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Effect of Oxethazaine HCl on Control of Food and Water Intake in the Rat

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POBER, Z. AND W. K. CALHOUN. *Effect of oxethazaine HCl on control of food and water intake in the rat.* PHARMAC. BIOCHEM. BEHAV. 3(1) 69–74, 1975. – Oral administration of the local anesthetic oxethazaine HCl was used to modify eating and drinking patterns in the rat. The addition of the oxethazaine HCl to the diet (0.5 gm/100 gm diet) markedly reduced food intake. Similarly, administration of the drug by gastric intubation (gavage) also reduced food intake. This reduction was of short duration. The inhibition of water intake associated with administration of the drug was shown to be a secondary effect of reduced food intake, rather than a direct action of the drug. The data indicate that local anesthesia of the gastrointestinal tract results in an inhibition of food intake.

Food intake Water intake Topical anesthetics Gastrointestinal receptors

CONTROL of food intake is a function of the several sensory inputs into the central nervous system. The gastrointestinal tract has long been implicated as one of these inputs. The first suggestion that the gastrointestinal tract influences food intake was made by Cannon and Washburn [2]. They demonstrated that perception of epigastric hunger pangs coincided with periodic contractions of the empty stomach. Later, Carlson [3] suggested that these contractions produced the entire complex known as hunger. Grossman and Stein [8] showed that denervation of the gastrointestinal tract resulted only in loss of the sensation of epigastric pangs, which is only a small portion of the total response known as hunger. This entire area has been discussed in detail by Janowitz [9].

Janowitz and Grossman [10] have shown that some of the mechanisms regulating food intake are within the stomach. They demonstrated that gastric loading in proper sequence with oral factors produced cessation of feeding in dogs. Ehman *et al.* [4] showed that the intestine has a definite role in the regulation of food intake. They demonstrated that introduction of inert materials (purified cellulose) or hypertonic solutions (NaCl or glucose) into the intestine results in a depression in food intake. This indicates that intestinal distension is a factor in the regulation of food intake. Campbell and Davis [1] did find that glucose in physiological concentrations inhibited food intake. While they could not show whether the glucose was acting on intestinal lumenal receptors or hepatic receptors, they did show that the response was to the glucose rather than simple distension. The alteration in food intake can be accomplished by other nutrients. Fat, for example, can reduce food intake in the rat [13]. This is possible through the action of cholecystokinin. Gibbs *et al.* [6] have shown that cholecystokinin administered parenterally will reduce food intake. However, cholecystokinin is released from the intestine in response to amino acids as well as fat [11]. While there is no evidence to show that protein in the intestine controls food intake, it has been shown that intestinal receptors are activated by protein to stimulate gastric secretion [15]. It was shown that protein in the intestine stimulates gastric secretion while similar osmolar concentrations of saline are inactive. Sircus [18] found that local anesthesia of the intestine will prevent the gastric secretory response to protein. Sharma [17] has shown the existence of intestinal chemoreceptors that respond to the nutrient portion of the food, but the role of these chemoreceptors in food intake regulation remains to be determined.

Oxethazaine HCl was used to try to determine the role of intestinal receptors on food intake because it is not absorbed in physiologically active quantities from the gastrointestinal tract [5]. In this study we have demonstrated that intragastric administration of oxethazaine HCl produces marked decreases in food intake.

METHOD

General

Experiments were performed on male, Sprague-Dawley derived rats (Carworth), housed individually in wire-bottomed, stainless steel cages. Continuous lighting was provided to reduce diurnal variation in eating. The animals were fed a ground commercial rat food (Purina). Water was permitted ad lib. The room temperature was maintained at 22°C with a 50% humidity.

Experiment 1: Local Anesthetic in Diet

Twenty-four animals, approximately 120 g in weight, were randomly divided into 2 groups. Food intake, water consumption, and body weight were measured daily during a 9-day period. The control group was fed the standard diet, while the treated group received the same diet on Day 1 and 0.5% oxethazaine HCl (Wyeth Laboratories) in the diet on the remaining eight days.

