Hindbrain catecholamine neurons mediate consummatory responses to glucoprivation

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Abstract

Previous work using the retrogradely transported immunotoxin, saporin (SAP) conjugated to a monoclonal antibody against dopamine-β-hydroxylase (DBH; DSAP), to selectively lesion norepinephrine (NE) and epinephrine (E) neurons projecting to the medial hypothalamus, demonstrated the essential role of these neurons for appetitive ingestive responses to glucoprivation. Here, we again utilized this lesion to assess the importance of these same neurons for the consummatory phase of glucoprivic feeding. To test consummatory responses, milk was infused intraorally through a chronic cheek fistula until rejected. Appetitive responses were tested in the same rats using pelleted food. Feeding responses to insulin-induced hypoglycemia, 2-deoxy-D-glucose (2DG)-induced blockade of glucose utilization, mercaptoacetate (MA)-induced blockade of fatty acid oxidation, 0.9% saline, and 18-h food deprivation were assessed. Unlike unconjugated SAP controls, the DSAP rats did not increase their food intake in response to glucoprivic challenges in either the pelleted food or the intraoral feeding tests. However, the DSAP rats did not differ from SAPs in their ingestive responses to food deprivation and blockade of fatty acid oxidation. The selective impairment of glucoprivic feeding responses indicates that DSAP did not impair the underlying circuitry required for either appetitive or consummatory ingestive responding but eliminated the mechanism for control of this circuitry specifically by glucoprivation. Results suggest that both appetitive and consummatory responses to glucoprivation are controlled and coordinated by multilevel terminations of the same catecholamine neurons.
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1. Introduction

Appetitive and consummatory responses are distinct components of ingestive behavior. Appetitive responses include complex behaviors involved in searching for and ingesting food, while consummatory responses include reflexes necessary for accepting, rejecting, chewing, and swallowing food once it is in the mouth [1–3]. The underlying neural circuits are not fully understood for either phase of ingestion. However, norepinephrine (NE) and epinephrine (E) neurons appear to contribute to the arousal of appetitive responses because they potently stimulate feeding behavior when administered directly into the hypothalamus [4–6]. Moreover, NE or E neurons appear to be required specifically for appetitive feeding responses induced by glucoprivation because selective immunotoxin

lesions of hindbrain NE and E neurons with projections to the hypothalamus eliminate glucoprivic feeding in home-cage feeding tests requiring appetitive behaviors [7]. The importance of hindbrain catecholamine neurons for the consummatory phase of the glucoprivic feeding response has not been assessed. However, these neurons not only provide dense innervation of forebrain areas implicated in the elicitation of ingestive behaviors, but also innervate hindbrain sites involved in visceral and gustatory functions [8–15]. Thus, the distribution of NE and E terminals is compatible with a role for these neurons in both the appetitive and the consummatory phases of ingestion.

In the present work, we examined the importance of NE and E neurons for the consummatory phase of the glucoprivic feeding response. We specifically examined the role of NE and E neurons with projections to the medial hypothalamus, which have been shown to be essential for the appetitive response to glucoprivation [7]. These neurons were lesioned by medial hypothalamic microinjection of the selectively internalized and retrogradely

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transported ribosomal toxin, saporin (SAP) conjugated to a monoclonal antibody against dopamine-β-hydroxylase (DBH; DSAP) [16,17]. Decerebration studies in rats have shown that neural circuits within the hindbrain are sufficient for the elicitation of consummatory responses and for the potentiation of these responses by glucoprivation [18]. Therefore, if NE or E neurons that project to the medial hypothalamus have hindbrain projections responsible for the elicitation of the consummatory phase of glucoprivic feeding, then, the same hypothalamic DSAP injection should effectively eliminate both phases of the glucoprivic feeding response. However, if noncatecholaminergic neurons, or catecholaminergic neurons that do not project to the medial hypothalamus, elicit the consummatory response to glucoprivation, then, the consummatory response should survive this DSAP lesion.

For the assessment of consummatory responding, milk was delivered intraorally during the test, and the amount of liquid food that the rat would accept before rejecting it actively or allowing it to drip out of the mouth was measured. Appetitive responses were assessed in the same rats by measuring the intake of pelleted chow in home-cage feeding tests. Glucoprivation was induced by systemic administration of 2-deoxy-D-glucose (2DG), a competitive inhibitor of glycolysis [19], and by hypoglycemic doses of insulin. To evaluate the specificity of DSAP lesion effects for glucoprivic ingestive responses, the rats were also tested for appetitive and consummatory responses to mercaptoacetate (MA)-induced blockade of fatty acid oxidation [20] and to mild (overnight) food deprivation. Previous work has shown that MA-induced feeding utilizes a neural pathway distinct from that required for glucoprivic feeding [7,21–26] and is not impaired by injections of DSAP into the paraventricular nucleus of the hypothalamus (PVH) in feeding tests requiring appetitive responses [7].

