Research report

Accumbens dopamine mechanisms in sucrose intake

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Abstract

Extracellular levels of dopamine (DA) and monoamine metabolites were measured in the nucleus accumbens (NAcc) during sucrose licking using microdialysis in freely moving rats. The converse relationship also was tested. Using bilateral reverse microdialysis, D1 and D2 receptor antagonists (SCH23390, sulpiride) and the DA uptake blocker nomifensine were introduced into NAcc while measuring both ingestive behavior and neurochemistry. Licking of 0.3 M sucrose caused a 305% (±69%) increase in NAcc DA compared with water intake. Reverse microdialysis of nomifensine at a dose that increased accumbens DA levels (1484±346%) led to an increase of sucrose intake (152.5±5.4%). Concurrent infusions of the D1 and D2 blockers with nomifensine brought sucrose ingestion back near to control levels (114.8±3.7%). The higher dose of the D2 antagonist sulpiride also increased DA levels and sucrose intake. In contrast, the lower dose of the D2, and both doses of the D1 antagonist had no chemical or behavioral effects. These results showed release of NAcc DA in response to sucrose licking and the converse, an augmentation of the behavior by uptake blockade. The same data, however, failed to prove that tonic, local accumbens D1 and D2 receptor activity influenced this ingestive behavior. © 2001 Elsevier Science B.V. All rights reserved.

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1. Introduction

The nucleus accumbens (NAcc) is considered to be part of a neural substrate participating in behavioral reward [22,35,50]. In addition to the emphasis on brain self-stimulation and psychostimulant reward, considerable evidence indicates that both deprivation-induced feeding and instrumental responses for food lead to an increase in NAcc DA [14,24,29,32,45,48,49,53]. Although no one denies that eating is accompanied by NAcc DA release, a number of studies provide evidence undermining the hypothesis that DA mediates food reward [6,7,34,37].

The DA activation associated with feeding could reflect many aspects of the process (e.g. appetitive, consummatory-motor, pre- and postabsorptive components), including the incentive-motivating effect of orosensory stimulation [2,23]. Indeed, a highly preferred food reliably elicits an increase in NAcc DA [27,30,31] and also increases somato-dendritic DA release in the ventral tegmental area, where the mesoaccumbens pathway originates [55]. For rats, foods high in sugar are highly preferred [39]. Postigestive factors play an important role in this preference [40], but rats also sham feed sugar solutions in a concentration dependent manner [12,47]. Thus, the orosensory effects of this stimulus are sufficient to elicit ingestion.

Furthermore, when sucrose is the stimulus, sham and real feeding are influenced similarly by manipulations of DA receptors. Specifically, systemic injections of D1 and D2 antagonists reduced both real and sham licking of sucrose [8,11,12,16,38,43]. In all of these experiments, dopaminergic drugs were injected systemically (i.e., i.p. or s.c.), rather than directly into the brain. Although a follow-up study suggested a central rather than a peripheral site of action [11], the involvement of specific brain areas has not been examined using sucrose licking as a measure.

Moreover, to our knowledge, direct dopamine and metabolite assays have been carried out in only two
sucrose-intake studies and both used tissue homogenates. Initially Smith et al. [42] found an increase in DA turnover in the hypothalamus after sham feeding of sucrose. Later the same group failed to find significant changes in DOPAC/DA ratios in any of the investigated brain areas — the hypothalamus, NAcc, striatum, amygdala, pyriform cortex, and olfactory tubercle [46]. These contradictory results may indicate the limitations of ex vivo dopamine measures in behavioral paradigms. First, the ex vivo measures reflect only a single-point condition after sucrose intake. Second, samples from tissue homogenates provide a sum of both the intra- and extracellular pool of DA and metabolites. Therefore, the release and re-uptake of DA cannot be monitored directly with this method. If discrepancies between presynaptically stored and released DA are behaviorally relevant [30,31], this latter technical pitfall may lead to a misinterpretation of the results.

Although several studies measured or manipulated NAcc DA using conditioned stimuli that had been paired with ‘sweet’ solutions ([19]; for review, see Ref. [3]), only one experiment used unpaired, intraorally applied saccharin in naive rats. In this circumstance, NAcc DA increased [26]. A similar experiment using sucrose obtained by licking has not been published.

