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A Comparison of the Effects of Atropine on Real-Feeding and Sham-Feeding of Sucrose in Rats[1]

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NISSENBAUM, J. W. AND A. SCLAFANI. *A comparison of the effects of atropine on real-feeding and sham-feeding of sucrose in rats.* PHARMACOL BIOCHEM BEHAV **29**(2) 231–238, 1988.—In Experiment 1 the influence of atropine methyl nitrate on the sham-feeding response of adult female rats to a sucrose solution was determined. Atropine (1 or 5 mg/kg) reliably suppressed the sham-intake of sucrose when the drug was administered 30 or 0 min prior to, or 17 min after the start of the feeding session. The suppressive effect was less, however, when the drug was administered 30 min before testing compared to the other two injection-test conditions. In Experiment 2 atropine failed to reliably decrease the real-feeding of a sucrose solution whether it was injected 30, 15, or 0 min prior to testing. These results were replicated in Experiment 3; atropine (0 min injection-test interval) reduced the sham-intake but not the real-intake of a sucrose solution. However, atropine decreased the rate of feeding under both real- and sham-feeding conditions. The fact that atropine reduced feeding rate but not meal size in the real-feeding condition was attributed to the drug's lack of effect on postingestive satiety. The present findings along with other recent results indicate that (1) the injection-test interval is a potentially important variable in studies involving atropine; (2) results obtained with sham-feeding animals do not always generalize to real-feeding animals; and (3) cholinergically-mediated cephalic responses are of questionable importance in the control of meal size.

Atropine methyl nitrate Sham-feeding Sucrose Vagus nerve Cephalic responses

IN recent years there has been a renewed interest in the role of peripheral neural and endocrine events in the control of food intake. One technique employed in this research is the sham-feeding preparation. In this preparation an animal is prepared with an esophageal or more often a gastric fistula such that ingested food (i.e., a liquid diet) drains out the esophagus or stomach as the animal feeds [18]. The gastric sham-feeding rat has served as a biobehavioral assay to determine the effects of hormones and drugs on food intake. Using this preparation, Lorenz *et al.* [7] and Weingarten and Watson [22] reported that atropine methyl nitrate (AMN), a peripheral cholinergic blocking drug, decreases the sham-feeding of palatable diets. These results indicate that peripheral cholinergic systems modulate feeding behavior which is consistent with recent studies on vagal mechanisms and food intake (see [5]). Of particular interest are the reports that (1) the vagus nerve mediates cephalic insulin release via its cholinergic connections with the endocrine pancreas; (2) the magnitude of the cephalic insulin response varies as a function of diet palatability; and (3) blocking the cephalic response by vagotomy eliminates the animal's differential feeding response to foods of different palatabilities [8, 9, 12]. Thus, AMN treatment may reduce food intake because it blocks the cephalic insulin response.

Atropine has long been known to suppress food intake but this response has in the past been attributed to a nonspecific effect of the "dry mouth" induced by the drug [19]. This explanation, though, does not explain the sham-feeding data since liquid diets were used in these studies. However, atropine does not always suppress the sham-feeding of liquid diets. That is, in contrast to the suppressive effect observed in the two sham-feeding studies cited above [7,22], Berthoud and Jeanrenaud [1] reported that atropine did not reduce the sham-feeding of liquid diets. Furthermore, whereas atropine has been reported to suppress the sham-feeding of sucrose solutions [22], Sclafani and Xenakis [17] observed that atropine injections produced relatively small and not always reliable reductions in the "real-feeding" of sucrose solutions. More recently, Radhakrishnan and Sharma [13] reported that atropine does not suppress the real-feeding of

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sucrose, glucose, or saccharin solutions.

In view of these inconsistent results, the present study reexamined the effects of atropine on the sham- and real-feeding response to sucrose solutions. One variable emphasized was the temporal relationship between drug administration and the onset of feeding. In the sham-feeding studies that reported an atropine feeding-suppressive effect the drug was administered either just prior to or following the onset of feeding [7,22], whereas in the sham- or real-feeding studies which failed to obtain a robust drug effect, the drug was injected 15–30 min prior to feeding onset [1, 13, 17].

EXPERIMENT 1

METHOD

Subjects

Six adult female rats (CD Strain, Charles River Breeding Laboratories, Wilmington, MA) weighing between 240–305 g were used. The rats were individually housed in wire cages in a colony room maintained at approximately 21°C and on a 12:12 light-dark cycle.

Procedure

The rats were anesthetized with Chloropent (0.32 ml/100 g b.wt.) and were fitted with stainless steel gastric cannulas according to previously described procedures [16]. Purina rat chow and water were available ad lib until the animals recovered their preoperative body weight. The rats were then maintained on a milk diet six days/week, and chow on the remaining day; water was available ad lib. The milk diet consisted of 384 ml evaporated milk (Pathmark Brand), 0.8 g vitamin mix (Bio Serv), and 400 ml water. The rats were given restricted amounts of milk so as to maintain them at 85% of their ad lib body weight; the food ration was presented 1 hr after the end of daily sham-feeding tests.