2. Materials and methods

2.1. Animals

Adult male Sprague–Dawley rats were obtained from Simonsen Laboratories (Gilroy, CA) and individually housed in suspended wire-mesh cages, under standard AAALAC-approved conditions, in a temperature-controlled room (21 ± 1 °C), illuminated between 0700 and 1900 h. Throughout the experiment, the rats had ad libitum access to pelleted rat chow (Harlan Teklad F6 Rodent Diet W, Madison, WI) and tap water, except as noted below. Prior to surgery, the rats were handled daily for at least 1 week and habituated to the laboratory environment and to the testing procedures. The Washington State University Institutional Animal Care and Use Committee, which conforms to National Institute of Health (NIH) rules and regulations, approved all experimental animal protocols.

2.2. Preparation of animals

The rats were prepared for experimentation by stereotaxic injection of SAP or DSAP into the PVH using techniques described previously [27]. The rats were anesthetized with equithesin, a choral hydrate-pentobarbital anesthesia cocktail [28] prepared in our laboratory (8.5 g chloral hydrate, 4.24 g magnesium sulfate, 1.772 g pentobarbital sodium, 30.1 ml of 95% ethyl alcohol, and 67.6 ml of propylene glycol, brought to a total volume of 200 ml by addition of sterile double-distilled H2O), administered intraperitoneally at a dose of 0.1 ml/100 g BW. We have noted an interaction between the DSAP lesioning potency and the type of anesthetic used. In our experience, DSAP produces the most consistent lesion when administered to rats anesthetized with equithesin. The rats were given bilateral microinjections of DSAP (Chemicon International, Temecula, CA; 42 ng/200 nl in phosphate buffer, pH 7.4, $n = 8$) or SAP (Advanced Targeting Systems, Carlsbad, CA; 8 ng/200 nl in phosphate buffer, pH 7.4, $n = 8$) using stereotaxically positioned drawn glass capillary micropipettes (30-μm tip diameter) connected to a Picospritzer II. Injection coordinates were 1.0 mm posterior to bregma, ± 0.5 mm lateral from midline, and 7.2 mm below the dura mater. Two weeks were allowed for the transport of the immunotoxin and degeneration of the affected neurons [16,17] prior to the initiation of experimentation. SAP- and DSAP-injected rats were again anesthetized and implanted with a cheek fistula designed and constructed at Washington State University (Fig. 1) for the intraoral infusions of liquid food.

For the cheek fistula implantations, the rats were anesthetized by intramuscular injection (0.1 ml/100 g BW) of a Ketaset/Rompun/Acepromazine cocktail (consisting of 5 ml ketamine, 100 mg/ml, Fort Dodge Animal Health, Fort Dodge, IA; 2.5 ml Rompin, 20 mg/kg, Bayer Corporation Agriculture Diversity Animal Health, Shawnee Mission, Kansas; 1 ml Acepromazine Maleate, 10 mg/kg, Boehringer Ingelheim Vetmedica, St. Joseph, MO; and 1.5 ml 0.9% saline, Abbot Laboratories, North Chicago, IL). Fistulas were implanted through the skin of the cheek, just lateral and rostral to the first premolar. At this site, the fistula did not penetrate or compromise the muscles of mastication. The implanter was comprised of 26-gauge tubing welded into the lumen of a 2-in. long, 16-gauge needle with 0.75 in. of 26-gauge tubing exposed. The exposed 26-gauge tubing was then placed into the lumen of the cheek fistula, and the implanter was passed through the skin of the cheek, followed by the fistula. The cheek fistula was placed so that the interior surface was located snugly against the inside of the cheek and locked into place by a threaded washer attached to the exterior of the fistula. The fistula was allowed to remain open when not in use. When the fistula was needed for intraoral infusion, the exterior washer was removed, and food remnants were cleared from the lumen of the fistula using 26-g stainless steel tubing. An infusion tip was screwed onto the fistula and connected to a syringe pump
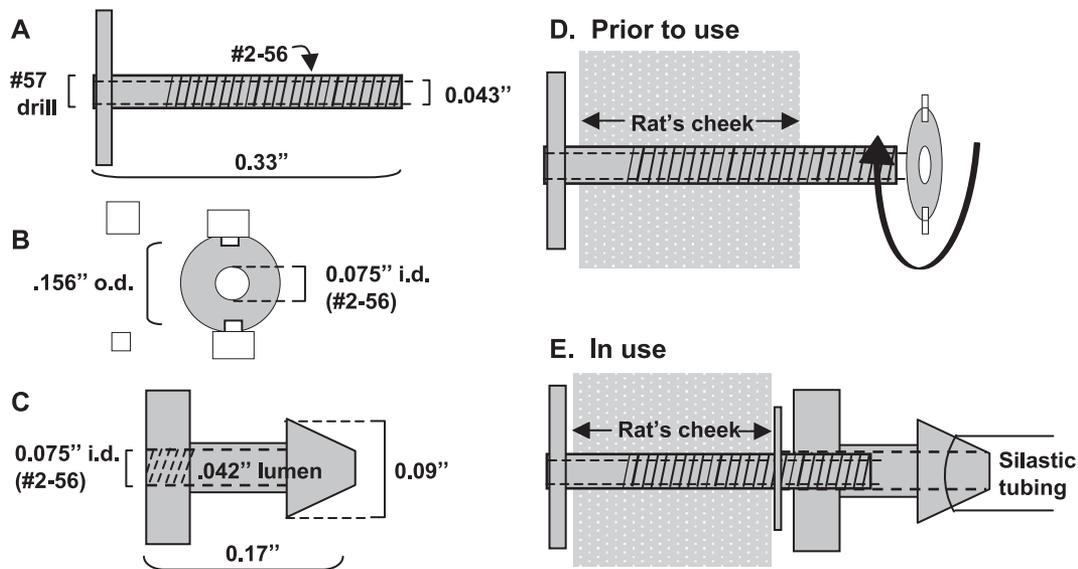
Fig. 1. Drawings depicting the design and implementation of the stainless steel cheek fistula used for the intraoral delivery of liquid food. The components of the fistula are shown in Diagrams A, B, and C. The fistula itself (Diagram A) was constructed from a stainless steel rod (0.07-in. diameter, 0.33 in. long), drilled through the center (#57 drill) to create a 0.043-in. diameter lumen, and threaded (#2–56 threading) along its exterior surface. A washer (0.02 in. thick) was permanently affixed to the intraoral end of the rod. A threaded (#2–56) washer (Diagram B) was used on the externalized threaded portion of the fistula to hold the fistula in place when not in use. The washer was notched to facilitate manipulation of the washer with forceps. The infusion tip (Diagram C) contained a central lumen compatible in diameter with the fistula. It was threaded (#2–56) so that it could be screwed onto the externalized portion of the fistula during the infusion of liquid food. The opposite end of the infusion tip (0.09 in., largest diameter) to facilitate the attachment of the silastic tubing used to deliver the food. Diagram D shows the fistula assembly at the time of implantation and between experiments. The external washer is affixed at the time of fistula insertion and remains in place even when the infusion tip is attached. Diagram E shows the fistula assembled for infusion, with the infusion tip attached.