The aim of the present experiments was to determine if accumbens DA mechanisms mediate natural sucrose licking. First, we studied the effect of 20-min of sucrose or water licking on accumbens DA and metabolite levels using microdialysis in mildly deprived rats. Next, to test the effect of higher DA levels on sucrose intake, the DA reuptake blocker nomifensine was perfused directly into the NAcc through the microdialysis probe. Finally, to test the respective contribution of the NAcc DA receptors in natural and nomifensine-induced sucrose licking, D1 and D2 receptor antagonists (SCH23390 and sulpiride) were infused alone and in combination with nomifensine.

2. Methods

2.1. Surgeries and microdialysis

All experimental protocols were approved by the local Animal Care and Use Committee and followed NIH guidelines. Thirty male, Sprague–Dawley rats (275–325 g, Charles River) were housed individually on a 12-h light/dark schedule (lights on 07:00 h). The rats were kept on a standard laboratory diet (Rodent Diet-W 8604, Harlan Teklad, Madison, WI, USA).

For surgery, subjects were anesthetized with pentobarbital (50 mg/kg, i.p.) after a pre-treatment with atropine-sulphate (0.15 mg/kg i.p.). Bilateral, 21-Gauge stainless steel guide shafts aimed at the caudomedial NAcc were implanted stereotaxically as follows: A 10.4 mm, L 1.0 mm, V 4.0 mm, with reference to the interaural line, midsagittal sinus, and the level skull, respectively [28]. Guide shafts were kept patent with 26-Gauge stylets. Microdialysis probes in the NAcc extended 5 mm beyond the guide shafts.

Probes were constructed with silica glass tubing (37 μm diameter I.D. Polymicro Tech.) inside a 26-Gauge stainless steel tube with a microdialysis tip of cellulose tubing (Spectrum Co. 20 kDa molecular weight cutoff, 0.2 mm outer diameter by 2 mm long) sealed at the end with epoxy cement [15]. Probes were perfused with an artificial cerebrospinal fluid (ACSF; 145 mM NaCl, 2.7 mM KCl, 1.2 mM CaCl₂, 1.0 mM MgCl₂, 2.0 mM Na₂HPO₄, pH 7.4) at a flow rate of 1.0 μl/min through a quartz-lined dual channel microdialysis swivel (model 375/D/22QE, Instech Laboratories, Inc.). Probes were inserted and fixed in place at least 18 h before each experiment. The next day, 20-min samples were collected and measured either directly or kept frozen (−80°C).

2.2. Reverse microdialysis of dopaminergic drugs

Microdialysis was conducted on each animal in three sessions, once per day. Both hemispheres were assayed and infused simultaneously. Each 3 day session began the morning after probe implant and consisted of a 3-h collection period. Nomifensine maleate (25 μM), R(+)-SCH23390 HCl (10 or 20 μM), and S(−)-sulpiride (5 or 10 μM) were administered via the microdialysis input line for 40 min, except in one subset of rats in which only nomifensine was infused for 60 or 80 min, n = 4. The combinations of blockers (SCH23390 10 μM+sulpiride 10 μM) and their co-infusion with nomifensine (25 μM), all used 40-min applications. All chemicals were from RBI (Natick, MA) and dissolved in ACSF. In order to avoid neurochemical or behavioral effects that might be triggered by onset or offset, the infusions started 20 min prior to presentation of the sucrose or water, and continued for an additional 20 min during the licking session. Infusions of ACSF served as controls. The doses of drugs were chosen based on results of previous studies [17,21,33] and on our preliminary dose–response assessments.

2.3. Behavioral protocols and experimental groups

Before and after the surgery, the rats were housed in separate cages in the animal facility and handled daily. Five to 7 days before the tests, the rats were transferred into the microdialytic cages (model ENV-007, MED Associates, placed in sound attenuating cubicles with 30-dB noise speakers and ventilating fans) and put on a restricted feeding schedule from the second day. Between noon and 18:00 h, both food and water were available ad libitum. After an overnight deprivation (16 h), at 10:00 h the rats had a 20-min access to water or 0.3 M sucrose alternating every other day. This same schedule continued throughout the test, and served to reduce the anticipatory and contrast effects. The 4–6 day training period was sufficient to
provide stable fluid intakes in the daily 20-min sessions, but did not induce schedule-induced alterations in intakes or in baseline DA levels as revealed in the behavioral and neurochemical records, respectively.

