984.
670. **Van Eden CG, Buijs RM.** Functional neuroanatomy of the prefrontal cortex: autonomic interactions. *Prog Brain Res* 126: 49–62, 2000.
671. **Vietor I, Rusnak M, Viskupic E, Blazicek P, Sabban EL, Kvetnansky R.** Glucoprivation by insulin leads to trans-synaptic increase in rat adrenal tyrosine hydroxylase mRNA levels. *Eur J Pharmacol* 313: 119–127, 1996.
672. **Viskupic E, Kvetnansky R, Sabban EL, Fukuhara K, Weise VK, Kopin IJ, Schwartz JP.** Increase in rat adrenal phenylethanolamine *N*-methyltransferase mRNA level caused by immobilization stress depends on intact pituitary-adrenocortical axis. *J Neurochem* 63: 808–814, 1994.
673. **Vogt M.** Norepinephrine and epinephrine in the central nervous system. *Pharmacol Rev* 6: 31–32, 1954.
674. **Von Euler U, Ikkos D, Luft R.** Adrenaline excretion during resting conditions and after insulin in adrenalectomized human subjects. *Acta Endocrinol* 38: 441–448, 1961.
675. **Von Euler US.** A specific sympathomimetic ergone in adrenergic nerve fibres (sympathin) and its relations to adrenaline and noradrenaline. *Acta Physiol Scand* 12: 73–96, 1946.
676. **Wallace DM, Magnuson DJ, Gray TS.** The amygdalo-brainstem pathway: selective innervation of dopaminergic, noradrenergic and adrenergic cells in the rat. *Neurosci Lett* 97: 252–258, 1989.
677. **Wang J, Bannon MJ.** Sp1 and Sp3 activate transcription of the human dopamine transporter gene. *J Neurochem* 93: 474–482, 2005.
678. **Wang P, Kitayama I, Nomura J.** Tyrosine hydroxylase gene expression in the locus coeruleus of depression-model rats and rats exposed to short- and long-term forced walking stress. *Life Sci* 62: 2083–2092, 1998.
679. **Warthan MD, Freeman JG, Loesser KE, Lewis CW, Hong M, Conway CM, Stewart JK.** Phenylethanolamine *N*-methyl transferase expression in mouse thymus and spleen. *Brain Behav Immun* 16: 493–499, 2002.
680. **Watanabe Y, McKittrick CR, Blanchard DC, Blanchard RJ, McEwen BS, Sakai RR.** Effects of chronic social stress on tyrosine hydroxylase mRNA and protein levels. *Brain Res* 32: 176–180, 1995.
681. **Watson F, Deavall DG, Macro JA, Kiernan R, Dimaline R.** Transcriptional activation of vesicular monoamine transporter 2 in the pre-B cell line Ea3.123. *Biochem J* 337: 193–199, 1999.
682. **Wei J, Ramchand CN, Hemmings GP.** Possible association of catecholamine turnover with the polymorphic (TCAT)n repeat in

the first intron of the human tyrosine hydroxylase gene. *Life Sci* 61: 1341–1347, 1997.
683. **Weinshilboum R, Axelrod J.** Serum dopamine-beta-hydroxylase activity. *Circ Res* 28: 307–315, 1971.
684. **Weinshilboum RM, Kvetnansky R, Axelrod J, Kopin IJ.** Elevation of serum dopamine-beta-hydroxylase activity with forced immobilization. *Nat New Biol* 230: 287–288, 1971.
685. **Wesselingh SL, Li YW, Blessing WW.** PNMT-containing neurons in the rostral medulla oblongata (C1, C3 groups) are transneuronally labelled after injection of herpes simplex virus type 1 into the adrenal gland. *Neurosci Lett* 106: 99–104, 1989.
686. **Westlund KN, Bowker RM, Ziegler MG, Coulter JD.** Noradrenergic projections to the spinal cord of the rat. *Brain Res* 263: 15–31, 1983.
687. **Winkler H, Apps DK, Fischer-Colbrie R.** The molecular function of adrenal chromaffin granules: established facts and unresolved topics. *Neuroscience* 18: 261–290, 1986.
688. **Wolfovitz E, Grossman E, Folio CJ, Keiser HR, Kopin IJ, Goldstein DS.** Derivation of urinary dopamine from plasma dihydroxyphenylalanine in humans. *Clin Sci* 84: 549–557, 1993.
689. **Wong DL.** Epinephrine biosynthesis: hormonal and neural control during stress. *Cell Mol Neurobiol* 26: 891–900, 2006.
690. **Wong DL, Anderson LJ, Tai TC.** Cholinergic and peptidergic regulation of phenylethanolamine *N*-methyltransferase gene expression. *Ann NY Acad Sci* 971: 19–26, 2002.
691. **Wong DL, Her S, Tai TC, Bell RA, Rusnak M, Farkas R, Kvetnansky R, Shih JC.** Stress-induced expression of phenylethanolamine *N*-methytransferase: normal and knock out animals. In: *Stress Neural, Endocrine and Molecular Studies*, edited by McCarty R, Aguilera G, Sabban EL, Kvetnansky R. London: Taylor and Francis, 2002, p. 129–135.
692. **Wong DL, Lesage A, Siddall B, Funder JW.** Glucocorticoid regulation of phenylethanolamine *N*-methyltransferase in vivo. *FASEB J* 6: 3310–3315, 1992.
693. **Wong DL, Siddall BJ, Ebert SN, Bell RA, Her S.** Phenylethanolamine *N*-methyltransferase gene expression: synergistic activation by Egr-1, AP-2 and the glucocorticoid receptor. *Brain Res* 61: 154–161, 1998.