by silastic tubing, which was supported above the rat's head so that the rat could move freely about the cage. The weight of the fistula was 242 mg and the weight of the infusion tip was 132 mg.

2.3. Feeding tests

The rats were tested for feeding in response to 2DG, insulin-induced hypoglycemia, MA, physiological saline, and food deprivation. During the tests, liquid food was presented, using the intraoral fistulas, or the standard pelleted food was presented on the cage floor.

2.4. Tests requiring appetitive responses

Glucoprivation was induced by subcutaneous injection of 2DG (Sigma, St. Louis, MO; 200 mg/kg), or a hypoglycemic dose of regular insulin (human rDNA origin, Eli Lilly and Company, Indianapolis, IN; 2 U/kg). It has been well documented that the glycemic responses to 2DG and insulin do not differ between PVH DSAP- and SAP-injected rats [7,27], and hence, glycemia was not monitored in these feeding experiments. The blockade of fatty acid oxidation was induced by an intraperitoneal injection of MA (thioglycolic acid, Sigma; 68 mg/kg). Saline (0.9%) was used as the control injection. For deprivation-induced feeding, food was withheld for 18 h, beginning just prior to dark onset. After injections or deprivation, the rats were immediately given a weighed quantity of pelleted rat chow, and the remaining pellets and spillage were measured 4 h later. Previous work has demonstrated that the intake of solid food in response to these stimulus conditions occurs primarily within the 3-h period following the presentation of food [21]. Consecutive feeding tests were separated by a 5-day interval. Each rat was tested twice for saline-, 2DG-, and insulin-induced feeding and once for MA- and deprivation-induced feeding.

2.5. Tests of consummatory responding

Prior to the intraoral feeding tests, the rats were trained to drink the liquid food (a 40% solution of lactose-free milk in tap water) from drinking tubes attached to the wall of their home cage. The rats were given unlimited access to the milk, along with their regular food and water, in three 2-h daytime training sessions during a 2-week period. During this period, the rats were also habituated to the infusion apparatus. When bottle training was complete, the rats were given milk intraorally during two 2-h training sessions, in which milk was infused as described below for the test sessions. All rats readily consumed the milk, regardless of how it was presented, after their first exposure to it. Rats adjusted to the intraoral infusions within 5–10 min of the first infusion and, typically, sat quietly during the infusions.

For tests of intraoral feeding, drug doses and deprivation conditions were the same with those described above

for solid food tests, except that the milk infusions were begun 30 min after drug injection. For the infusion of liquid food, the intraoral fistulas were connected with silastic tubing (1.57 mm inner diameter × 3.18 mm outer diameter) to a 60-cc syringe mounted on a syringe pump. Intraoral feeding tests were conducted under direct individual observation using an open-topped, $8 \times 8 \times 12$ in. wire-mesh cage where the rat was clearly visible. Paper under the cage helped visualize the spillage of the milk. During the test, the liquid food was infused through the silastic tubing at a rate of 0.95 ml/min. The milk was infused in successive 5-min cycles, separated by a 1-min rest, until the rat allowed the milk to drip out of its mouth for a continuous 30-s period. At this point, the rat was determined to have physically rejected the milk, and the test was terminated. Pilot studies conducted to determine infusion parameters indicated that rats would not resume drinking for at least 15 min after a 30-s period of rejection. The total volume of milk ingested was recorded at the end of the test. Each rat was tested twice for saline-, 2DG-, and insulin-induced feeding and once for MA- and deprivation-induced feeding, with a 5-day interval between tests.