Rats in the first group \( (n=6) \) received no infusions other than ACSF. Their probes served only for sample collection from the NAcc during sucrose or water licking on two consecutive days in the same animal.

The second group \( (n=10) \) was trained as the first group, but nomifensine was infused prior to and during sucrose or water licking through the microdialysis probes while the samples were taken simultaneously for DA and metabolite assay. In this way, each rat received two infusions on two consecutive days, randomly started on a ‘sucrose-day’ or a ‘water-day’. The third day, again without nomifensine, the sucrose or water session served as a control (in comparison with baseline values) to check for toxic effects, as well as pharmacological or behavioral sensitization due to the drug infusions. In a subset of six rats, bilateral nomifensine infusions started 20 min before the onset of licking session and lasted 20 min during the session. In four additional rats, nomifensine infusions occurred on day 1 and day 3, but began 40 or 60 min before the sucrose-licking sessions.

In a third group \( (n=14) \), the neurochemical and behavioral effects of dopaminergic drugs were tested. In six of these rats, separate tests on different days determined the effects of bilateral reverse dialysis of a combination of D1 and D2 receptor antagonists alone or added to nomifensine on extracellular DA and metabolites in the NAcc and on sucrose licking. The first day of the test, rats were perfused with ACSF to obtain baseline values. On the second and third days, drugs were assigned randomly. Thus, a test day with nomifensine + antagonists was either followed or preceded with infusions of the two antagonists alone. In a second group \( (n=8) \), the D1 and D2 antagonists were tested independently (two concentrations each). After a control day with ACSF, either SCH23390 or sulpiride was infused in two doses (lower followed by higher) on consecutive days. Only one drug was tested in each rat.

In order to compare the effect of different drugs on the same rat’s intake and on the neurochemistry of the same probe, the training regimen had to be changed in this third group. These rats only had access to sucrose during the daily 20-min licking periods throughout the experiment (i.e., 4–6 days prior to, and 3 days with dialysis).

2.4. Neurochemical assay

Dopamine, dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), and 5-hydroxy-indoleacetic acid (5-HIAA) were analyzed by reverse phase high-performance liquid chromatography with coulometric detection. Samples \( (20 \mu l) \) were injected directly into a Rheodyne injector (model: 9125 with 50 \( \mu l \) sample loop) leading to a 15-cm column with 3 mm bore, 3 \( \mu m \) C18 packing (ESA MD-150). The mobile phase contained 60 mM sodium phosphate, 100 \( \mu M \) EDTA, 1.24 mM heptanesulfonic acid (Sigma Chemical Co.), and 6% (v/v) methanol at pH 3.6. Once separated, compounds were measured with a Coulomem II system (ESA, Inc., Chelmsford, MA; analytic cell: model 5014B, electrode 1: \( -175 \text{ mV} \); electrode 2: \( +175 \text{ mV} \); guard cell: model 5020: \( +300 \text{ mV} \)). The detection limit for DA was approximately 0.4 pg/20 \( \mu l \) standard sample.

2.5. Statistical analyses and histology

In most cases, the probes were functional for 3 days. In case of probe failure, however, the data obtained from that rat were excluded from the statistics. In addition, rats with only one functional probe were not tested for the behavioral effect of infusions. Absolute, basal recovery of DA and metabolites varied considerably between subjects. For this reason, peak areas (analyzed on a PC computer with a Chromatographic Data System, ESA501) were converted to a percent of the mean of three baseline samples taken 1 h before treatment. Data were analyzed by two-way ANOVA with repeated measures followed by post-hoc Newman–Keuls tests when justified (Statistica 5.0). Assessment of dose–responses and interactions required analysis of covariance and linear-regression analyses (Statistica 5.0). Behavioral data were analyzed with one-way ANOVAs and \( t \)-tests (Statistica 5.0). Normally \( n \)-values reflect the number of subjects except for the neurochemical results where \( n \)-values correspond to the actual number of series of samples used for that particular analysis. Although samples were taken bilaterally, for technical reasons, in some instances data from unilateral series of microdialysates were used for statistics (i.e., loss or contamination of individual samples during chromatography).