694. **Wong DL, Tai TC, Wong-Faull DC, Claycomb R, Kvetnansky R.** Adrenergic responses to stress: Transcriptionaln and post-transcriptional changes. *Ann NY Acad Sci* 1148: 249–256, 2008.
695. **Wong DL, Tai TC, Wong-Faull DC, Claycomb R, Kvetnansky R.** Genetic mechanisms for adrenergic control during stress. *Ann NY Acad Sci* 1018: 387–397, 2004.
696. **Wong DL, Tank AW.** Stress-induced catecholaminergic function: transcriptional and post-transcriptional control. *Stress* 10: 121–130, 2007.
697. **Wong DL, Zager EL, Ciaranello RD.** Effects of hypophysectomy and dexamethasone administration on central and peripheral *S*-adenosylmethionine levels. *J Neurosci* 2: 758–764, 1982.
698. **Wurtman RJ, Axelrod J.** Control of enzymatic synthesis of adrenaline in the adrenal medulla by adrenal cortical steroids. *J Biol Chem* 241: 2301–2305, 1966.
699. **Xu L, Chen X, Sun B, Sterling C, Tank AW.** Evidence for regulation of tyrosine hydroxylase mRNA translation by stress in rat adrenal medulla. *Brain Res* 1158: 1–10, 2007.
700. **Xu W, Liu L, Mooslehner K, Emson PC.** Structural organization of the human vesicular monoamine transporter type-2 gene and promoter analysis using the jelly fish green fluorescent protein as a reporter. *Brain Res* 45: 41–49, 1997.
701. **Yang C, Kim HS, Seo H, Kim CH, Brunet JF, Kim KS.** Paired-like homeodomain proteins, Phox2a and Phox2b, are responsible for noradrenergic cell-specific transcription of the dopamine beta-hydroxylase gene. *J Neurochem* 71: 1813–1826, 1998.
702. **Yang C, Kim HS, Seo H, Kim KS.** Identification and characterization of potential *cis*-regulatory elements governing transcriptional activation of the rat tyrosine hydroxylase gene. *J Neurochem* 71: 1358–1368, 1998.
703. **Yehuda R, McEwen B.** *Biobehavioral Stress Response: Protective and Damaging Effects.* New York: NY Acad. Sci., 2004, p. 331.
704. **Yin JC, Tully T.** CREB and the formation of long-term memory. *Curr Opin Neurobiol* 6: 264–268, 1996.
705. **Young JB, Landsberg L.** Catecholamines and the adrenal medulla. In: *Williams Textbook of Endocrinology*, edited by Wilson JD, Foster DW, Kronenberg HM, Larsen PR. Philadelphia, PA: Saunders, 1998, p. 665–728.
706. **Yuksel S, Yurekli M.** The effect of adrenomedullin (ADM) on tyrosine hydroxylase (TH) enzyme activity and blood pressure in cold exposed rats. *Endocr J* 50: 553–559, 2003.
707. **Zafar HM, Pare WP, Tejani-Butt SM.** Effect of acute or repeated stress on behavior and brain norepinephrine system in Wistar-Kyoto (WKY) rats. *Brain Res Bull* 44: 289–295, 1997.
708. **Zagon A, Smith AD.** Monosynaptic projections from the rostral ventrolateral medulla oblongata to identified sympathetic preganglionic neurons. *Neuroscience* 54: 729–743, 1993.
709. **Zardetto-Smith AM, Gray TS.** Organization of peptidergic and catecholaminergic efferents from the nucleus of the solitary tract to the rat amygdala. *Brain Res Bull* 25: 875–887, 1990.
710. **Zellmer E, Zhang Z, Greco D, Rhodes J, Cassel S, Lewis EJ.** A homeodomain protein selectively expressed in noradrenergic tissue regulates transcription of neurotransmitter biosynthetic genes. *J Neurosci* 15: 8109–8120, 1995.
711. **Zhang L, Rao F, Wessel J, Kennedy BP, Rana BK, Taupenot L, Lillie EO, Cockburn M, Schork NJ, Ziegler MG, O'Connor DT.** Functional allelic heterogeneity and pleiotropy of a repeat polymorphism in tyrosine hydroxylase: prediction of catecholamines and response to stress in twins. *Physiol Gen* 19: 277–291, 2004.
712. **Ziegler MG, Bao X, Kennedy BP, Joyner A, Enns R.** Location, development, control, and function of extra-adrenal phenylethanolamine *N*-methyltransferase. *Ann NY Acad Sci* 971: 76–82, 2002.
713. **Ziegler MG, Kennedy BP, Houts FW.** Extra-adrenal nonneuronal epinephrine and phenylethanolamine-*N*-methyltransferase. *Adv Pharmacol* 42: 843–846, 1998.
714. **Zigmond RE.** The long-term regulation of ganglionic tyrosine hydroxylase by preganglionic nerve activity. *Federation Proc* 39: 3003–3008, 1980.
715. **Zubieta JK, Heitzeg MM, Smith YR, Bueller JA, Xu K, Xu Y, Koeppe RA, Stohler CS, Goldman D.** COMT val158met genotype affects mu-opioid neurotransmitter responses to a pain stressor. *Science* 299: 1240–1243, 2003.
716. **Zucker M, Weizman A, Rehavi M.** Repeated swim stress leads to down-regulation of vesicular monoamine transporter 2 in rat brain nucleus accumbens and striatum. *Eur Neuropsychopharmacol* 15: 199–201, 2005.
717. **Zukowska-Grojec Z.** Neuropeptide Y. A novel sympathetic stress hormone and more. *Ann NY Acad Sci* 771: 219–233, 1995.
718. **Zukowska-Grojec Z.** Neuropeptide Y: an adrenergic cotransmitter, vasoconstrictor, and a nerve-derived vascular growth factor. *Adv Pharmacol* 42: 125–128, 1998.