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Effects of Atropine Methyl Nitrate on Sham Feeding in the Rat

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WEINGARTEN, H. P. AND S. D. WATSON. *Effects of atropine methyl nitrate on sham feeding in the rat.* PHARMAC. BIOCHEM. BEHAV. 17(4) 863–867, 1982.—The purpose of this experiment was to clarify the effects of administration of peripheral cholinergic blocking agents on sham feeding. Rats were implanted with chronically indwelling gastric cannulae and allowed to sham feed sucrose solutions varying in their degree of diet palatability. Prior to the sham feeding tests, rats received an intraperitoneal injection of either atropine methyl nitrate (250 μg/kg) or a control substance, physiological (0.9%) saline. The results indicated that atropine methyl nitrate inhibited sham feeding at all levels of diet palatability tested. This reduction in food intake level following drug administration was apparent from the initiation of the sham feeding bout and did not result from a more rapid cessation of feeding under the drug condition. Further, similar doses of AMN did not reduce sham drinking of water in water-deprived animals. The implications of these findings to a cholinergic involvement in food intake control systems are discussed.

Sham feeding Cholinergic blockade Gastric cannula Palatability Cephalic phase Food intake Atropine Hunger

ALTHOUGH the suppression of food intake following administration of peripheral cholinergic blocking agents has been recognized for some time, views regarding the mechanism underlying this drug effect have recently altered. Originally, it was believed that peripheral anticholinergics inhibited feeding only indirectly by interfering with responses that played a permissive role in feeding. Specifically, it was proposed that these drugs suppressed salivary secretions, thus producing a dry mouth and, therefore, difficulty with chewing and swallowing [6,8]. In contrast to such nonspecificity interpretations, more recent empirical findings and theoretical speculations suggest a more direct involvement of cholinergic systems in ingestion and, therefore, a more specific consequence of peripheral cholinergic blockade on food intake control mechanisms. For example, it is suggested that neurally-mediated anticipatory and cephalic phase responses may serve a critical function in the elaboration of hunger and satiety [4,5]. The particular metabolic event which has received most attention in this context, neurally-mediated insulin secretion, is cholinergic [11,12] and many other (but not necessarily all) of these responses depend on the integrity of physiological systems which have acetylcholine as their neurotransmitter. One of the most specific statements regarding the cholinergic function in the control of food intake is based upon the recent demonstration that atropine methyl nitrate (AMN) inhibits sham feeding, but not sham drinking, in the rat [3]. This result was taken as evidence that the blockade of some peripheral cholinergic mechanism was involved with the elicitation of satiety.

The focus of this paper is to examine the role of cholinergic systems in feeding by further analyzing this latter experimental finding of a suppression of sham feeding following AMN administration. Several ambiguities surround the initial demonstration of inhibition of sham feeding with AMN-treatment. First, another study using a larger effective dose of AMN failed to obtain a reduction in the amount of sham feeding [1]. Second, it has recently been demonstrated that the magnitude of sham feeding depends critically on the palatability of the diet used during the feeding tests [9]. Thus, it is unclear whether an AMN-induced inhibition of sham feeding represents a general characteristic of this drug treatment or whether this reported effect is peculiar to the level of diet palatability used in that original experiment. In fact, this possibility may help to explain the conflicting results in the two previous experiments examining the outcome of AMN administration. Third, many of the details of how this inhibitory phenomenon may operate are still unclear.

EXPERIMENT 1

The following experiment examines in detail the effects of AMN on the sham feeding of a variety of substances varying in palatability.

METHOD

Subjects

Subjects for this experiment were six male Long-Evans

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hooded rats weighing between 220–300 g at the beginning of the experiment. They were housed individually in a room maintained at 21°C and on a 14:10 light:dark cycle. Water was available ad lib and food was provided according to the protocol described below.

Surgery

The surgery involved implantation of a chronically indwelling gastric cannula into each rat. Rats were deprived of food for 24 hours prior to surgery and were anesthetized by sodium pentobarbital (Somnotol—45 mg/kg) for this operation.

Following an initial midline laparotomy, the stomach was exposed and two concentric purse string sutures, approximately 1 cm in diameter were sewn into the fundic portion of the nonglandular stomach. A tiny slit was made in the gastric wall encircled by these sutures and one end of the cannula was inserted into the stomach through this tear. The gastric fistula was completed by exteriorizing the free end of the cannula through the abdominal wall and skin. The fistula was kept closed normally by a screw which threaded into the shaft of the cannula. Complete details of cannula design and implantation surgery have been described previously [10].

