Pharmacology Biochemistry & Behavior, Vol. 39, pp. 1025–1028. © Pergamon Press plc, 1991. Printed in the U.S.A. 0091-3057/91 $3.00 + .00

BRIEF COMMUNICATION

Failure to Detect Increases in Brain Dopamine Metabolism in Rats Sham Feeding Sucrose and Corn Oil

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Received 15 February 1991

WEATHERFORD, S. C., D. GREENBERG, L. D. MELVILLE, C. JEROME, J. GIBBS AND G. P. SMITH. *Failure to detect increases in brain dopamine metabolism in rats sham feeding sucrose and corn oil.* PHARMACOL BIOCHEM BEHAV **39**(4) 1025–1028, 1991.—In a recent study we found that when rats sham fed 6% sucrose, 10% sucrose, and 100% corn oil, the rank order of inhibitory potency for D-1 and D-2 receptor antagonists was 6% sucrose > 10% sucrose > 100% corn oil. In a complementary study, sham-feeding rats preferred 100% corn oil > 10% sucrose > 6% sucrose as measured by two-bottle preference tests. The preferences are evidence for the rank order of reward value of these solutions. In the present study we tested the hypothesis that the relative antagonist potencies were due to differential release of DA, dependent on the reward value of the sham-fed solution. Dopamine metabolism, estimated by the ratio of dihydroxphenylacetic acid (DOPAC) to DA, was measured in forebrain-DA terminal fields during sham feeding of 100% corn oil, 6% sucrose, and 10% sucrose. The results did not support our hypothesis: no increase in DA metabolism was observed after the sham feeding of any solution.

Sham feeding Fats Sweet taste DOPAC/DA ratio Food reward Preference Control of eating

RATS prefer foods high in fat and sugar (2, 4, 11). Postingestive metabolic effects clearly play a role in the preference for both nutrients (8,12), but studies showing that rats sham feed sugar solutions and oils (3, 7, 20) in a concentration-dependent manner, demonstrate that the orosensory qualities of these nutrients are sufficient to stimulate ingestion in the absence of postingestive cues. Since sham feeding permits the orosensory effects of nutrients to act on eating, but minimizes (13) or excludes (22) the postingestive effects, the sham feeding of sugar solutions and oils is strong evidence that the orosensory effects of these nutrients are positively reinforcing for the oromotor acts of licking and ingestion.

Several studies support the hypothesis that central dopamine (DA) systems mediate the rewarding effects of sugars (3, 10, 14–16) and fats (17,18) in rats. This hypothesis has been most rigorously investigated in the case of sweet taste and is supported by the finding that sham fed sucrose increases dopamine (DA) metabolism in the forebrain (15). In addition, selective D-1 and D-2 receptor antagonists decrease the sham feeding of sucrose (9,10) and corn oil (17,18), suggesting that central dopaminergic activity at these receptors is necessary for the normal positive reinforcing effect of sucrose and other sweet solutions and for fats.

In a recent study, we found a differential potency of the selective D-1 antagonist, SCH 23390 (5), and the selective D-2 antagonist, (−) raclopride (6), for decreasing the sham intake of 100% corn oil, 10% sucrose and 6% sucrose, such that the rank order of inhibitory potency for both antagonists was 6% sucrose > 10% sucrose > 100% corn oil (17). In a second study (17) we found that the rank order of preference for these three liquids was 100% corn oil > 10% sucrose > 6% sucrose as measured by two-bottle preference tests. Thus the inhibitory potency of both antagonists was inversely related to the reward value of the liquids that were sham fed.

This result is consistent with the dopamine hypothesis of the positive reinforcing effect of orosensory stimulation by nutrients (16,21) in that the hypothesis predicts that a more reinforcing stimulus would be more difficult to antagonize because more DA would be released at relevant receptor sites. In the present experiment we test this hypothesis by measuring DA metabolism, using the ratio of dihydroxyphenylacetic acid (DOPAC) to DA in forebrain DA terminal fields of rats that sham fed these three

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liquids. A preliminary report of these results has appeared (19).

METHOD

Twenty-four male Sprague-Dawley rats (Taconic Farms, Germantown, NY) weighing 210–278 g were used in this experiment. Rats were housed and tested in individual hanging plastic cages in a temperature-controlled environment (21 ± 1°C) with a 12:12 light/dark cycle (lights on 0800). Rats were maintained on Purina rat chow and tap water.