Experiment 2: Local Anesthetic by Gastric Intubation (gavage)

Because oxethazaine HCl was observed by the authors both to have a bitter taste and produce a localized area of loss of sensation when placed on the tongue, in all further experiments the local anesthetic was administered by gastric intubation (gavage). The materials were injected by syringe through a 20 ga intubation needle passed into the stomach of each rat at the time of administration. It was expected that this would eliminate the influence of oropharangeal factors on food intake. Thirty-two animals of approximately 100 g were randomly divided into 2 groups, A and B. During a 4-day period food intake was measured from 7:00 a.m. to 4:00 p.m., providing a 9-hour test period (test day) followed by a 15-hour recovery period. During the test day the animals were given 1.0 ml distilled water at 2-hour intervals from 7:00 a.m. to 3:00 p.m. by gavage. On Day 2 group A received 1 ml of a 5 mg/ml solution of oxethazaine HCl at 2-hour intervals (total – 25 mg/animal) in place of the water. On Day 4 group B received similar treatment.

Experiment 3: Duration of Action

Sixty-four animals of approximately 120 g were randomly divided into 6 groups of 10 or 11 each. On the first day of the experiment the animals were given 1.0 ml of water at 2-hour intervals by gavage during the 9-hour test day. After a 15-hour recovery period, the control group received 1.0 ml of water at 2-hour intervals. The remainder of the animals received 4.0 mg/100 g body weight oxethazaine HCl in either 1, 2, 3, 4, or 5 divided doses, according to Table 1.

TABLE 1

SCHEDULE OF ADMINISTRATION OF OXETHAZAINE HCl*

	Time from start of test day (hours)				
	0	2	4	6	8
Control	W†	W	W	W	W
Group 1	4.0	W	W	W	W
Group 2	2.0	2.0	W	W	W
Group 3	1.33	1.33	1.33	W	W
Group 4	1.0	1.0	1.0	1.0	W
Group 5	0.8	0.8	0.8	0.8	0.8

*In mg/100 g body weight, administered in 1.0 ml of distilled water

†1.0 ml distilled water

Experiment 4: Dose-response Relationship

The dose-response relationship was determined in a series of thirteen trials using 6 to 8 animals per trial. The animals were given oxethazaine by gavage at 2-hour intervals during the test day, while the control groups received a similar volume of water. The animals ranged in weight from 140 to 260 g. Mean food intake for each treated group was compared to a control group of similar weight, tested at the same time. The dose range varied from 1.0 to 10.0 mg/100 g body weight.

Experiment 5: Influence on Water Intake

In order to determine whether oxethazaine HCl had a direct action on water intake, 68 animals of approximately 120 g were randomly divided into 4 groups of 17. Two groups received 4.0 mg oxethazaine HCl/100 g body weight intragastrically at 2-hour intervals for 8 hr by gavage. The other 2 groups received an equal volume of water. One of the groups receiving the drug and one receiving the water were, in addition, given 1.5 g of sucrose/100 g body weight as a 75% solution by gavage at the beginning of the test period. The animals not receiving the sucrose were given an equivalent amount of water. Food and water intakes were measured for the 8 hour test period.

RESULTS

The addition of oxethazaine HCl to the diet (0.5%) in young growing rats resulted in a reduction in food intake, as compared to the control group (Fig. 1). During the first day on the treated diet, the animals lost on the average 5.9 g, as compared to an increase of 1.2 g in the control group. However, this was a temporary effect, since the treated animals started to gain weight by the second day of treatment, although at a slower rate than the controls. The regression coefficient for the growth curve of the treated animals was 1.79 as compared to 5.16 for the controls. Food efficiency was significantly lower ($p<0.01$) using Student's t Test in the oxethazaine-treated animals, 0.17 g increase in body weight/g of feed, as compared to 0.36 g increase in body weight/g of feed in the control animals. Water consumption in the rats receiving the oxethazaine was 15.2 ± 0.71 ml per rat per day as compared with 19.1 ± 0.4 ml for the control group. There was a statistically significant difference ($p<0.05$) using analysis of variance between food intake in the treated and untreated animals. Similar differences were observed in the water consumption of the treated and untreated rats.

Administration of oxethazaine (25.0 mg/animal) by gavage (Fig. 2) resulted in a marked decrease in food intake during the test day. The drug appeared to have some residual effect through the recovery period for Group B. However, no such effect was observed with Group A.

The effect of varying the number of administrations of the local anesthetic on food intake while holding the total dose constant is shown in Fig. 3. Division of the total dose during the test day at 2-hour intervals resulted in the greatest decrease in food intake. The highly significant ($p<0.001$) regression coefficient of 0.479 indicates that the greatest effect was obtained with the most frequent administration of the drug.