Apparatus and Testing Procedure

To prepare a rat for a sham feeding test its gastric fistula was opened by removing the screw which was threaded into the cannula. Residual stomach contents were removed by gentle aspiration applied through the open cannula. A 19.1 mm long stainless steel collecting tube was then screwed into the cannula. A 15 cm plastic drainage tube was force fit onto this collection tube and with this drainage system in place any liquid food ingested orally drained freely out of the stomach and down the collecting tube by gravity flow.

Sham feeding tests took place with the rats housed in rectangular acrylic plastic cages (21.5 cm long × 11 cm wide × 10 cm high, suspended on 20 cm high stilts). The floor of these cages was constructed of longitudinal stainless steel rods separated by one cm except for the two center rods which were spaced 1.6 cm apart to allow the collecting drainage tube to pass through the floor. Test solutions were contained in graduated cylinders mounted on the outside front wall of the test cage. The licking spout of the graduated cylinder protruded approximately 2.5 cm into the test cage through a hole in the front wall of the cage.

Protocol

Rats were permitted a two-week period to recover from the gastric surgery. During this time they were maintained ad lib on Purina Rat Chow pellets.

On the final day of recovery, 85% of the current body weight of each rat was calculated. During the next seven days, each rat was reduced to its individually determined 85% body weight level by controlling the size of its daily food ration. Animals were maintained at this 85% deprivation level (allowing for a growth factor of 2 g/day) for the remainder of the experiment. During this weight reduction phase, and throughout the period of sham feeding tests, rats were maintained on a nutritionally adequate evaporated milk-based diet. The use of a liquid diet permitted effective removal of stomach contents necessary for the sham feeding tests.

The next 12 days of the experiment constituted a training period during which rats became accustomed to the procedures used for sham feeding and, more importantly, learned to lick reliably in the test chamber. During the training phase rats were placed daily in the test cages and permitted to sham feed a 16% (weight/volume) sucrose solution for 30 minutes. Five minutes prior to being placed into the test cage rats received an intraperitoneal injection of physiological (0.9%) saline at a dose of 0.083 ml/100 g body weight. By the end of training all rats sampled the solution in the test cage by licking at the drinking spout within the first 30 seconds of being placed into that chamber.

Testing began following the training period. During testing rats were exposed to three diets differing in their sucrose content—6%, 16% or 30% sucrose (weight/volume) solutions—and tested under two different drug pretreatment conditions—atropine methyl nitrate (AMN) or physiological saline (SAL). Thus there were a total of 6 different test conditions (i.e., 6% SAL; 6% AMN; 16% SAL; 16% AMN; 30% SAL; 30% AMN). On any given day rats were tested under only one of the six possible test situations. The test sequence was random within the constraint that, within a 6-day block, rats were exposed to all six conditions. Rats were tested under each condition on three separate occasions.

The AMN injection was prepared by dissolving the drug powder (Sigma, Inc., St. Louis, MO) in sterile physiological saline. The drug was prepared so that the dose used (250 μg/kg body weight) corresponded to an injection volume of 0.083 cc/100 g body weight. On AMN days, rats were injected (IP) five minutes prior to being placed in the sham feeding cages. On SAL days, equivolume injections of physiological saline were administered to animals five minutes prior to testing. Sham feeding tests were 30 minutes in duration and fluid intakes were recorded every five minutes throughout the test session.

RESULTS AND DISCUSSION

A two-factor analysis of variance was used to assess the effects of drug administration, diet palatability, and the corresponding interaction, on the total amount sham fed during the 30-minute feeding tests. These data are presented graphically in Fig. 1. AMN significantly inhibited the magnitude of sham feeding, $F(1,25)=33.02$, $p<0.01$; at all levels of sucrose, rats sham fed less following AMN pretreatments than after saline injections. The analysis also revealed that the amount consumed depended on the sucrose content of the diet, $F(2,25)=26.29$, $p<0.01$. Animals exhibited a steady increment in the amount consumed with increases in the sucrose concentration of the diet. Although AMN depressed the overall level of intake, a nonsignificant Diet × Drug interaction component, $F(2,25)=1.26$, $p>0.05$, indicated that the tendency to consume more of a sweet diet was unaffected by the administration of AMN.