2.6. Immunohistochemistry

After testing was completed, the rats were killed by lethal dose of pentobarbital sodium (Abbott Laboratories, North Chicago, IL; 300 mg/kg) and perfused transcardially with phosphate-buffered saline followed by 4% formalin (both pH 7.4). Brains were removed, postfixed at room temperature for 2 h, and cryoprotected overnight in 25% sucrose. Coronal sections (40 μm) of the brainstem were immunoreacted for DBH to verify the DSAP-induced lesion.

Immunohistochemical staining was done by using standard avidin–biotin–peroxidase techniques described previously [29]. Briefly, sections were treated with 50% ethanol for 30 min, then washed (3×5 min) in 0.1 M PB, and incubated for 45 min in 10% normal horse serum (Gibco BRL Life Technologies, Grand Island, NY) made in Tris sodium phosphate buffer (TPBS, pH 7.4) with 0.05% thimerosol. The blocking solution was removed from the tissue, and the sections were incubated in mouse monoclonal anti-DBH (1:100,000; Chemicon) made in 10% normal horse serum–TPBS. After 48 h, the primary antibody was removed, and the sections were washed and incubated in biotintylated donkey antimouse IgG (1:500; Jackson ImmunoResearch Laboratories, West Grove, PA) made in 1% normal horse serum–TPBS. After 24 h, the tissue was washed (3×10 min), incubated with Extravidin-peroxidase (1:1500 in TPBS; Sigma) overnight, washed again (3×10 min), and reacted for visualization of DBH-stained neurons by using nickel-intensified diaminobenzidine (nickel-DAB) in the peroxidase reaction to produce a black reaction product. The sections were then mounted on slides and were cover slipped for microscopic evaluation. The antibodies used in the experiment were titrated prior to use to

determine optimal concentrations. Standard controls for the specificity of primary antibodies were used, including the incubation of the tissue with normal instead of immune serum and the preincubation of the immune serum with the antigen prior to its application to the tissue.

The distribution of NE (A1–A6) and E (C1–C3) cell bodies has been described in detail [9,12,30–33]. To determine the effectiveness of PVH DSAP injections in lesioning NE and E neurons, DBH-ir cell bodies were quantified at representative levels through hindbrain cell groups A1, A2, and C1–C3, which provide the major NE/E innervation of the PVH. In the area of overlap of A1 and C1, nearly all NE and E cell bodies project to the medial hypothalamus. Cell groups A5 and A7 do not innervate the PVH, and our previous work has shown that these cell groups are not damaged by PVH DSAP injections, and these groups were not analyzed quantitatively in this study. One of three sets of hindbrain sections from each rat was used for quantification. Immunoreactive cells in five anatomically matched sections, sampling the extent of each of the cell groups (specified below), were counted bilaterally for each rat. The mean number of cells per section was calculated for each cell group for each rat and was averaged across the rats in each treatment group. All immunoreactive cells were counted, regardless of the presence of a cell nucleus. No correction factor for double counting was applied due to the use of relatively thick sections for the quantification. For purposes of quantification, DBH-ir cell bodies in the ventrolateral medulla were considered as three separate groups, referred to here as A1, A1/C1, and C1, anatomically defined as described below. The approximate anatomical levels (millimeter, caudal to bregma) of each quantified area were determined from Paxinos and Watson [34] and are included below. Cells designated as A1 were those found between the decussation of the pyramids and the calamus scriptorius (14.60–14.30 mm). At this level of the ventrolateral medulla, the DBH-ir cell bodies are predominantly NE containing. Cells designated as A1/C1 were those found between the calamus scriptorius and obex (the rostral border of the area postrema; 14.30–13.68 mm), where NE and E cell bodies have an overlapping distribution. Cells referred to as C1 were those located between the obex and the caudal border of the facial nucleus, a region containing predominantly E cell bodies (13.68–11.80 mm). In the most rostral portion of C1, most cells project to the spinal cord, not to the PVH, and were not damaged by the PVH DSAP injection. In this study, the regions of C1 containing the spinally and the hypothalamically projecting cell populations were counted together (i.e., not distinguished). A2 cells were counted in the nucleus of the solitary tract from the level of the calamus scriptorius through the caudal half of the area postrema (14.30–13.80 mm), caudal to the appearance of C2 neurons. C2 cell bodies were counted within the nucleus of the solitary tract rostral to the obex (12.8–12.5), and C3 neurons were counted medial to the nucleus of the solitary tract, at the level of the rostral terminus of the inferior olive. Hypotha-

Table 1
Numbers of catecholamine cell bodies were significantly reduced by PVH DSAP injection in cell groups with projections to the PVH

Cell group	SAP	DSAP
A2	50.4 ± 2.3	19.3 ± 0.8 *
A1	33.3 ± 1.5	8.5 ± 0.5 *
A1/C1	34.9 ± 1.5	4.9 ± 0.4 *
C1	66.4 ± 1.5	27.8 ± 2.1 *
C2	37.4 ± 1.8	20.9 ± 0.7 *
C3	22.2 ± 0.8	11.6 ± 0.6 *

Data are expressed as mean $\pm$ S.E.M.
 * $P < .01$, SAP vs. DSAP.

lamic sections were examined for the presence of DBH-ir terminals. However, because immunoreactivity does not provide a reliable basis for the quantification of terminals, DBH-ir terminals were not quantified.