A dose-response curve from several experiments is shown in Fig. 4. The alteration in food intake occurs over the entire range tested from 1.0 mg/100 g body weight to

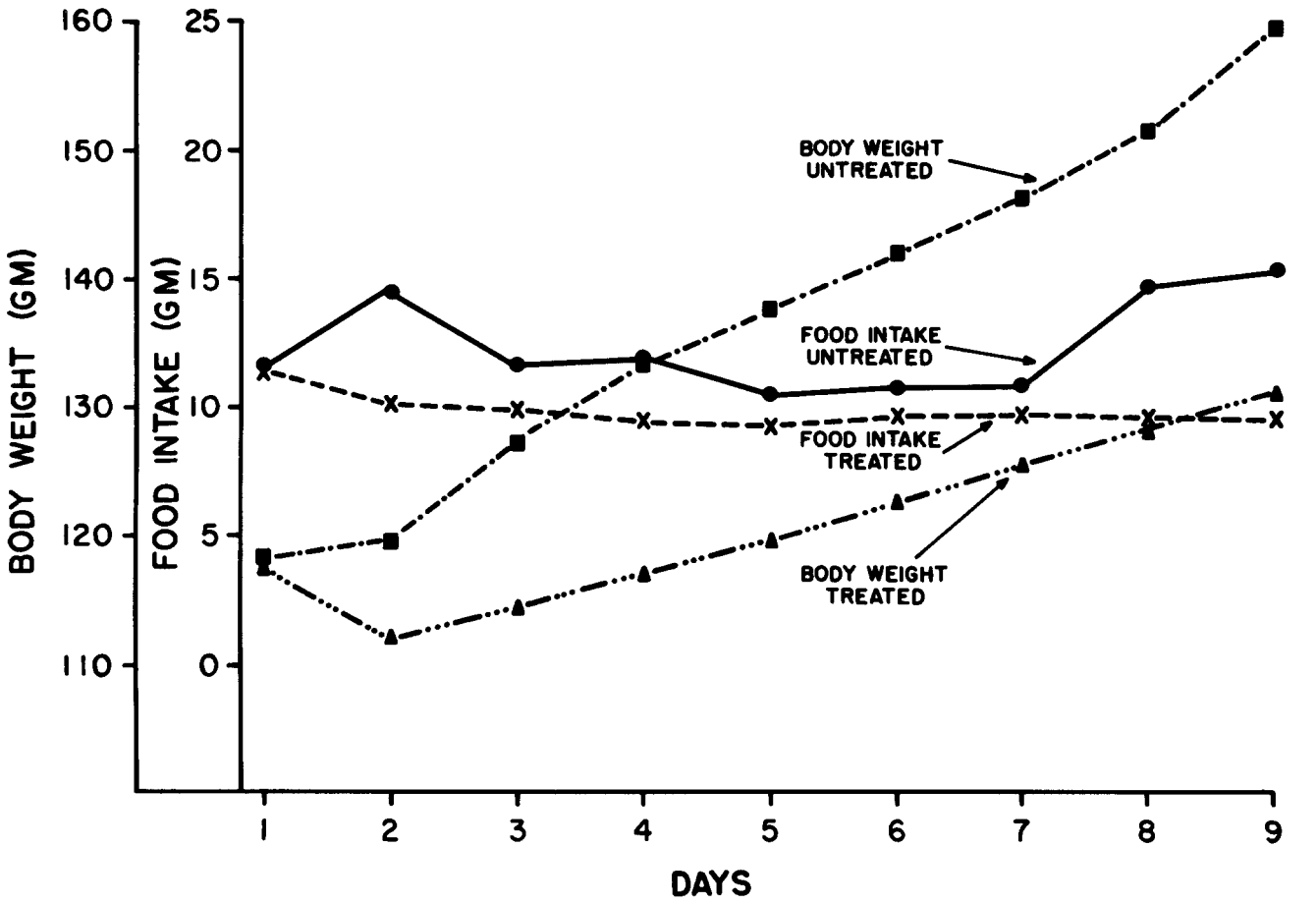

FIG. 1. Effect of addition of oxethazaine HCl to the diet (0.5%) on food intake in the rat. Treatment started on Day 2.