As part of another study, the rats were first tested for their sham-feeding response to saccharin solutions 30 min/day [16]. At the start of the present experiment the rats were trained to sham-feed a 32% sucrose (w/v) solution 30 min/day using the previously described procedure [16]. The rats were removed from their home cage, their gastric cannulas were opened, and their stomachs were flushed clean with three or more gastric washes of 5 ml isotonic saline. The rats were then placed into test cages identical to their home cages and presented with the sucrose solution. At the end of the test session the rats' gastric cannulas were closed and the animals were returned to their home cage. Drainage pans below the test cages were weighed before and after the sham-feeding tests. In most cases the amount of drainage collected equalled or exceeded the amount of solution consumed. In a few instances the amount collected was slightly less (1–4 ml) than the amount consumed, but visual inspection of the cannulas indicated that they were patent. (Note that even when the amount of drainage collected exceeds the amount consumed some of the ingested food may have been absorbed [15].) The rats were tested six days/week during the middle of the light part of the day-night cycle.

In the first test (Test 1) the rats were pretreated with AMN 30 min prior to the sham-feeding session, which was the injection-test interval used by Sclafani and Xenakis [17]. The AMN was dissolved in isotonic saline and was injected (IP) in 1 and 5 mg/kg doses; isotonic saline (1 ml/kg) was injected on control days. The order of testing was saline, 1 mg/kg AMN, saline, 5 mg/kg AMN. At the end of the 30-min sham-feeding session the intake of the 32% sucrose solution was recorded to the nearest 0.5 ml.

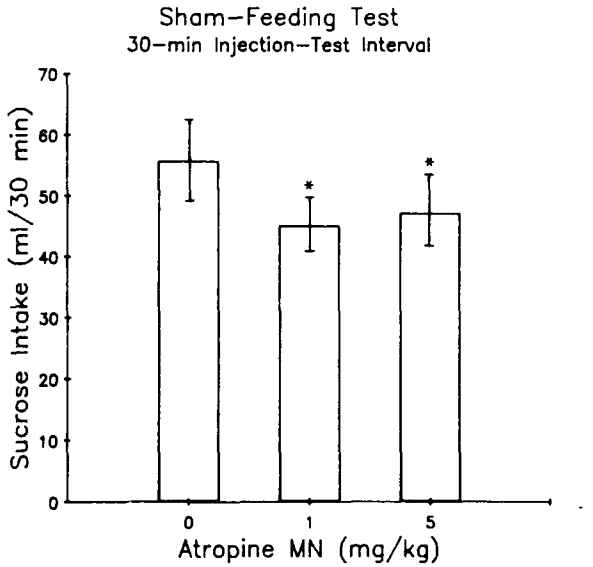

FIG. 1. Mean (±SE) intake of 32% sucrose solution in sham-feeding Test 1. Atropine methyl nitrate (1 or 5 mg/kg) or saline (0 mg/kg) was injected 30 min prior to the sham-feeding test. Asterisks indicate significant ($p<0.05$) differences relative to the saline condition.

In Test 2 the rats were tested using a paradigm similar to that of Lorenz *et al.* [7]. The animals were placed in the test chambers and allowed to sham-feed the 32% sucrose solution for 17 min. They were then injected with saline or AMN and were returned to the test chamber for an additional 60 min of sham-feeding. Intake was measured at the end of the first 30 min and at the end of the 60 min period. The order of testing was saline, 1 mg/kg AMN, saline, 5 mg/kg AMN.

Test 3 was a repeat of Test 1 in that the rats were injected with saline or AMN 30 min prior to sham-feeding. In Test 4 the animals were injected with saline or AMN immediately prior to the 30-min sham-feeding period; this approximated the 5-min injection-test interval used by Weingarten and Watson [22]. In both Tests 3 and 4 only the 1 mg/kg dose of AMN was used; saline tests were conducted on the day prior to the AMN tests.

The sucrose solution intakes during the saline and atropine tests were compared using a repeated measures analysis of variance followed by Newman-Keuls tests for individual comparisons (Tests 1 and 2) or dependent *t*-tests (Tests 3 and 4). The statistical tests were evaluated at the 0.05 level of significance.

RESULTS

In Test 1 AMN was administered 30 min prior to sham-feeding. At both the 1 and 5 mg/kg doses AMN produced small but significant ($p<0.05$) reductions in sucrose intake compared to the saline control condition (Fig. 1). The 1 and 5 mg/kg doses suppressed sucrose intake by 17.8% and 14.3%, respectively, which did not reliably differ.

In Test 2 AMN was injected after the rats had been sham-feeding for 17 min. Sucrose intake during the subsequent 30-min period was significantly ($p<0.05$) suppressed at

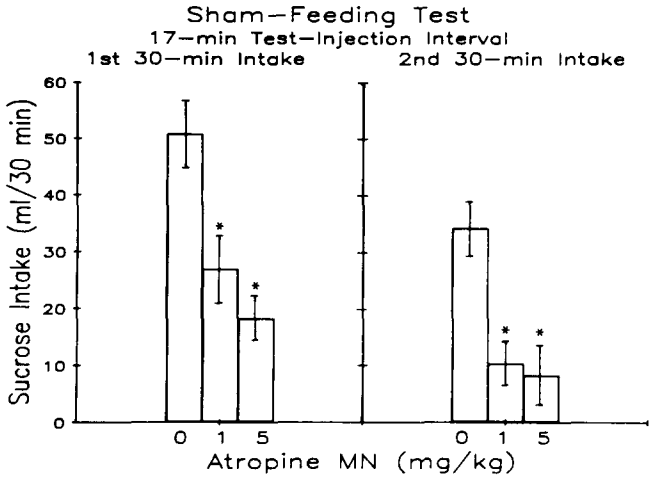

FIG. 2. Mean (±SE) intake of 32% sucrose solution during the first and second 30-min periods of sham-feeding Test 2. Atropine methyl nitrate (1 or 5 mg/kg) was injected 17 min after the rats had started sham-feeding and intake during the next 60 min is presented in the figure. Asterisks indicate significant ($p<0.05$) differences relative to the saline condition.