Further information into the effects of AMN on sham feeding was obtained by analyzing the pattern of feeding behavior during the 30-minute sessions under both AMN and SAL conditions. For each sucrose concentration, the group mean intakes during consecutive 5-minute time bins throughout the sham feeding sessions were calculated and are presented in Fig. 2. Separate analyses of variance were conducted for each diet in order to ascertain the influence of AMN on the magnitude of feeding and whether the drug administration affected the pattern of feeding within the session. As expected from the previous analysis, these statistical tests corroborated that AMN suppressed feeding regard-

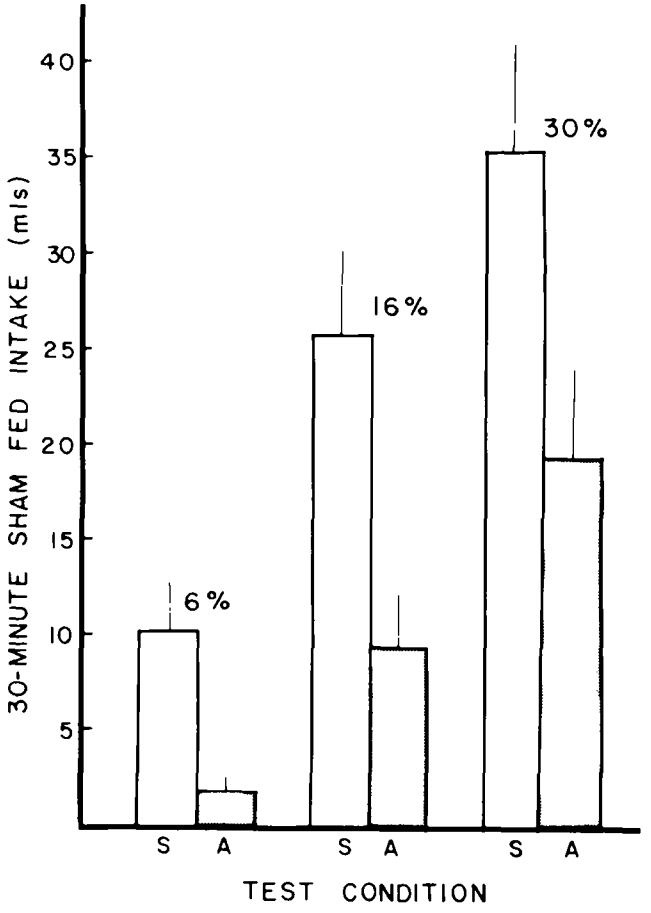

FIG. 1. For each sucrose concentration, the group average (N=6) total sham fed intake following saline (S—white bars) and AMN (A—shaded bars) pretreatments. Vertical lines represent one standard error of the mean.

