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Atropine Methyl Nitrate Inhibits Sham Feeding in the Rat

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LORENZ, D., P. NARDI AND G. P. SMITH. *Atropine methyl nitrate inhibits sham feeding in the rat.* PHARMAC. BIOCHEM. BEHAV. 8(4) 405–407, 1978. – Atropine methyl nitrate inhibited sham feeding of a liquid diet in a dose-related manner. Identical doses of atropine methyl nitrate had no effect on sham drinking of water. This differential effect is evidence that the inhibition of sham feeding was not due to peripheral anticholinergic disruption of licking, salivating or swallowing. The results suggest that peripheral blockade of cholinergic receptors of the muscarinic type is a mechanism for eliciting satiety for food in the rat.

Atropine methyl nitrate Anticholinergic Muscarinic receptor Satiety Sham feeding Sham drinking

ATROPINE methyl nitrate inhibits food intake [3,4]. The proposed mechanism of the anorexic action is thought to be indirect: peripheral, anticholinergic blockade of salivary secretion prevents normal mastication and swallowing of dry food [3,4]. This dry mouth hypothesis is supported by the report that atropine methyl nitrate (AMN) did not inhibit the intake of wet mash [2]. We report here, however, that AMN inhibits sham feeding of liquid diet, but not sham drinking of water, in a dose-related manner. This is critical evidence against the dry mouth hypothesis and suggests that peripheral anticholinergic blockade activates an inhibitory mechanism specific for feeding behavior.

EXPERIMENT 1

Method

Animals and procedure. Adult male albino rats (Sprague-Dawley, Hormone Assay Co., Chicago, IL), weighing between 350–450 g, were housed and tested in individual wire cages in a room maintained at about 24°C and lit from 0700 to 1900 hr. Under Chloropent anesthesia (2–3 ml/kg, IP, Fort Dodge, IA) each rat had a gastric cannula implanted along the greater curvature in the rumen of the stomach, as described by Antin *et al.* [1]. Seven to 10 days after surgery, rats were placed on a 17-hr food deprivation schedule. All tests were performed during the light phase (1000 to 1200 hr). Fifteen min before the start of a test, the screw occluding the gastric cannula was removed, gastric contents were flushed out with tap water, and an extension drainage tube was attached to the cannula of each rat. Rats were then returned to their cages ready to sham feed.

Liquid diet (25% v/v, No. 116 EC, GIBCO, Grand Island, NY) was offered for 77 min. Sham intake was recorded every 5 min throughout the test. Behavior was sampled for 10 sec every 5 min and the occurrence of resting behavior was noted. Resting behavior was defined as a relaxed, immobile posture with or without the head and trunk of the body positioned on the cage floor. The eyes could be open or closed. The occurrence of resting behavior was considered the terminal item in the behavioral sequence of satiety [1]. Since resting never occurs in sham feeding rats injected with saline [1], if AMN inhibited sham feeding and elicited resting behavior, it would suggest that AMN had produced satiety and not just disrupted feeding.

Atropine methyl nitrate (AMN 2, 10, 50, 250, 1250 μg/kg, No. A-0382, Sigma, St. Louis, MO) was dissolved in 1 ml of saline (0.9%) and administered intraperitoneally 17 min after sham feeding began. All rats were tested twice with each dose. Injections of AMN alternated with 1 ml injections of saline (0.9%) in a crossover design.

Gastric drainage was collected in plastic containers placed beneath the cages and measured after each test. If the volume of gastric drainage was less than 100% of the volume sham fed, the test was discarded.

At the end of the test gastric cannulas were closed and rats were given the 25% liquid diet to eat for 1 hr. Purina pellets were then provided until 1700 hr when food was removed in preparation for the next day's test.

Results

AMN inhibited sham feeding in a dose related manner (Fig. 1). The threshold dose was 10 μg/kg. The largest dose

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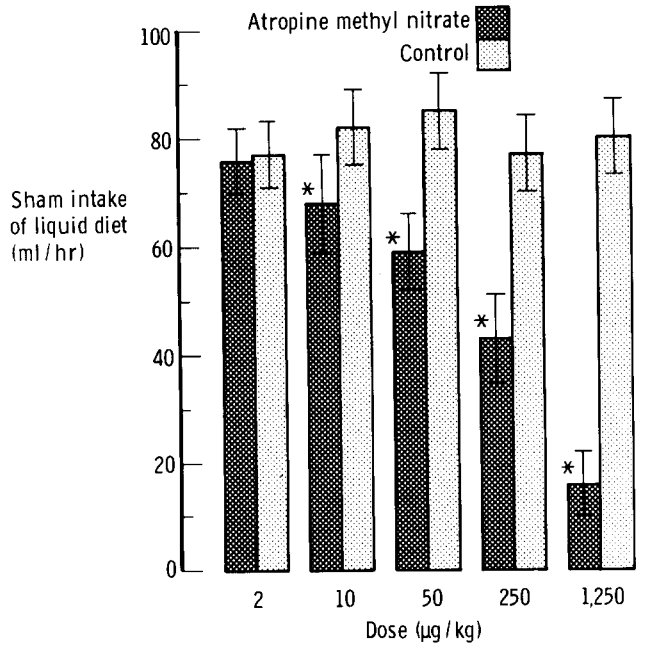

FIG. 1. Sham intakes of 11 rats after intraperitoneal administration of saline or 5 doses of atropine methyl nitrate. *Denotes significant difference ($p<0.025$, matched pairs t-test). Each rat was tested twice with all doses of atropine methyl nitrate.