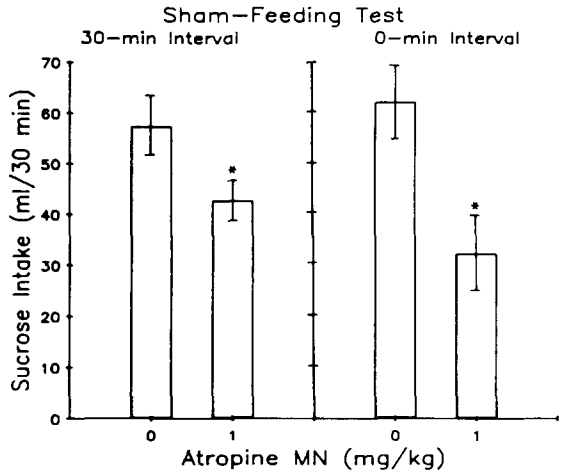

FIG. 3. Mean (±SE) intake of 32% sucrose solution in sham-feeding Test 3 (left panel) and Test 4 (right panel). Atropine methyl nitrate (1 mg/kg) was injected 30 min (Test 3) or 0 min (Test 4) prior to the sham-feeding test. Asterisks indicate significant ($p<0.05$) differences relative to the saline condition.

both dose levels (Fig. 2). The 1 and 5 mg/kg doses suppressed sham-feeding 47.6% and 64.2%, respectively, which did not reliably differ. Sham-feeding was suppressed even more ($p<0.05$) during the second 30-min period following AMN treatment, i.e., 70.4% and 78.3% at the 1 and 5 mg/kg doses, respectively. Over the entire 1-hr period the 1 and 5 mg/kg doses suppressed sucrose intake 56.8% and 69.6%, respectively.

In Test 3 the rats were retested with 1 mg/kg AMN injected 30 min prior to sham-feeding. Sucrose intake was significantly ($p<0.05$) suppressed compared to the saline baseline (Fig. 3). The suppression was comparable to that obtained with the 1 mg/kg dose in Test 1 (23.4% vs. 17.8%,

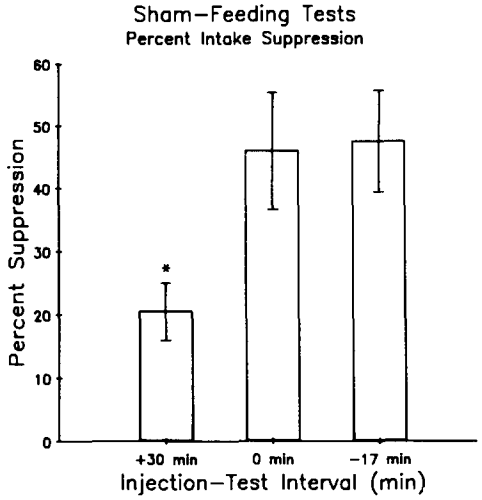

FIG. 4. Mean (±SE) percent suppression in sucrose solution intake relative to the saline condition following atropine methyl nitrate (1 mg/kg) injections as a function of the injection-test interval. In the +30 min condition (Tests 1 and 3) the rats were injected with atropine 30 min prior to sham-feeding; in the 0 min condition (Test 4) they were injected 0 min prior to sham-feeding and in the −17 min condition (Test 2) they were injected 17 min after they had started to sham-feed. Asterisk indicates that the percent suppression in the +30 min condition was significantly ($p<0.05$) less than that in the 0 min and −17 min conditions.

n.s.). Test 4 employed a 0-min interval between drug injection and sham-feeding, and the AMN injection (1 mg/kg) reduced ($p<0.05$) sucrose intake by 46.1% compared to the saline baseline (Fig. 3).

Comparison of the results obtained in the four tests indicated that injecting AMN (1 mg/kg) 30 min prior to sham-feeding (+30 min condition, Tests 1 and 3) produced a significantly ($p<0.05$) smaller suppression in sucrose intake than that produced by injecting AMN at the beginning of the sham-feeding session (0-min condition, Test 4) or after sham-feeding had started (−17-min condition, Test 2; Fig. 4). The suppression in sham-feeding obtained in the latter two conditions was comparable.

DISCUSSION

The results of this experiment confirm two previous reports that AMN depresses sham-feeding behavior in rats [7,22]. The present results further demonstrate, however, that the feeding suppressive effect is significantly influenced by the temporal relationship between drug administration and behavioral testing. That is, feeding suppression was greatest when the AMN was administered at or after the onset of sham-feeding, and was less when it was administered 30 min prior to sham-feeding. This finding may at least partially explain the discrepancy between published reports concerning the feeding suppressive effects of atropine. In those studies in which AMN reduced food intake the drug was injected just before or after the start of testing [7,22], whereas in those studies in which food intake was not reliably suppressed the drug was injected 15 to 30 min prior to testing [1, 13, 17] (but see Experiment 2).