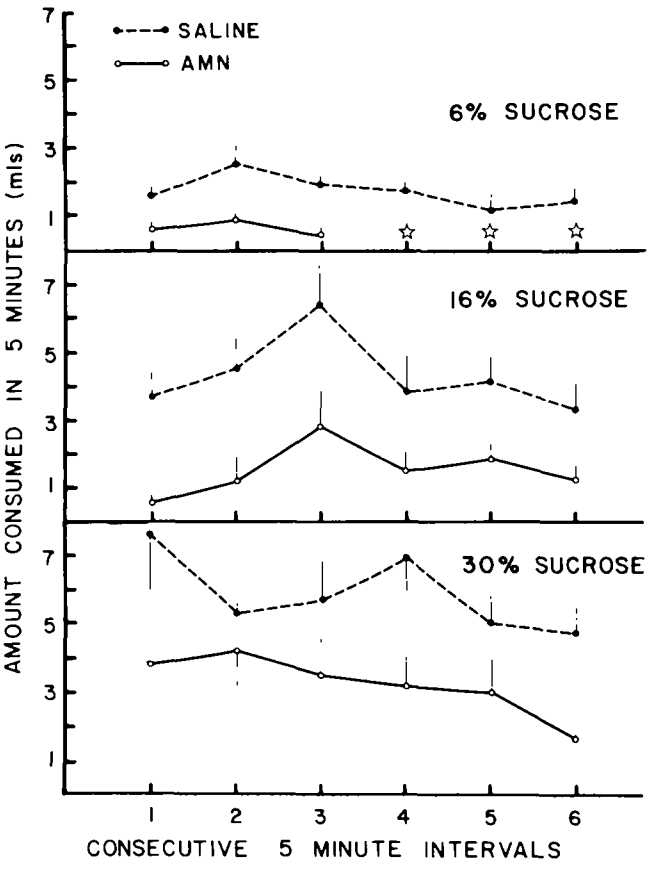

FIG. 2. For each sucrose concentration, the group average sham fed intakes during consecutive 5-minute time bins throughout the test session following both saline and atropine methyl nitrate (AMN) pretreatments. Stars in top panel indicate that no rat consumed any 6% sucrose during any test in the time bins indicated. Vertical lines represent one standard error of the mean.

less of the sucrose content of the food—6% $F(1,55)=58.00$, $p<0.01$; 16% $F(1,55)=71.60$, $p<0.01$; 30% $F(1,55)=28.25$, $p<0.01$. However, this analysis and examination of Fig. 2 also indicated that the AMN-induced inhibition of overall food intake level did not result from a more rapid cessation of feeding under the AMN conditions relative to SAL. To the contrary, the AMN effect was apparent immediately upon the initiation of the sham feeding bout. For every diet tested, rats sham fed less under the influence of AMN during the first 5 minutes of eating than in the corresponding time period after SAL injections. Furthermore, the fact that none of the Diet × Time interactions were significant (for each sucrose level, $p>0.05$) indicated that, although AMN affected the overall level of eating, it did not alter the pattern of ingestion within the session.

EXPERIMENT 2

The previous experiment indicated that AMN resulted in a reduction of sham feeding regardless of the palatability of the diet being consumed. One of the limitations of this finding is that, although rats were food-deprived and ingesting sucrose solutions, these rats, in fact, are drinking. The present study examines the specificity of the AMN suppression of intake by investigating whether this drug also inhibits the sham drinking of water in water-deprived animals.

METHOD

Subjects

Subjects for this experiment were 4 male Long-Evans hooded rats weighing an average of 356 g at time of gastric surgery. They were housed under conditions identical to those described in Experiment 1 except that water available on the schedule detailed below.

Protocol

Initially, a chronically indwelling gastric cannula was implanted into each rat. Rats were permitted a three week recovery period with water and food available ad lib.

Throughout the period of sham feeding tests rats had food available ad lib in the home cage. However, water was rationed so that all rats were 20-hr water deprived at the time of sham feeding.

Sham feeding tests were conducted in a manner identical

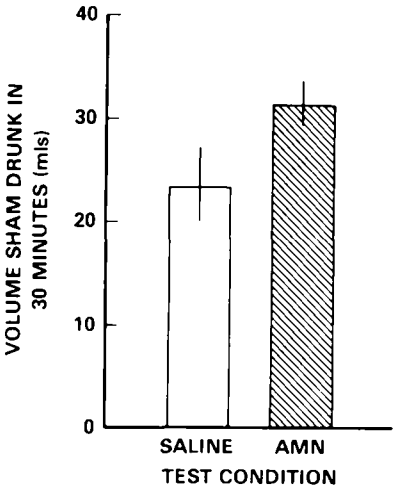

FIG. 3. Average amount sham drunk in 30 minutes (N=4) on the last 2 days of testing with saline preinjections and on the following 2 days with preinjections of atropine methyl nitrate (AMN). Vertical bars represent one standard error of the mean.

to those described in Experiment 1 except that rats were water-deprived and, during testing, were permitted to sham drink water. As in Experiment 1, rats were trained initially to lick reliably. On these training days, they were injected with physiological saline 5 min prior to sham drinking. The effects of AMN on sham drinking were assessed once the magnitude of sham drinking on saline days had stabilized. The influence of AMN was examined by injecting rats with a dose of AMN (250 μg/kg) 5 min prior to their sham drinking test. On all days, sham drinking tests were 30 min long and fluid intake during consecutive 5 min time periods throughout the session were recorded.