Histology was performed to verify placement of the microdialysis probes in the NAcc. Data from rats with misplaced probes were excluded from the statistics as well as from the presentation of data. Subjects received an overdose of sodium pentobarbital (100 mg/kg, i.p.) and were perfused transcardially with 0.9% saline followed by 10% formalin. Brains were removed, frozen, and serially sectioned at 50 \( \mu m \). Slices were stained with cresyl Lecht violet.

3. Results

3.1. Effect of licking sucrose on NAcc neurochemistry

Average, absolute basal recoveries of DA, DOPAC, and HVA were 0.05\( \pm 0.02 \), 19.4\( \pm 2.8 \), 8.1\( \pm 1.2 \), respectively (pmol/20 \( \mu l \) sample\( \pm \text{S.E.M.} \), not corrected for probe recovery). Intakes of sucrose and water were 18\( \pm 1 \) ml (mean\( \pm \text{S.E.M.} \), range 12–25 ml) and 4\( \pm 2 \) ml (range 1–7 ml), respectively.
As it is shown in Fig. 1, neither DA nor its metabolites were affected significantly by water licking (open circles in Fig. 1). In contrast, in the same rats DA increased significantly in response to sucrose in the 80-min sample (305±69%, F(1,18)=9.56, P<0.01, n=10). Sucrose licking also caused a significant increase in metabolite levels compared with water intake (DOPAC: F(8,144)=8.52, P<0.01, n=20, HVA: F(8,144)=5.48, P<0.01, n=20).

3.2. Behavioral and neurochemical effects of blockade of DA reuptake

Nomifensine infused bilaterally in the NAcc for 20 min prior to and 20 min during the licking session caused a significant 152±5% increase in sucrose intake (t(5)=6.34, P<0.01, n=6; Fig. 2, bottom left), but did not have any effect on water intake (t(5)=0.36 NS, n=6). In four rats, pre-session nomifensine infusions were extended to 40 and 60 min. These longer infusions resulted in lower sucrose intakes than did the standard 20-min pre-infusions (40-min: 118±6%, n=4; 60-min pre-infusions: 86±7%, n=4, Fig. 2). Although these latter two intake values did not differ significantly from the baseline, taken with the 20-min value, sucrose intake showed a strong negative correlation with the duration of the nomifensine infusion (r=−0.96, F(1,8)=87.98, P<0.001, n=10). In contrast, there was no correlation between sucrose intake and the magnitude of DA increase (Fig. 2 top, r=−0.22, F(1,8)=0.34, NS, n=10).

3.3. Behavioral and neurochemical effects of D1 and D2 antagonists

The changes in sucrose intake produced by intraaccumbens infusions of dopaminergic drugs are summarized in

Fig. 1. Extracellular levels of dopamine (top) and metabolites dihydroxyphenylacetic acid (DOPAC, middle) and homovanillic acid (HVA, bottom), expressed as a percentage of mean baseline levels before, during, and after 20-min presentations (from 60 to 80th min; dashed bracket) of 0.3 M sucrose solution (filled circles, n=6) and water (open circles, n=6).

Fig. 2. Effect of different durations of nomifensine infusions (25 μM) on NAcc DA release (closed squares with error bars on the top) and on the concurrent 20-min sucrose intake (closed columns at the bottom). Intakes are expressed as a percentage of the mean baseline sucrose intake of the same rats perfused with ACSF on other days. Nomifensine was infused for 20, 40, 60 min prior to ('Duration of pre-session infusions') and 20 min during the licking session. For statistics and more explanation, see the text.
Fig. 3. Neither dose of SCH23390 had an effect on sucrose intake but the high dose of sulpiride increased consumption to 120±3% of baseline (t(3)=6.24, P<0.01, n=4). Coinfusion of the high dose of SCH23390 with the low dose of sulpiride (D1+D2) brought the sucrose intake back to normal (105±3%, n=6). Nomifensine alone increased intake substantially (Figs. 2 and 3, coarse crosshatch bar). Coadministration of the antagonists with nomifensine also reduced the sucrose intake to near normal (115±4%, t(5)=2.47, P<0.05, n=6).