Histological sections used in the figures were captured by using a Coolsnap digital camera (Media Cybernetics, Silver Spring, MD) and Metamorph imaging software (Universal Imaging, Downingtown, PA). Adobe Photoshop was used to assemble plates of multiple images and to match the brightness of images on the same plate.

2.7. Statistics

Feeding responses were analyzed by two-way repeated-measures ANOVA, followed by the Bonferroni pairwise

multiple-comparison procedure. The numbers of immuno-reactive cell bodies in each of the quantified areas were compared between the SAP and DSAP groups using *t* tests. The differences were considered significant if $P < .05$.

3. Results

The rats recovered rapidly from intracranial SAP and DSAP injections and remained healthy and vigorous during the entire course of experimentation (approximately six months). The DSAP-injected rats were indistinguishable from the controls with respect to general motor function and home-cage behavior. Most cheek fistulas remained in place throughout the six months of experimentation, without producing any signs of discomfort, infection, or inflammation or interfering with normal food intake or body weight gain. In the few cases where the fistulas were lost, the animals had to be eliminated from the testing due to an unavoidable delay in the fabrication of replacement fistulas.

3.1. Immunohistochemistry

The results of the quantification of NE and E cell bodies in PVH SAP- and DSAP-injected rats are shown in Table 1 and Fig. 2. PVH DSAP injections destroyed
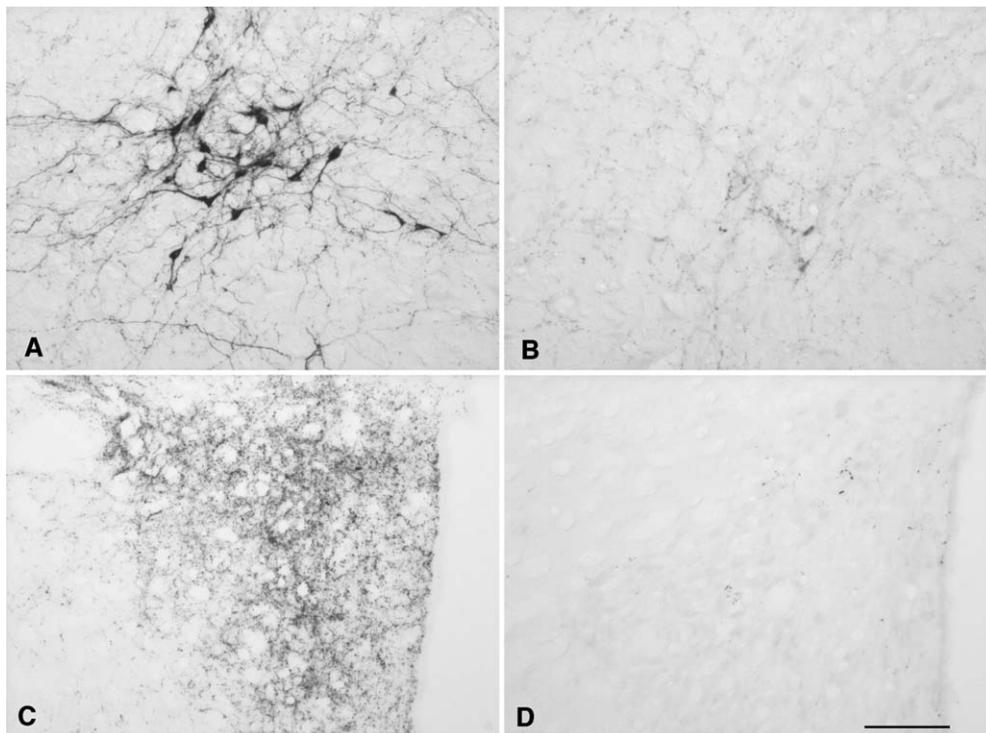