Surgical Procedure

All rats, except the control group, were implanted with chronic stainless steel gastric cannulas according to previously described methods (22) and were allowed a minimum of two weeks to recover.

Testing Procedure

All rats were placed on a 17-h, overnight, food-deprivation schedule and were trained to sham feed one of three liquids: 100% corn oil (n = 5), 6% sucrose (n = 6) or 10% sucrose (n = 6). At 0900 the screw cap occluding the cannula was removed and the stomach was gently lavaged with tap water until the gastric drainage was free of food particles. To facilitate drainage and collection of gastric contents, a Silastic drainage tube surrounded by a flexible metal spring was threaded into the shaft of the cannula. Rats were returned to their cages and the drainage tube was passed through a longitudinal gap in the wire-mesh floor, thus allowing the rat free movement throughout the experimental period. A plastic tray was placed below the rats' cages to collect gastric drainage. Rats were then offered 100% corn oil (Mazola, Englewood Cliffs, NJ; blended with Tween-80; Sigma Chemical Co., St. Louis, MO; 0.75 ml Tween-80 per 100 ml corn oil), 6% sucrose (Sigma Chemical Co., St. Louis, MO; w/v in distilled water) or 10% sucrose (w/v in distilled water) in calibrated drinking tubes (Wahmann) for a 30-minute sham feeding test. At the conclusion of the test, 30-minute sham intake was noted, the drainage tube was removed and the screw cap was replaced. Pellets were returned immediately after the test and were available until the next deprivation period began. Tap water was available at all times except during the test.

Rats were tested five days a week under these conditions until sham-intake had stabilized (>15 training sessions). Sham intakes for each group stabilized at 34 ± 3 ml for 100% corn oil; 37 ± 2 ml 6% sucrose; and 47 ± 3 ml 10% sucrose.

In the final week of testing rats were allowed to sham feed their respective solutions for three consecutive days. On the third test day rats were allowed to sham feed their assigned solution for 9 min, at which time intakes were recorded and the rats were immediately decapitated. Rats that served as control animals (N = 7) were decapitated at the end of the 17-h overnight deprivation period.

Sample Preparation and Neurochemical Measurements

The brains were rapidly removed and dissected on a chilled platform according to a method described previously (15). The regions obtained were olfactory tubercle, nucleus accumbens, hypothalamus, amygdala-pyriform cortex and striatum.

The tissue samples were weighed and stored on dry ice for approximately 3 h and processed as described previously (15) with the exception that 0.5% L-cysteine was used as the antioxidant.

Protein analyses were performed on all of the tissue pellets according to the procedure of Bradford (1).

Regional brain analysis of DOPAC, HVA and DA was performed by reverse phase high performance liquid chromatography with electrochemical detection. The system consisted of a Waters model 510 pump (Waters Assoc., Milford, MA) linked to a Waters "WISP" Automatic Sample Processor, model 710A and a Waters M7 30 recorder-integrator. Electrochemical measurements were made by a Bioanalytical Systems LC-4B detector with a glassy carbon electrode, and the potential set at +0.75 V with the sensitivity set at 10 nA/V full scale. The mobile phase was 0.1 M phosphate buffer, containing 0.1 mM EDTA and 0.35 mM 1-octyl sulfonic acid and 15% methanol. The analytical column used was a C-18, 10 μm Radial PAK cartridge (Waters, Milford, MA).

Statistics

Statistical analyses were performed using a one-way analysis of variance.

RESULTS

DA, DOPAC and the ratio of DOPAC/DA did not increase in any group of sham-feeding rats relative to non-sham feeding controls in any brain region examined (see Table 1).

The protein expressed as a percentage of tissue weight, of the various regions was: olfactory tubercle = 4.41 ± 0.30% (N = 24), nucleus accumbens = 8.44 ± 0.66% (N = 23), hypothalamus = 6.92 ± 0.31% (N = 22), amygdala-pyriform = 5.03 ± 0.28% (N = 24), and striatum = 6.68 ± 0.36% (N = 24).

DISCUSSION

The ratio of DOPAC/DA did not change significantly in any brain region examined in rats that had sham-fed sucrose or corn oil for nine minutes. This result does not support the hypothesis that the differential potency of D-1 and D-2 antagonists for decreasing sham intake of these three liquids is due to a differential release of DA dependent on the reward value of the sham-fed solution.