With or without receptor antagonists, nomifensine increased DA levels (Fig. 4, top, 80th min: 1338±239%, n=7, and 1484±346%, n=10, respectively); the difference between the groups was not significant. When infused without nomifensine, the blockers also increased DA, but much less so (80th min: 183±19%, t(9)=3.95, P<0.01 n=10). Although DOPAC and HVA levels increased in all groups, this effect was more pronounced in presence of blockers (Fig. 4, middle and bottom charts). The infusions did not affect the 5-HIAA levels (not shown).

Except for the higher dose of sulpiride causing a significant increase in DA (80th min: 206±37%, t(6)=4.28, P<0.01, n=7), prior to sucrose availability the D2 antagonist had no effect on the levels of DA or its metabolites (Fig. 5). In contrast, NAcc reverse dialysis of sulpiride before and during sucrose intake resulted in lasting (>60 min) and dose-dependent increases in DOPAC and HVA levels (Fig. 5, circles, F(1,7)=6.97,
stimulating effect on DA release. During sucrose intake, however, both doses of the D2 antagonist increased the DA turnover compared with ACSF controls (D2L, +151%, t(6)=4.35, P<0.01, n=7; D2H, +180%, t(6)=5.02, P<0.01, n=7). Furthermore, coinfusion of the low dose of SCH23390 with the high dose of sulpiride (D1+D2) diminished DA turnover prior to sucrose intake, but had no effect on the DA release or metabolism during sucrose ingestion.

3.4. Histology

Histological analysis revealed that most microdialysis probes were located in the caudal part of the NAcc — planes A: 10.6–9.7 of Paxinos and Watson [28], medial to the anterior commissure (L: 0.6–1.4) in 26 of 30 animals. The results from the four rats with misplaced probes were discarded. The tracks of the probes were always identified, but precise localization of the tip of the dialytic membrane was more difficult. The dialytic membranes are narrow and thus produce less damage than the remainder of the probe. In addition, the membranes often bent slightly and thus the tips ended at an angle in adjacent sections. Overall, the probes ended from the medial shell to the medial core of
the NAcc. In nine subjects, the microdialysis probe on one side was displaced slightly in the anteroposterior plane compared with the contralateral tip, i.e., A 9.7–9.5 or A 10.7–11.0. The neurochemical data obtained from these sites, however, were within the range of the more centrally located probes in the corresponding sample groups.

4. Discussion

4.1. Effect of licking sucrose on NAcc neurochemistry

The experiments revealed an increase in NAcc DA release and metabolism in response to licking a 0.3 M sucrose solution by overnight-deprived rats. In contrast to findings with longer deprivations or trained licking [53,54], in the present experiment, licking water caused no changes in NAcc DA activity. It is worth noting, however, that the intake of water and sucrose differed, i.e., the rats consumed 15–24 ml of sucrose but only 1–8 ml of water. Therefore, an effect of total motor activity on DA release cannot be excluded, and was not assessed in these experiments. Another possible explanation is that, since ‘water-days’ were alternated with ‘sucrose-days’, the highly preferred sucrose solution suppressed the rewarding properties of water even though the rats were fluid deprived for 16 h.

The temporal resolution of microdialysis in general and the 20-min sampling rate used here in particular, does not allow us to exclude the effect of anticipation on DA release [4]. Nevertheless, there were no significant differences in baseline samples between rats that trained on sucrose exclusively (third experiment) and those that received sucrose or water alternately (groups 1 and 2).

Because the fluid was ingested, a further distinction between the caloric and hedonic components of sucrose awaits the results of sham-feeding experiments.

4.2. Effect of blockade of DA reuptake on sucrose intake

Reverse microdialysis of the DA reuptake blocker nomifensine in the NAcc caused an increase in sucrose intake. Since water licking did not change with nomifensine infusions in the same rats, this effect seems specific to the stimulus, i.e., sucrose. The behavioral effect of nomifensine was almost completely reversed by coinfusion of D1 and D2 receptor antagonists indicating the DA-specificity of the increased sucrose licking.