Fig. 2. Photomicrographs of coronal sections showing DBH immunoreactivity (black precipitate) in rats given bilateral PVH microinjections of SAP (Panels A and C) or DSAP (Panels B and D). By its retrograde action, PVH DSAP profoundly reduced cell bodies in the A1/C1 area of the ventrolateral medulla (Panel B) compared with the SAP control (Panel A). The cell body number was also reduced in other hypothalamically projecting cell groups (see Table 1). The PVH of the DSAP rat (Panel D) is virtually devoid of DBH-ir terminals compared with SAP controls (Panel C). Scale bar = 200 μm.
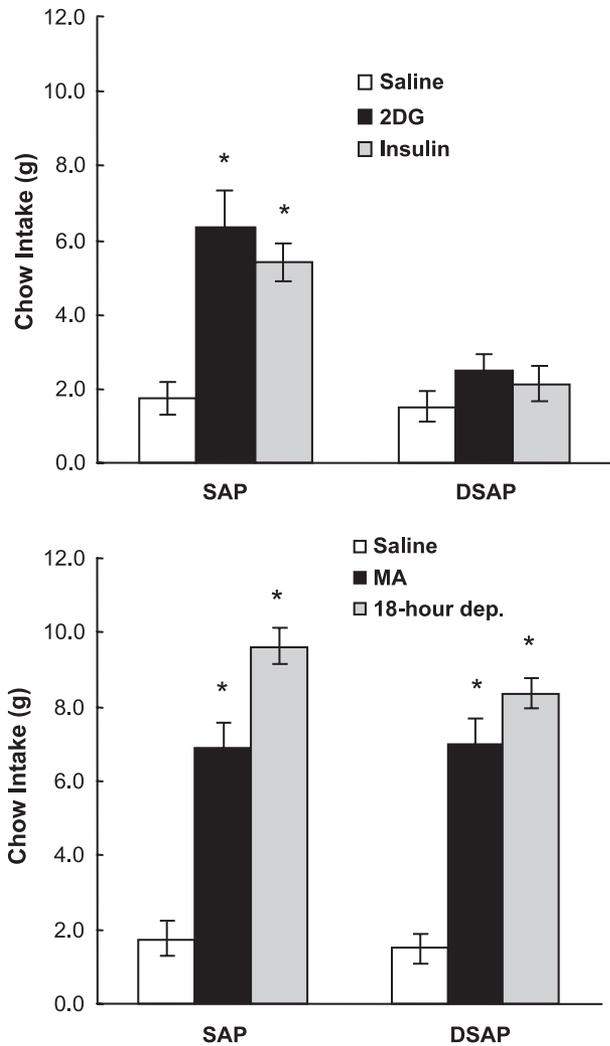
Fig. 3. Top: Total 4-h intake of pelleted rat food following subcutaneous injection of saline (1 ml/kg), 2DG (200 mg/kg), or insulin (2 units/kg) in SAP control and DSAP-immunolesioned rats. DSAP rats did not increase their food intake in response to either 2DG-induced glucoprivation or to hypoglycemic doses of insulin. Bottom: Total 4-h intake of pelleted food following saline (1 ml/kg), MA (68 mg/kg), or overnight food deprivation (18 h) in SAP controls and DSAP-immunolesioned animals. DSAP rats increased their food intake significantly in response to both MA and food deprivation. DSAP impaired the responses to glucoprivation selectively. Data are expressed as mean 4-h food intake $\pm$ S.E.M. (*$P < .001$ vs. intake after saline).

DBH-ir terminals in the PVH and medial hypothalamus. By its retrograde action, PVH DSAP profoundly reduced cell body numbers in the A1/C1 area of the ventrolateral medulla, where the majority of NE and E cell bodies project to the medial hypothalamus. Cell body number was also reduced in other hypothalamically projecting cell groups (see Table 1). In the quantified sections, cell numbers in DSAP rats were significantly reduced ($P < .01$ vs. SAP) to 38% of SAP control for A2, 26% for A1, 20% for A1/C1, 42% for the rostral and middle levels of C1, 57% for C2, and 52% for C3. The magnitude of the cell loss was related to the proportion of cells in a

particular group that are known to innervate the medial hypothalamus.

3.2. Appetitive feeding responses

For the feeding measures from these four experiments, see Fig. 3. There were seven DSAP-treated and five SAP-treated rats. For 2DG-induced glucoprivic feeding, the interaction was significant [$F(1,10) = 11.92$, $P = .006$], and so was the main effect of 2DG [$F(1,10) = 27.99$, $P = .0004$] and of DSAP [$F(1,10) = 11.92$, $P = .006$]. For insulin-induced glucoprivic feeding, the interaction was significant [$F(1,10) = 10.16$, $P = .01$], and so was the main effect of insulin [$F(1,10) = 20.65$, $P = .001$] and of DSAP [$F(1,10) = 11.85$, $P = .006$]. Neither
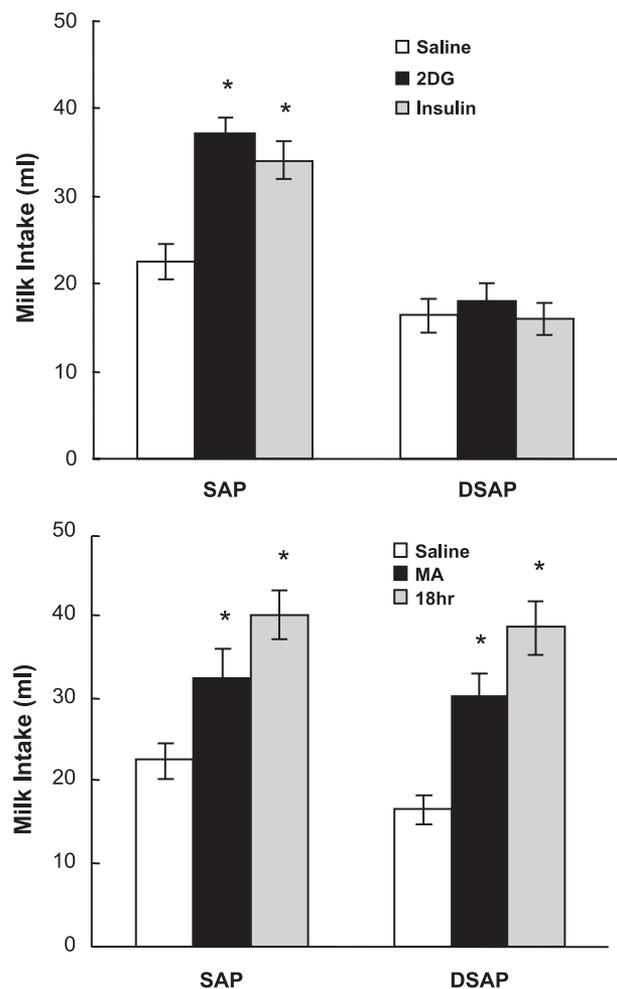