(1250 µg/kg) inhibited sham feeding 77%. The inhibition of sham feeding was accompanied by an increased incidence of resting behavior (Table 1). Since resting behavior is the terminal item in the behavioral sequence of satiety, this result suggests that AMN not only inhibits sham feeding but also elicits satiety. A more complete behavioral analysis, however, will be required to establish this point. It is possible that AMN inhibits sham feeding by disrupting the normal motor integration necessary for licking or swallowing liquid food. If this were so, AMN should also inhibit sham drinking of water. This was tested in the next experiment.

TABLE 1

EFFECT OF ATROPINE METHYL NITRATE ON RESTING BEHAVIOR

Atropine (µg/kg)	2	10	50	250	1250
Number of rats resting	0	2	3	7	11

Number of rats resting means the number of rats that rested in at least 1 out of the 2 tests after a given dose of atropine methyl nitrate. No rat rested after saline injections. All rats rested after 1250 µg/kg.

EXPERIMENT 2

Method

Animals. Seven adult male albino rats, weighing between 300–435 g were equipped with chronic gastric cannulas.

Procedure

After body weights returned to preoperative levels, rats were placed on a 22-hr water deprivation schedule with Purina rat pellets available at all times except during the test period. All tests were performed during the light phase (1000–1200 hr). Fifteen minutes before the start of the test, the screw occluding the gastric cannula was removed, the gastric contents were flushed out with saline (0.9%), and an extension drainage tube was attached to the cannula of each rat. Rats were then returned to their cages ready to sham drink.

Deionized water was offered for 77 min. The rats were given an intraperitoneal injection of either AMN (2, 10, 50, 250, or 1250 µg/kg) dissolved in 1 ml of saline (0.9%) or saline alone 17 min after sham drinking began. The doses of AMN were administered in random sequence every other day in a crossover design. Sham water intakes and a 10-sec sample of behavior were recorded every 5 min in an identical manner as described for the sham feeding tests.

For an unknown reason ingested water did not drain out the gastric fistula by gravity as well as liquid diet did. To overcome this problem, the gastric extension tubes were aspirated with a vacuum pump at regular intervals. This did not disrupt sham drinking behavior and the recovery of gastric contents averaged 98% of the volume of water ingested. The small volumes of ingested water that did not drain out the gastric fistula were not sufficient to inhibit sham drinking.

Rats were required to sham drink throughout a 77-min period without stopping for more than one 5-min interval before being tested with AMN. Two rats were eliminated on the basis of this criterion.

Results

AMN did not inhibit sham drinking (Table 2). AMN also did not elicit resting behavior in a dose-related manner (resting occurred once in 1 rat after 10 µg/kg and once in another rat after 20 µg/kg).

TABLE 2

EFFECT OF ATROPINE METHYL NITRATE ON SHAM DRINKING

Dose of Atropine (µg/kg)	Intake after Atropine (ml/hr)	Intake after Saline (ml/hr)
2	70±10.5	84±15.8
10	65±11.3	83±14.9
50	87± 6.3	70±10.0
250	82±10.1	82±12.3
1250	69± 8.6	84±13.8

Mean sham intakes (±SE) of water (ml/hr) after IP administration of atropine methyl nitrate or equivolumetric saline in 4 or 5 rats. No dose of atropine had a significant effect on sham drinking (matched pairs t-test, 2-tailed).

DISCUSSION

Identical doses of AMN inhibit sham feeding, but not sham drinking. This differential effect of AMN extends earlier results because it was obtained under conditions in which apparently identical motor acts were required for ingesting liquid food or water. It is possible that this differential effect on sham feeding and drinking is due to the longer deprivation prior to sham drinking than sham

feeding (22 vs 17 hr), but we doubt it. We interpret this differential effect as evidence that the inhibition of sham feeding was not due to peripheral anticholinergic disruption of licking or swallowing. It was also not the result of anticholinergic blockade of salivation because it is hard to imagine how a dry mouth could inhibit liquid food intake.

Sham feeding was very sensitive to AMN: 10 μg/kg produced a 16% inhibition under these test conditions. This dose is much smaller than doses used to inhibit normal feeding [3,4]. The reason(s) why the sham feeding rat is so much more sensitive to inhibition by AMN is not known, but the sensitivity of the sham feeding rat makes it a good preparation to analyze the inhibitory effect of AMN.

AMN not only inhibited sham feeding, it also elicited resting behavior in a dose related manner over the same dose range. This combination of inhibition of sham feeding and elicitation of resting behavior suggests, but does not prove, that peripheral blockade of cholinergic receptors of the muscarinic type is a mechanism for eliciting satiety for food in the rat.

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