Why does the injection-test interval influence the feeding suppression produced by atropine? The simplest explanation

would be that atropine is a short-acting drug such that by 30 min post-injection its effects are significantly attenuated. This interpretation is not supported, however, by the results obtained in Test 2 in which the feeding suppressive effect of the atropine was greater during the second half than during the first half of the 1-hr test. Previous studies also demonstrate that atropine has long-lasting effects on ingestive behavior. For example, atropine, at doses similar to those used in the present study, was found to reliably depress 24-hr chow intake [17]. Yet, in the same study atropine had minimal effects on sucrose solution intake 30 min post-injection. Thus, some factor other than a decline in drug activity would appear to be responsible for the temporal effects observed in the present experiment.

It may be that it is the onset of the cholinergic blockade produced by atropine rather than the chronic effects of cholinergic blockade that are responsible for the suppression in sham-feeding behavior. That is, the rapid onset of cholinergic blockade, and the associated changes in smooth muscle and endocrine activity produced by the atropine may result in visceral afferent feedback to the brain that is interpreted as satiety (or reduced hunger). This sensory feedback may be of short duration such that feeding is maximally suppressed when meal onset follows shortly after atropine administration. Alternatively, the animal may quickly adapt to the sensory feedback such that by 30 min post-injection it has a minimal impact on sham-feeding. Note that it could be argued that atropine produces aversive rather than satiating effects since atropine has been used to condition taste aversions in rats [24]. This interpretation is not supported, however, by the findings that atropine, while it inhibits sham-feeding in hungry rats, does not inhibit sham-drinking in thirsty rats [7,22].

The influence of the injection-test interval on feeding response to atropine has received little or no attention in previous studies. VanderWeele and Talbert [20] recently reported, however, that the effect of atropine on the feeding suppression produced by glucagon is dependent upon the interval between atropine administration and glucagon injection. That is, atropine blocked the feeding suppression produced by glucagon when it was administered 10 min prior, but not when it was administered 20 or 30 min prior to the glucagon. These findings, while consistent with the present results with respect to identifying the injection-test interval as an important variable, would seem to conflict with the present results with respect to the nature of atropine's effect on food intake. That is, whereas atropine reduced feeding suppression in the glucagon experiment, it produced a feeding suppression in the present experiment. However, the two studies differed in a number of ways not the least being that real-feeding was studied in the glucagon experiment and sham-feeding was studied in the present experiment (see Experiment 2). In any event, in view of the VanderWeele and Talbert findings and the present results, the injection-test interval should be considered as a potentially important variable in future studies involving atropine.

EXPERIMENT 2

The findings of Experiment 1 revealed that the suppressive effect of atropine on sham-feeding behavior is dependent upon the injection-test interval. The second experiment assessed whether the effect of atropine on real-feeding behavior is also influenced by temporal factors. For this purpose, rats were initially tested as in the study of Sclafani and Xenakis [17]; that is, they were minimally food deprived, and tested with a 20% sucrose solution. In the second part of the experiment the animals were tested under a food deprivation condition similar to that used in Experiment 1.

METHOD

Subjects

Eleven adult female rats (CD Strain) weighing between 240–295 g were used. The rats were housed as in Experiment 1.

Procedure

The rats were trained to drink a 20% sucrose solution in their home cage during 30 min/day sessions at midday. Food, but not water, was removed 1 hr prior to sucrose presentation. (Since the animals would have consumed little or no food during this 1 hr period they essentially had food available ad lib.) Following this initial training period the rats were divided into three subgroups, matched for sucrose intake and body weight. Each animal was injected with 1 mg/kg AMN on three different days: at 30, 15, or 0 min prior to sucrose presentation, with the order of testing being counterbalanced across the three subgroups. On the day preceding each AMN injection the rats were injected with saline; on the day following the AMN treatments the rats were not injected, but were tested with the sucrose solution. Following these tests, the animals were food restricted and maintained at 85% of their ad lib body weight. They were then tested with AMN again using the procedure described above. The daily food ration was given to the animals 1 hr after the daily sucrose solution tests.

The sucrose solution intakes during the saline and atropine tests were compared using a repeated measure analysis of variance. The sucrose intakes following the three saline treatments (0, 15, and 30 min injection-test intervals) did not differ and thus were averaged to yield a single saline value.

RESULTS

AMN failed to reliably suppress the intake of the 20% sucrose solution during the first test series when the animals had food ad lib (Fig. 5). Atropine tended to reduce sucrose intake more under the 0-min injection-test condition (24.4% suppression) than under the 15-min (6.3% suppression) and 30-min (9.8% suppression) conditions, but these differences failed to be significant.

In the second test series the rats were food-deprived, and they consumed approximately twice as much sucrose solution as they did during the first series. Yet, despite the higher baseline level, AMN again failed to reliably decrease sucrose intake (Fig. 6). Although the rats tended to consume less sucrose as the atropine injection-test interval decreased from 30 to 0 min (from 7.5% to 17.3% to 17.9% suppression) this effect was not reliable. Comparison between the results obtained in the food ad lib and food deprived tests indicated that AMN did not differentially affect sucrose intake under the two deprivation conditions.