Fig. 4. Top: Cumulative intake of 40% lactose-free milk, infused through an intraoral fistula, beginning 60 min after the injection of saline (1 ml/kg), 2DG (200 mg/kg), or insulin (2 units/kg) in SAP control and DSAP-immunolesioned rats. DSAP rats did not increase their intake of milk either in response to 2DG-induced glucoprivation or to hypoglycemic doses of insulin. Bottom: Consumption of the same diet beginning 60 min after saline (0.9%, 1 ml/kg), MA (68 mg/kg) injection, and overnight food deprivation in SAP and DSAP rats. Amount consumed did not differ between SAP and DSAP rats in any of these tests. Data are expressed as mean intraoral intake in ml $\pm$ S.E.M. (*$P < .001$ vs. intake after saline).

the interaction nor the main effect of DSAP was significant for either MA- or deprivation-induced feeding.

3.3. Consummatory feeding responses

For the feeding measures from these four experiments, see Fig. 4. There were five DSAP-treated and five SAP-treated rats. For 2DG-induced glucoprivic feeding, the interaction was significant [$F(1,8) = 6.30$, $P = .036$], and so was the main effect of 2DG [$F(1,8) = 15.20$, $P = .005$] and of DSAP [$F(1,8) = 11.10$, $P = .01$]. For insulin-induced glucoprivic feeding, the interaction was significant [$F(1,10) = 22.57$, $P = .001$], and so was the main effect of insulin [$F(1,8) = 19.16$, $P = .002$] and of DSAP [$F(1,8) = 17.30$, $P = .003$]. Again, neither the interaction nor the main effect of DSAP was significant for either MA- or deprivation-induced feeding.

4. Discussion

The selective immunotoxic lesion of hindbrain catecholaminergic cell bodies with projections to the medial hypothalamus abolished the feeding response to glucoprivation when the rats' standard pelleted food was used as the test diet, as reported previously [7]. The major new finding is that these same lesions also abolished the glucoprivation-induced intake of a liquid food infused intraorally through a chronic cheek fistula. These two feeding tests require distinct components of the ingestive response. The feeding test using pelleted chow requires complex appetitive behaviors involved in searching for, acquiring, and ingesting food. The intraoral delivery of food bypasses the appetitive phase of ingestion and provides a testing protocol that relies primarily on consummatory responding, which is more highly reflexive in nature and facilitates the acceptance, chewing, and swallowing of food once it is in the mouth [1,3,35–40]. The fact that the DSAP lesion abolished feeding in both types of tests indicates that hindbrain NE and E neurons are required for both the appetitive and the consummatory phases of the ingestive response to glucoprivation.

The same DSAP-lesioned rats that did not exhibit either appetitive or consummatory responses to either a hypoglycemic dose of insulin or intracellular blockade of glucose metabolism had normal appetitive and consummatory responses to mild food deprivation and MA-induced blockade of fatty acid oxidation. These results therefore indicate that the DSAP lesion did not impair the neural circuits required for the execution of consummatory responses. It was still possible to arouse these responses using other stimuli for ingestion. Although the presence of these normal responses in DSAP rats is not surprising, since DSAP-lesioned rats are healthy and maintain normal (or sometimes elevated) body weights [7,27,41], they reinforce the selectivity of the DSAP lesion and provide a remarkable demonstration that neural circuits subserving consummatory responding are controlled by multiple stimulus-specific pathways.

The involvement of hindbrain NE/E neurons in glucoprivic feeding is well supported by a variety of experimental results. NE and E are potent orexigens [6,42], as is neuropeptide Y (NPY; [43]), which is coexpressed by the majority of NE and E neurons providing afferent innervation to the hypothalamus [44]. In addition, NE and E cell bodies are located in hindbrain sites shown to be glucoreceptive and capable of stimulating food intake and other glucoregulatory responses when selectively glucodeprived [45,46]. Moreover, these neurons are activated by glucoprivation, as shown by an increase in the turnover of NE during glucoprivation [47,48] and by their increased expression of Fos-ir [29]. More recently, we have shown that the DSAP-induced lesion of NE/E neurons with projections to the medial hypothalamus reduces or eliminates glucoprivation-induced increases in Fos-ir [7] and c-*fos* mRNA in the PVH [27], as well as the glucoprivation-induced increases in arcuate nucleus NPY and AGRP mRNAs [41]. Thus, NE/E neurons provide a critical linkage between the hindbrain glucoreceptive and the forebrain areas also involved in the execution of feeding behavior.