RESULTS AND DISCUSSION

The amount sham drunk by rats in the 30-min test sessions stabilized by about the seventh training trial. In order to compare the effects of AMN on a stable sham drinking background sham feeding tests with saline preinjections were continued for 5 more days. Figure 3 shows the average total water consumption in 30 min on the last two days of saline trials (Days 11 and 12) and on the first two experiences with AMN pretreatments (Days 13 and 14). Injecting animals with AMN prior to sham drinking did not reduce the amount consumed. In fact, the amount drunk after AMN pretreatments tended to be greater than that observed following saline administration. A correlated t-test comparing water intake following either saline or AMN preinjection was performed and these results coupled with an examination of Fig. 3 revealed that administration of the peripheral cholinergic blocker produced an increased water consumption which approximated a significance level, $t(3)=2.77$, $p\cong0.07$ (two-tailed).

These data reveal that in contrast to the suppressive effects of AMN on sham feeding, the same peripheral cholinergic blockade does not inhibit (and, in fact, tends to enhance) sham drinking of water in water-deprived animals.

GENERAL DISCUSSION

These experiments reveal that the administration of a peripheral anticholinergic agent, atropine methyl nitrate, results in the inhibition of sham feeding in the rat. These data support a previous report of a reduction in the magnitude of sham feeding as a result of this pharmacological treatment [3] and further reinforce their observations that this suppression of intake is specific to hungry rats sham feeding liquid diets and does not generalize to an inhibition of sham drinking situation with thirsty animals. The particular results obtained in this experiment also provide some further details into the nature of this drug effect and, therefore, permit additional speculation as to the mechanisms underlying this drug-induced suppression and the role of peripheral cholinergic systems in feeding.

One outcome of this study is the indication that the inhibitory effect of anticholinergics on sham feeding is evident regardless of the palatability of the diet being ingested. In this experiment, a reduction in the amount consumed following AMN injection was observed at all levels of sucrose tested. This suggests that the reduction of intake following disruption of peripheral cholinergic systems by AMN is a generalized effect and is not dependent on, or peculiar to, the nature of the foodstuff used during the feeding tests. The nature of the results obtained here also eliminate from consideration one possible explanation of the AMN-induced suppression of feeding. Since sham feeding only activates oral sensory factors the response profile of animals sham feeding a variety of diets differing in palatability indicates their reactivity to changes in the stimulus properties of food. We have reported previously [9], and have replicated here, that under normal conditions one obtains a steadily increasing level of intake with increases in palatability (in this case, increases in the sucrose concentration of the solution). This normal excitation of feeding in response to enhanced levels of diet palatability is unaffected by the administration of AMN. Under the influence of this drug, although reduced in absolute level, animals exhibited the identical profile of consumption across the sucrose concentrations as under the saline control conditions. Thus, whatever the mechanism underlying this reactivity to hedonically positive taste cues, it appears to be unrelated to cholinergically mediated events occurring in the periphery.

The present findings do provide some information as to the mechanism underlying the AMN drug effect, and, therefore, lead to certain speculations on the role of cholinergic visceral events in the control of feeding. Originally, the suppressive action of AMN on sham feeding was tentatively interpreted as indicating that peripheral cholinergic blockade elicits or contributes to satiety [3]. The present data suggest a somewhat different characterization of this drug-related phenomenon. If AMN inhibits sham feeding by eliciting or potentiating pregastric satiety cues, as has been speculated [3], then one might expect to see a more rapid cessation of sham feeding after AMN administration relative to saline conditions. This prediction stems from the suggestion that agents which operate via satiety mechanisms should be expected to exert their effects when satiety becomes activated, i.e., near the terminal aspects of a meal [2]. However, as the within session analyses reveal in this experiment, no such tendency is observed. In fact, there is no alteration in the pattern of consumption with AMN, compared to saline trials, once the sham feeding bout has begun. This observation, coupled with the fact that the reduction of food intake is

apparent immediately upon the initiation of feeding is consistent with speculation that anticholinergic agents inhibit some peripheral events (or disinhibit others) the net effect of which is to render organisms less hungry. How this may operate, or whether a formulation that AMN reduces hunger simply represents a semantic rearrangement of a hypothesis suggesting that AMN elicits satiety, awaits further elucidation of the role of peripheral cholinergic events in the elaboration of hunger and satiety. It is noteworthy, however, that the view that peripheral cholinergic blockade may reduce hunger is consistent with historical views as to the contribution of peripheral or visceral sensations to feeding [7].

ACKNOWLEDGEMENTS

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