DISCUSSION

These results confirm previous findings that AMN produces little or no decrease in the real-feeding of a sucrose solution [13,17]. The present experiment further demonstrates that reducing the interval between drug administra-

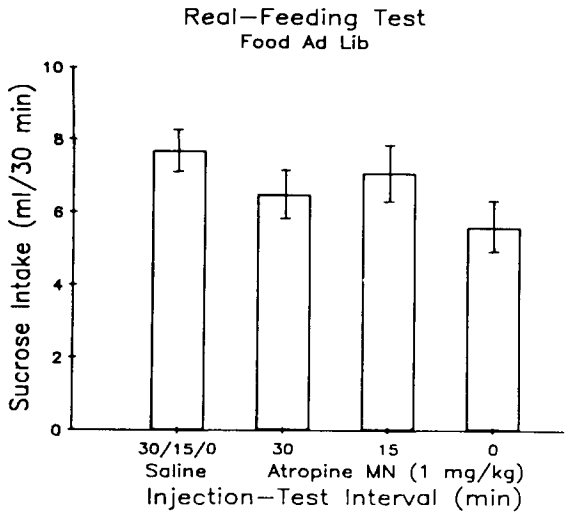

FIG. 5. Mean (±SE) intake of 20% sucrose solution in the real-feeding test conducted under the food ad lib condition. Saline or atropine methyl nitrate (1 mg/kg) was injected 0, 15, or 30 min prior to the feeding test. The saline score represents the mean intake under the three injection-test intervals.

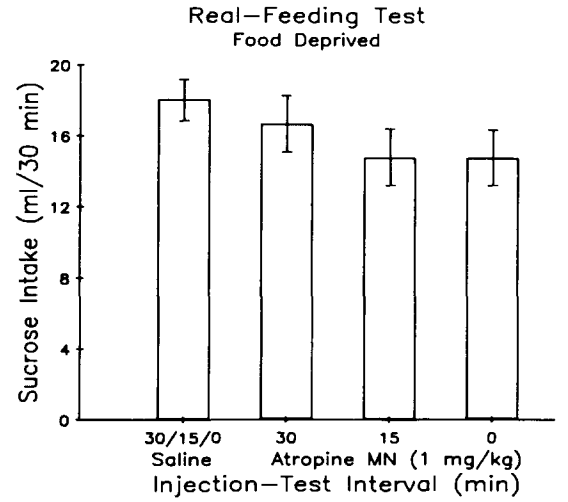

FIG. 6. Mean (±SE) intake of 20% sucrose solution in the real-feeding test conducted under the food deprived condition. Saline or atropine methyl nitrate (1 mg/kg) was injected 0, 15, or 30 min prior to the feeding test. The saline score represents the mean intake under the three injection-test intervals.

tion and testing from 30 to 0 min does not reliably increase the suppressive effect of atropine or sucrose intake. This latter finding contrasts with the results obtained in the sham-feeding tests of Experiment 1. Taken together, the data indicate that differences in the injection-test intervals used in previous studies do not account for the greater atropine suppressive effects observed in sham-feeding as opposed to real-feeding tests [13, 17, 22]. Note that although a 20% sucrose solution was used in the present experiment whereas a 32% solution was used in Experiment 1, it is unlikely that this difference explains the discrepant results obtained in the two experiments (see Experiment 3 and [22]).

Why does atropine suppress the sham-feeding but not the real-feeding of sucrose solutions? A major difference between the two feeding conditions is that much more food is consumed in sham-feeding tests than in real-feeding tests [18]. In the present study, for example, the rats sham-fed approximately three times more sucrose in Experiment 1 than they really-fed in Experiment 2 under comparable deprivation conditions (55–60 vs. 18 ml/30 min). Perhaps atropine suppresses sucrose intake only after a substantial amount has already been ingested. Consistent with this interpretation, note that despite the fact that atropine suppressed sucrose intake in the sham-feeding tests, the amount sham-fed following the atropine treatment (1 mg/kg dose) was greater than the amount really-fed following saline treatment (27 to 45 vs. 18 ml/30 min). Furthermore, in Experiment 1 (Test 2) the feeding suppressive effect of atropine was greater during the second 30 min of sham-feeding than during the first 30 min (Experiment 1, Test 2). Note also that in two previous studies in which atropine failed to suppress sham-feeding the baseline intakes of the rats were relatively low (28 and 6 ml, respectively) because of the particular conditions of the experiments (i.e., short-test session or the use of dietary obese rats) [1,21].

On the other hand, the sham-feeding results of Weingarten and Watson [22] argue that the amount of food ingested is not an important factor in atropine's feeding suppressive effect. In their experiment atropine produced equivalent reductions in the intakes of 6, 16, and 30% sucrose solutions even though the baseline intakes of the three solutions were quite different, e.g., the rats consumed 10 ml/30 min of the 6% solution versus 35 ml/30 min of the 30% solution. Furthermore, analysis of the rats' feeding pattern revealed that atropine suppressed sham-feeding beginning with the first 5 min of the test session [22].