Because decerebration, which disconnects the hindbrain from the forebrain, leaves the consummatory response to glucoprivation intact [18], it seemed reasonable to hypothesize that PVH DSAP injections, which destroy NE/E-afferent inputs to the forebrain [7], might also leave the consummatory response intact. The fact that the DSAP lesion abolished the glucoprivation-induced consummatory response indicates that this response depends on local hindbrain projections of the same NE or E neurons that innervate the medial hypothalamus. These are the only hindbrain neurons destroyed by the hypothalamically injected DSAP [7]. Thus, with respect to this particular consummatory response, the critical difference between the decerebration and the DSAP lesion is that decerebration leaves the hindbrain NE/E cell bodies and their hindbrain processes intact, while the PVH DSAP injection retrogradely destroys these cell bodies and all of their processes.

The fact that injections of orexigenic substances, such as NE, E, NPY, or Agouti gene-related protein, into the hypothalamus stimulate feeding [6,43,49] demonstrates that consummatory responses can be aroused and facilitated by descending projections, presumably to hindbrain visceral sensory and motor areas [10]. Results from our PVH DSAP lesion, which destroys the NE- and E-afferent input to the medial hypothalamus, do not, by themselves, preclude the possibility that the role of this afferent input is to stimulate descending projections required for glucoprivic facilitation of consummatory responding. However, the decerebration results indicate that projections descending from the forebrain are not essential for the glucoprivic stimulation of consummatory responses. Any effects of the forebrain on glucoprivation-induced consummatory responding would

have to be viewed as modulatory or integrative in nature, as they would be superimposed on activity generated locally in the hindbrain. More likely, the rostral projections of catecholamine neurons function to arouse appetitive mechanisms for food acquisition and to integrate appetitive and consummatory responses to glucoprivation.

The fact that decerebrate rats exhibit increased consummatory responding to glucoprivation [18] also dictates that the NE/E neurons, found here to be critical for the control of this consummatory response, must be activated directly either by glucoprivation or by local connections from hindbrain glucoreceptor cells, without dependence on descending connections, e.g., from hypothalamic glucose-sensitive or glucose-responsive neurons [50]. There is abundant support for the existence of glucoreceptor cells, capable of stimulating food intake, in the hindbrain. This support comes from cannulation studies that have mapped sites, where the local application of a glycolytic inhibitor produces a potent stimulus for feeding [51], and from studies showing that the acute blockade of the mesencephalic aqueduct, restricting the rostral to caudal flow of cerebral spinal fluid, eliminates the feeding response to lateral, but not to fourth ventricular, administration of a glucoprivic agent [52]. Furthermore, these studies, together, clearly indicate that hindbrain glucoreceptor cells can fully account for the feeding response to systemic or intracranial glucoprivation. Glucoreceptors in other sites are not required. In fact, the localized glucoprivation of hypothalamic and other forebrain sites has not proven to be effective for stimulation of feeding [45,52–54], except when the dose, volume, or route of administration of the glucoprivic agent permit its diffusion through the ventricular system to the hindbrain [52,55,56].

Neural activation arising from hindbrain glucoreceptors produces an integrated response aimed at the restoration of glucose homeostasis. This integrated response includes not only the arousal of the behavioral and reflexive components of ingestion examined here, but also critical endocrine responses that reshape metabolism to deal appropriately with the metabolic deficit. In another work, we have shown that medial hypothalamic DSAP injections also selectively impair the corticosterone response to glucoprivation, without impairing the basal circadian rhythm of corticosterone secretion or the corticosterone response to forced swimming [27]. The lengthening of estrous cycles in response to glucoprivation is also eliminated by this DSAP lesion, but the basal estrous cycle is not altered [57]. Important questions for future study are whether the same catecholamine neurons mediate all of the above responses to glucoprivation or whether functionally distinct populations are coactivated by glucoprivation to produce these responses. Another important question is how peptides, such as NPY, which are coexpressed and coreleased with NE and E [58–60], contribute to the stimulation of the appetitive and consummatory phases of ingestion and to other glucoregulatory responses aroused by these neurons.

In summary, the destruction of hindbrain NE and E neurons by medial hypothalamic injections of the retrogradely transported immunotoxin, DSAP, has revealed that NE or E neurons with projections to the medial hypothalamus are essential for the glucoprivic arousal of both the appetitive and consummatory phases of ingestion. Because decerebration does not eliminate consummatory responding to glucoprivation [3,18], our findings indicate that this response is mediated by hindbrain collaterals of some of the same catecholamine neurons that innervate the hypothalamus. The results from decerebrate rats also indicate that glucoreceptor cells controlling these catecholamine neurons are located in the hindbrain. Furthermore, catecholamine cell bodies and glucoreceptors controlling them appear to exist in close proximity because localized glucoprivation of areas containing the catecholamine cell groups stimulates feeding and other glucoregulatory responses [45,46]. Alternatively, these catecholamine neurons themselves may be glucoreceptive, simultaneously sensing and disseminating information regarding glucose availability.

Acknowledgements

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