Our negative result with 10% sucrose is not consistent with the results of Smith et al. (15) who found a significant increase in DOPAC/DA in the hypothalamus after 9 minutes of sham feeding 10% and 40% sucrose. But it is worth noting that 10% sucrose appears to be the "threshold" concentration for increasing the ratio of DOPAC/DA in the sham-feeding paradigm, because in two of three experiments 10% sucrose failed to increase the ratio. It may also be important that DOPAC/DA was reliably increased in rats that had sham fed a more rewarding 40% sucrose solution (20) in the study by Smith et al., while this ratio was not affected in our experiment in rats that had sham fed corn oil, which was also found to be preferred over 10% sucrose (17). Inasmuch as the reward value of corn oil relative to 40% sucrose is not known, it is not possible to determine if our negative result was because the reward value of corn oil is "subthreshold" or because the orosensory qualities of corn oil are not a stimulus for DA release.

It is possible, of course, that a more sensitive measure of DA release, e.g., in vivo microdialysis, may detect differences among these stimuli that we did not observe. But until such measurements are made, we consider that our results do not support the hypothesis that the correlation between relative preference and resistance to D-1 and D-2 antagonists is the result of differential release of dopamine at central synapses.

ACKNOWLEDGEMENTS

This research was supported by NIMH MH15455 and RSA MH00149 (G.P.S.).

TABLE 1

REGIONAL CONCENTRATION OF DOPAC AND DA, AND RATIOS OF DOPAC/DA AFTER 9 MINUTES OF SHAM FEEDING 100% CORN OIL, 6% SUCROSE, OR 10% SUCROSE

Group	DA ng/mg Protein	DOPAC ng/mg Protein	DOPAC/DA
		Hypothalamus	
Corn Oil	4.60 ± 0.60	0.60 ± 0.07	0.14 ± 0.010
6% Sucrose	5.07 ± 0.51	0.71 ± 0.17	0.14 ± 0.060
10% Sucrose	4.50 ± 0.32	0.55 ± 0.05	0.12 ± 0.006
Control	5.77 ± 1.03	0.69 ± 0.17	0.11 ± 0.008
		Nucleus Accumbens	
Corn Oil	90.66 ± 29.33	17.46 ± 5.07	0.20 ± 0.020
6% Sucrose	36.07 ± 11.55	7.21 ± 2.10	0.20 ± 0.010
10% Sucrose	57.68 ± 13.98	11.07 ± 3.00	0.18 ± 0.009
Control	70.45 ± 18.90	12.20 ± 4.00	0.17 ± 0.010
		Striatum	
Corn Oil	315.34 ± 50.15	31.87 ± 5.11	0.09 ± 0.003
6% Sucrose	291.47 ± 33.19	24.82 ± 1.31	0.09 ± 0.009
10% Sucrose	312.12 ± 20.52	32.34 ± 3.20	0.10 ± 0.005
Control	266.96 ± 25.25	25.88 ± 2.77	0.10 ± 0.004
		Amygdala and Pyriform Cortex	
Corn Oil	9.60 ± 2.47	1.47 ± 0.38	0.20 ± 0.04
6% Sucrose	11.34 ± 3.80	2.50 ± 0.86	0.23 ± 0.07
10% Sucrose	20.39 ± 0.56	3.26 ± 0.79	0.16 ± 0.02
Control	12.72 ± 3.60	1.97 ± 0.69	0.14 ± 0.02
		Olfactory Tubercle	
Corn Oil	128.92 ± 10.80	19.72 ± 1.90	0.15 ± 0.008
6% Sucrose	113.08 ± 08.70	17.15 ± 1.68	0.15 ± 0.008
10% Sucrose	122.94 ± 09.97	16.64 ± 2.20	0.13 ± 0.010
Control	104.83 ± 14.14	15.04 ± 2.20	0.14 ± 0.006

Data are mean ± SE; number of rats and sham-fed intakes (mean ± SE ml/9 min) in each group were corn oil (n=5) 10.6 ± 2.0, 6% sucrose (n=6) 11.0 ± 0.5, 10% sucrose (n=6) 15.3 ± 1.8, and control (n=7) 0.

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