EXPERIMENT 3

The results of the first two experiments indicate that atropine reduces sham-feeding but not real-feeding of sucrose solutions. The third experiment sought to replicate this differential drug effect using the same group of animals tested under both real- and sham-feeding conditions. In addition to measuring total 30-min intakes, feeding rates throughout the test sessions were monitored. As discussed above, sham-feeding may be suppressed more by atropine than is real-feeding because rats consume considerably more sucrose in the former condition than in the latter condition. This interpretation is challenged, however, by the observation of Weingarten and Watson [22] that atropine reduces the rate of sucrose sham-feeding beginning within the first five minutes of testing. Thus, a second purpose of the present experiment was to replicate this observation and to determine what, if any, effect atropine has on the rate of real-feeding.

METHOD

Subjects

Eleven adult female rats (CD Strain) weighing between 210–260 g were used. The rats were housed as in Experiment 1.

Apparatus

Feeding tests were conducted in four cages similar to the animals' home cages kept in a quiet room adjacent to the

vivarium. The sucrose solution was available through a stainless steel drinking tube attached to a graduated cylinder. The cylinder was mounted on a device that automatically positioned the drinking tube at the front of the cage at the start of the test session, and retracted it at the end of the session. A contact sensitive electronic drinkometer measured licking behavior, and licks per min were recorded by a microcomputer.

Procedure

The rats were fitted with gastric cannulas as in Experiment 1. Following recovery from surgery, the rats were given restricted amounts of Purina chow so as to maintain them at 85% of their ad lib body weight. The animals were trained to real-feed a 20% sucrose solution in the test cages 30 min/day for six days. On the following two days they were injected with saline 0 min before the feeding tests; the first saline day was considered a pretest and the data were not used. On the day after the second saline treatment the rats were injected with 1 mg/kg AMN 0 min before the feeding test. The next day the rats were given no injection but were tested with the sucrose solution.

In the second part of the experiment, the rats were trained to sham-feed the 20% sucrose solution 30 min/day for four days. They were then injected, on successive days, with saline and 1 mg/kg AMN 0 min prior to testing. Following a no-injection test day, the rats were retested with saline and 1 mg/kg AMN but the injections were given 5 min prior to the test following the procedure of Weingarten and Watson [22].

Sucrose solution intakes and total licks were recorded at the end of the daily 30-min tests. Using these data and the lick/min data, cumulative solution intakes per minute were determined. The cumulative intake data were evaluated using a repeated measure analysis of variance followed by tests of simple main effects. The data analysis is based on 10 or 11 subjects; data were lost from one rat in the real-feeding test due to an equipment malfunction.

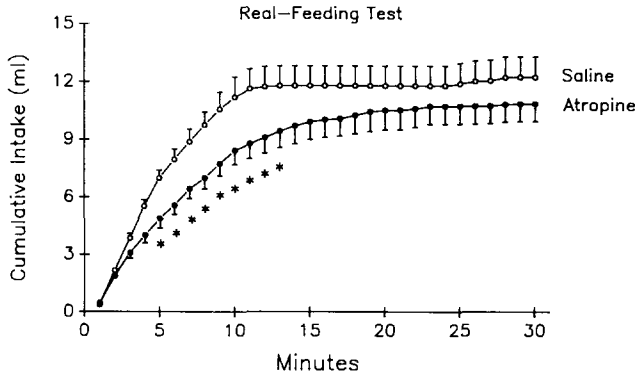

FIG. 7. Mean cumulative (±SE) intake of 20% sucrose in the real-feeding test. Saline or atropine methyl nitrate (1 mg/kg) was injected 0 min prior to the test session. Asterisks indicate significant ($p<0.05$) difference relative to the saline test.

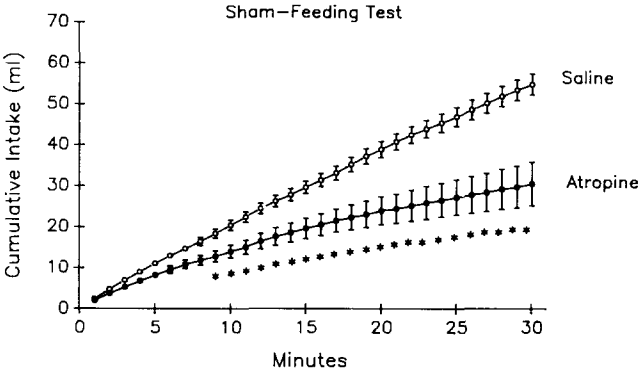

FIG. 8. Mean cumulative (±SE) intake of 20% sucrose in the sham-feeding test. Saline or atropine methyl nitrate (1 mg/kg) was injected 0 min prior to the test session. Asterisks indicate significant ($p<0.05$) difference relative to the saline test.

RESULTS

Compared to the saline treatment, AMN did not significantly suppress total 30-min sucrose solution intake in the real-feeding tests (12.2 vs. 10.9 ml/30 min). However, as illustrated in Fig. 7, AMN slowed down the rate of consumption such that the cumulative intakes of the sucrose solution were significantly ($p<0.05$) reduced, relative to the saline condition, during minutes 5 to 13 of the 30-min test.

In the first sham-feeding test, AMN reliably suppressed total 30-min sucrose intake compared to the saline treatment (30.5 vs. 55.0 ml/30 min, $p<0.01$). The cumulative intake data revealed that the AMN suppression was statistically significant ($p<0.05$) at 9 minutes and later (Fig. 8). By 9 minutes the rats had sham-fed 12.8 ml in the AMN test compared to 18.3 ml in the saline test.