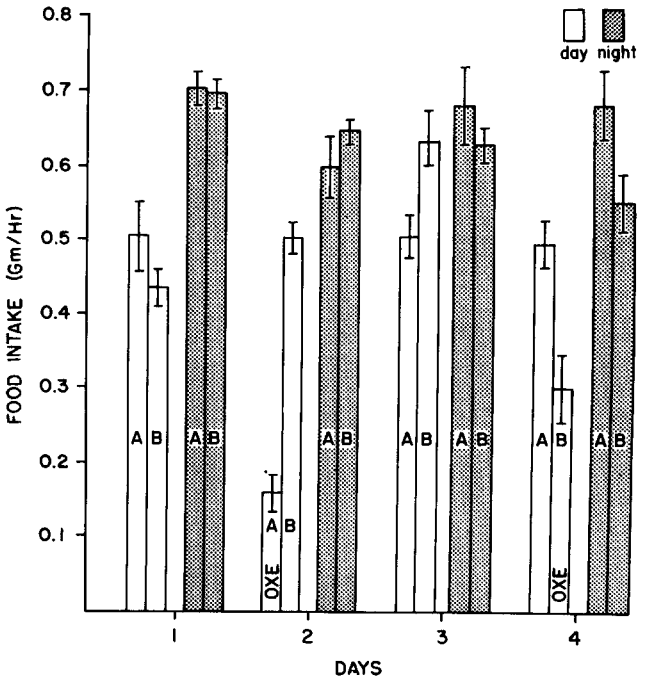

FIG. 2. Effect of oxethazaine HCl (25 mg/animal) on food intake in the rat. Group A received the oxethazaine HCl on Day 2, Group B on Day 4.

10.0 mg/100 g body weight. The curve represents the best fit third order curve calculated by polynomial regression.

The interrelationship between forced feeding and the effects of oxethazaine HCl (a sub-test of Experiment 5) are shown in Tables 2 and 3. Either forced feeding or giving oxethazaine by gavage produces a significant ($p<0.05$) reduction in food intake (Table 2). An even greater reduction is found when both factors are combined; however, the difference between treatment with either single factor, and the treatment with both, are not statistically different. The data on water intake on these same animals are shown in Table 3. Thus, it can be seen that inhibition of water intake is observed only in those animals treated with oxethazaine and not force fed.

DISCUSSION

The mechanism by which the gastrointestinal tract controls food intake has been examined in these experiments. The administration of oxethazaine HCl, either in the meal or by gavage, reduces food intake and weight gain in the rat. Other local and topical anesthetics, such as benzocaine, were tried. However, they produced drowsiness and lethargy indicating that they were acting on the central nervous system. No such side effect was seen with oxethazaine. The reduction in food intake may be due to direct action of the drug on intestinal receptors regulating food intake, or indirectly through reduction in either gastrointestinal secretion and/or motility. It is unlikely that any