With longer exposures, however, nomifensine reduced sucrose consumption or left it unchanged and produced no changes in the respective DA levels. This phenomenon may reflect pharmacological effects, but it may also have behavioral relevance. Sills and Vaccarino [41] showed that a low dose of amphetamine increased sucrose consumption in rats that normally are low responders. Under other conditions (i.e., higher responders, higher doses), amphetamine generally decreased sucrose consumption when injected into the NAcc.

Our results are consistent with studies in which cocaine and DA reuptake blockers enhanced brain stimulation reward [1,13,20,25]. Despite differences between cocaine self-administration and the role of NAcc DA in food reinforcement [5], one may conclude that DA reuptake blockade in the NAcc accentuates behavior supported by both artificial and natural rewards.

4.3. Behavioral and pharmacological effects of D1 and D2 antagonists

Infusions of the D1 receptor antagonist SCH23390 in either dose or the D2 receptor antagonist sulpiride in a low dose had no chemical or behavioral effect. Sulpiride in a higher dose, however, increased both DA release and sucrose consumption. In contrast to the observed increase in DA levels, the high dose D2 antagonist failed to increase sucrose intake in presence of the low dose D1 blocker. Therefore, one may assume that the behavioral effect is D1-mediated.

Under basal condition (i.e., without the high DA levels induced by nomifensine), combined infusion of the D1 and D2 blockers into the NAcc had no effect on sucrose intake. This militates against a tonic influence of D1 and D2 receptor activity on this ingestive behavior. This finding is consistent with the literature indicating that intraaccumbens DA manipulation disrupts different aspects of feeding behavior differentially. Specifically, when lab chow is concurrently available with reinforcement pellets in an operant chamber, NAcc DA antagonism or depletion decreases lever pressing for pellets but actually increases chow consumption [9,10,36]. Furthermore, DA receptor manipulations could be site specific within the striatum [44,51,52]. Infusion of quinpirole, a selective D2 receptor agonist, into the NAcc failed to affect food intake [44]. In contrast, perfusion of another selective D2 receptor agonist, bromocriptine, was effective if injected into the ventrolateral striatum [18]. Pretreatment with sulpiride perfusion that decreased food intake and increased DA release if infused alone inhibited the increase of food intake induced by bromocriptine [18]. These latter examples taken with the discrepancies between our present findings and those from peripheral injections of DA receptor antagonists (see Introduction) provide some rationale for reconsidering the relationship between accumbens DA and sucrose licking, if not feeding reward in general.

4.4. Interactions between D1 and D2 receptor antagonists during sucrose licking

Administered alone, both D1 and D2 antagonists diminish the sucrose-related DA release. This suppressive effect,
however, was not seen if the blockers were co-administered. An analysis of DA turnover revealed further evidence of altered receptor interactions during sucrose licking. Sulpiride dose-dependently enhanced DOPAC levels during sucrose intake, which resulted in higher DOPAC/DA ratios (Fig. 6). These findings agree with those found during feeding in hungry rats [48]. D1 receptors also appear to be involved, because the effect of sulpiride on sucrose-related turnover changes was blocked by a co-administration of the D1 antagonist. In contrast, infusion of D1 blocker alone failed to influence DA turnover or release. Thus, there are differences in the interaction of DA receptors under basal conditions and during sucrose licking.

5. Conclusions

The role of the NAcc DA in natural and artificial rewards has been debated in different contexts over a number of years. The present experiments were not designed to determine the role of NAcc DA in mediation of reward and reinforcement in general, or in food reward in particular. Neither the paradigm nor the method used here is suitable to that purpose. Our experiments were restricted to assessing how sucrose licking affects DA release in the NAcc, and vice versa, i.e., how artificially elevated DA levels influence this behavior.

The results demonstrate that DA release in the NAcc increased in response to spontaneous sucrose licking. On the other hand, interfering with DA receptors in NAcc failed to reduce sucrose intake, contrary to prior reports in which the antagonists were injected systemically. Local infusions of nomifensine which increase DA do increase sucrose intake. Together the two findings indicate that NAcc DA can amplify the sucrose intake, but the behavioral response itself is not under tonic DA control. A similar augmentation of behavior by DA-uptake blockade in the NAcc has been shown previously in cocaine- and self-stimulation studies. Whether this behavioral activation represents a change in the reward value of the stimulus or a facilitation of other components of the behavior, remains to be determined.

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