The results of the second sham-feeding test, in which the rats were injected 5 min prior to testing, were comparable to those obtained in the first sham-feeding test. AMN suppressed total 30-min sucrose intake compared to the saline treatment (33.4 vs. 58.9 ml/30 min, $p<0.01$). Also, the effect of atropine on cumulative sucrose intake was very similar to that observed in the first sham-feeding test (data not presented). Cumulative sucrose intake was reliably suppressed by 8 min at which time the rats had consumed 12.0 ml in the AMN test and 18.2 ml in the saline test. Inspection of the cumulative intake curves suggested that the suppressive effect on sucrose intake increased with time but an analysis of the within-session feeding rate indicated that this was not the case. That is, as illustrated in Fig. 9, atropine suppressed ($p<0.01$) sham-feeding rate (intake/5 min) throughout the 30-min session. Feeding rate declined ($p<0.01$) over time in both the saline and atropine tests, but the drug by time interaction was not significant.

DISCUSSION

The present findings replicate the results of the first two experiments that atropine significantly reduces the sham-intake but not the real-intake of sucrose solutions. In terms of total 30-min sucrose intake, atropine produced a nonsignificant reduction in sucrose consumption in the real-feeding test that closely matched that observed in Experiment 2; the suppressions in sucrose intake, relative to the saline condition, were 17.9% and 18.0%, respectively, in the two experiments. In the sham-feeding test, atropine produced a significant suppression in total 30-min intake that was very similar to that observed in Experiment 1 (Test 4); the suppression scores were 43.2% and 46.1%, respectively. Thus, the previously observed differential effects of atropine on sham- and

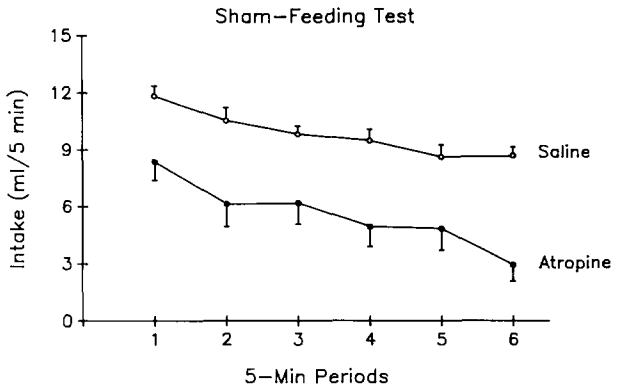

FIG. 9. Mean (±SE) intake of 20% sucrose during consecutive 5 min periods of 30 min sham-feeding test. Saline or atropine methyl nitrate (1 mg/kg) was injected 5 min prior to the test session.

real-feeding were not due to interexperiment procedural differences.

An analysis of the within-session feeding rates indicated that atropine suppressed sham-feeding beginning early in the test session which confirms the results of Weingarten and Watson [22]. The present results also revealed that atropine decreased sucrose intake early in the real-feeding test but that this suppressive effect disappeared later in the test session. These findings refute the hypothesis that atropine suppression of sucrose intake occurs only after the rat has consumed a substantial amount of sucrose. Rather, the results suggest a different explanation for the differential drug effect on real-feeding and sham-feeding behavior.

In the real-feeding situation, following saline treatment, the rats consumed sucrose for 10–11 min then abruptly stopped feeding. The cessation of feeding was presumably due to the inhibitory effect of postingestive satiety since in the sham-feeding situation the rats continued to feed throughout the 30-min test session. Following atropine treatment, the rats real-fed at a slower rate but they continued to feed until about 15 min into the test so that their total intake was not reliably less than their intake in the saline test. Taken together, these results indicate that atropine reduces feeding rate but has minimal effect on postingestive satiety. This would explain why the drug produces a greater reduction of sham-feeding than of real-feeding. In the sham-feeding condition postingestive satiety is eliminated, and the amount consumed is determined by the palatability-dependent feeding rate [23]. In the real-feeding condition, however, postingestive satiety rather than palatability-dependent feeding rate determines the intake of concentrated sugar solutions.

GENERAL DISCUSSION

The present study attempted to clarify the effects of atropine on real-feeding and sham-feeding behavior. Previous studies reported that atropine reduces the sham-intake but not the real-intake of palatable foods. Different injection-test intervals were used in these studies and it appeared possible that this procedural difference accounted for the discrepant results. The present findings revealed that variations in the injection-test interval significantly alter atropine's effect on sham-feeding but not its effect on real-feeding. Furthermore, under comparable test conditions atropine reliably reduced the sham-intake but not the real-intake of sucrose solutions. These results demonstrate that real-feeding and sham-feeding behavior are, in fact, differentially affected by atropine treatment.

In contrast to the effects obtained with atropine, a variety of other pharmacological agents have been found to suppress both real-feeding and sham-feeding (i.e., cholecystokinin, bombesin, insulin, pimozide, naloxone) [3, 4, 10, 11, 14]. Also, at least one agent inhibits real-feeding but not sham-feeding (i.e., glucagon) [2]; this effect was interpreted to mean that glucagon's feeding inhibitory effect requires the synergistic action of some postingestive stimulus absent during sham-feeding. Atropine is the only agent identified to date that, under comparable conditions, suppresses the sham-intake but not the real-intake of food. This finding along with other recent results [6] indicate that we cannot necessarily predict on the basis of the sham-feeding preparation what influence a treatment may have on real-feeding behavior. The recent observation that a gastric fistula does not completely block the digestion and absorption of ingested food also calls for care in the interpretation of sham-feeding results [15].

The finding that atropine reduces the rate of real- and sham-feeding provides some support for the hypothesis that cephalic responses, the cephalic insulin response in particular, modulates food palatability [8,9]. However, the failure of atropine to reliably reduce total intake in the real-feeding situation argues against the idea that cephalic phase responses are an important determinant of meal size. This hypothesis is also challenged by the report by Berthoud and Jeanrenaud [1] that atropine injections that were shown to inhibit the cephalic insulin responses to a liquid diet nevertheless did not reduce the sham-intake of the diet. These findings, however, do not exclude the possibility that cephalic responses have long-term influences on food intake secondary to their effects on the postingestive disposition of food.

REFERENCES

1. Berthoud, H. R. and B. Jeanrenaud. Sham feeding-induced cephalic phase insulin release in the rat. *Am J Physiol* **242:** E280–E285, 1982.
2. Geary, N. and G. P. Smith. Pancreatic glucagon fails to inhibit sham feeding in the rat. *Peptides* **1:** 163–166, 1982.
3. Geary, N. and G. P. Smith. Pimozide decreases the positive reinforcing effects of sham fed sucrose in the rat. *Pharmacol Biochem Behav* **22:** 787–790, 1985.
4. Gibbs, J., R. C. Young and G. P. Smith. Cholecystokinin elicits satiety in rats with open gastric fistulas. *Nature* **245:** 323–325, 1973.
5. Kral, J. G., T. L. Powley and C. Mc. C. Brooks (Eds.). *Vagal Nerve Function: Behavioral and Methodological Considerations*. Amsterdam: Elsevier, 1983.
6. Kraly, F. S. Vagotomy does not alter cholecystokinin's inhibition of sham feeding. *Am J Physiol* **246:** R829–R831, 1984.
7. Lorenz, D., P. Nardi and G. P. Smith. Atropine methyl nitrate inhibits sham feeding in the rat. *Physiol Behav* **8:** 405–407, 1978.
8. Louis-Sylvestre, J., I. Giachetti and J. LeMagnen. Vagotomy abolishes the differential palatability of food. *Appetite* **4:** 295–299, 1983.

9. Louis-Sylvestre, J. and J. LeMagnen. Palatability and preabsorptive insulin release. *Neurosci Biobehav Rev* **4:** Suppl 1, 43–46, 1980.
10. Martin, C. F. and J. Gibbs. Bombesin elicits satiety in sham feeding rats. *Peptides* **1:** 133–134, 1980.
11. Oetting, R. L. and D. A. VanderWeele. Insulin suppresses intake without inducing illness in sham feeding rats. *Physiol Behav* **34:** 557–562, 1985.
12. Powley, T. L. and H. R. Berthoud. Diet and cephalic phase insulin responses. *Am J Clin Nutr* **42:** 991–1002, 1985.
13. Radhakrishnan, V. and K. N. Sharma. Effect of selective gastric vagotomy on gustatory behavior in rats. *J Auton Nerv Syst* **16:** 127–136, 1986.
14. Rockwood, G. A. and L. D. Reid. Naloxone modifies sugar-water intake in rats drinking with open gastric fistulas. *Physiol Behav* **29:** 1175–1178, 1982.
15. Sclafani, A. and J. W. Nissenbaum. Is gastric sham-feeding really sham-feeding? *Am J Physiol* **248:** R387–R390, 1985.
16. Sclafani, A. and J. W. Nissenbaum. On the role of the mouth and gut in the control of saccharin and sugar intake: A reexamination of the sham-feeding preparation. *Brain Res Bull* **14:** 569–576, 1985.
17. Sclafani, A. and S. Xenakis. Atropine fails to block the overconsumption of sugar solutions by hypothalamic hyperphagic rats. *J Comp Physiol Psychol* **95:** 708–719, 1981.
18. Smith, G. P. and J. Gibbs. Postprandial satiety. In: *Progress in Psychobiology and Physiological Psychology, Vol 8,* edited by J. M. Sprague and A. N. Epstein. New York: Academic Press, 1979, pp. 179–242.
19. Stein, L. Anticholinergic drugs and central control of thirst. *Science* **139:** 46–48, 1963.
20. VanderWeele, D. A. and D. R. Talbert. Modulation of the effectiveness of glucagon to reduce food intake by anticholinergic and alpha-adrenergic drugs. *International Conference on the Physiology of Food and Fluid Intake,* Seattle, WA, 1986.
21. VanderWeele, D. A. and T. B. Van Itallie. Sham feeding is inhibited by dietary-induced obesity in rats. *Physiol Behav* **31:** 533–537, 1983.
22. Weingarten, H. P. and S. D. Watson. Effects of atropine methyl nitrate on sham feeding in the rat. *Pharmacol Biochem Behav* **17:** 863–867, 1982.
23. Weingarten, H. P. and S. D. Watson. Sham feeding as a procedure for assessing influence of diet palatability on food intake. *Physiol Behav* **28:** 401–407, 1982.
24. Weisman, R. N., L. W. Hamilton and P. L. Carlton. Increased conditioned gustatory aversion following VMH lesions in rats. *Physiol Behav* **9:** 801–804, 1972.