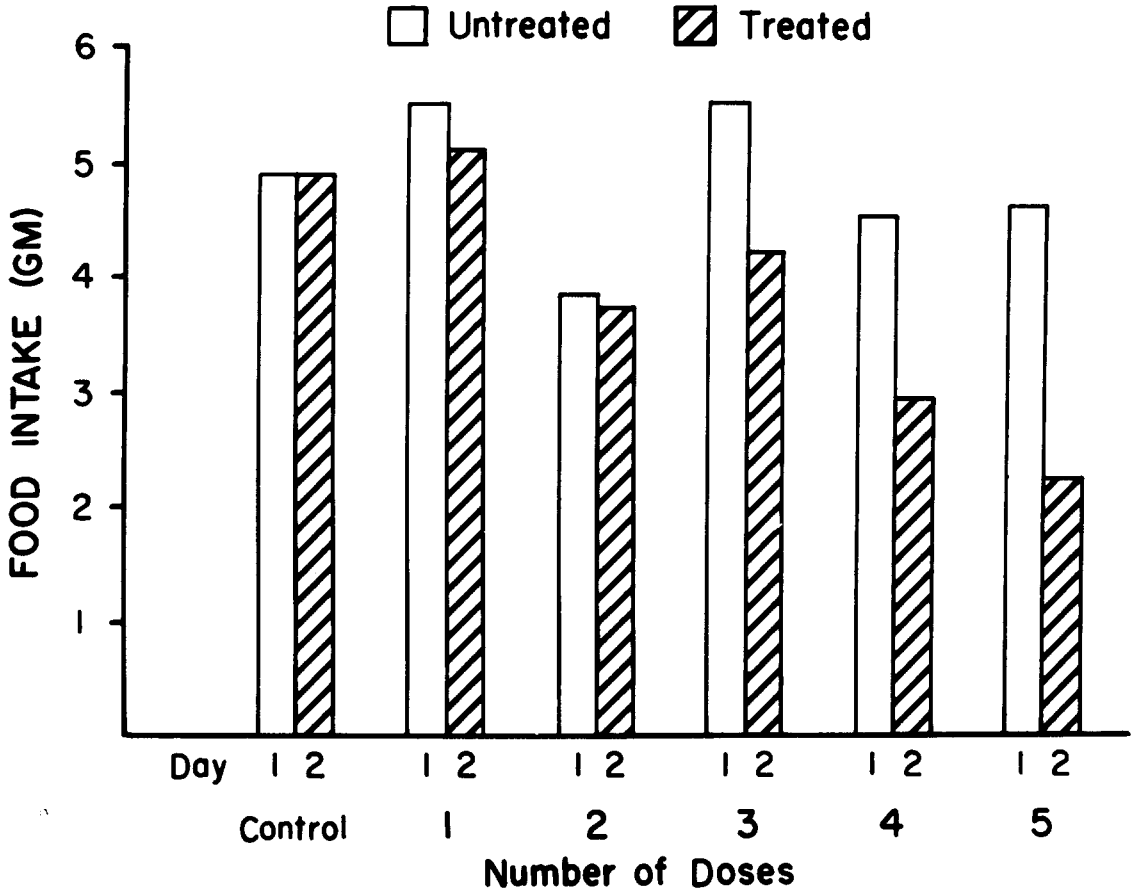

FIG. 3. Effect of dividing the total dose of oxethazaine HCl on food intake in the rat. Total dose was 4.0 mg/100 g body weight.

of the oxethazaine HCl remained on the surface of the gavage tube when withdrawn from the stomach. If present, this could produce lingual anesthesia. Any of the drug that might be left on the surface of the gavage tube would first be aspirated into the lung. Since the material is so toxic when given by this route, death or at least obvious central nervous system effects would be observed. Posey *et al.* [16] have shown that oxethazaine is similar to other local anesthetics in its inhibition of the release of gastrin which then results in a reduction in gastric secretion. However, it is unlikely that the drug acts on gastrointestinal secretions to reduce food intake, since the dose required to reduce gastrointestinal secretion [14] is much lower than that required to affect food intake in these experiments. While it is known that the oxethazaine does not affect gastrointestinal motility at the lower doses required to alter gastric secretion [14], no information is available on higher doses.

The data tends to indicate that the action of the drug is in the intestine rather than the stomach. Since oxethazaine HCl is a topical anesthetic, the concentration reaching the mucosal surface and the duration of contact would determine the activity of the drug. Increases in concentration above the maximum effective concentration for any topical anesthetic do not result in any greater analgesia or duration of action [7]. The maximum effective dose for oxethazaine HCl is 0.5% [5]. The results described here indicate that the inhibition of food intake is total-dose, and not concentration-dependent. This would indicate that factors other than the direct action of the drug on the mucosal surface must be evaluated, the most likely being a dilution factor. Dilution by food would not be important, as it is the concentration of the drug in water that is the critical factor. While there is a statistical difference in water intake, the actual dilution factor from the water drunk (see Table 3) would not be sufficient to explain the large changes in the dose response curve. Since oxethazaine HCl has been shown to inhibit gastric secretion, the dilution must take place in the intestine and not the stomach, thereby implicating the intestine, rather than the stomach, in the central mechanism.

Strominger [19] and later Lepkovsky *et al.* [12] demonstrated that food and water intake are interdependent. The forced feeding experiment was performed to determine whether the reduced water intake observed with oxethazaine treatment was a direct action of the drug, or an indirect one through reduced food intake. Water intake with forced feeding of sucrose solution and administration of oxethazaine was essentially the same as the control group. The animals without forced feeding, but with oxethazaine HCl, had lowered water intake as well as reduced food intake. The animals drank in response to the caloric or food intake, since, when the caloric intake was high through forced feeding, and the animals were treated with the local anesthetic, water intake was similar to the

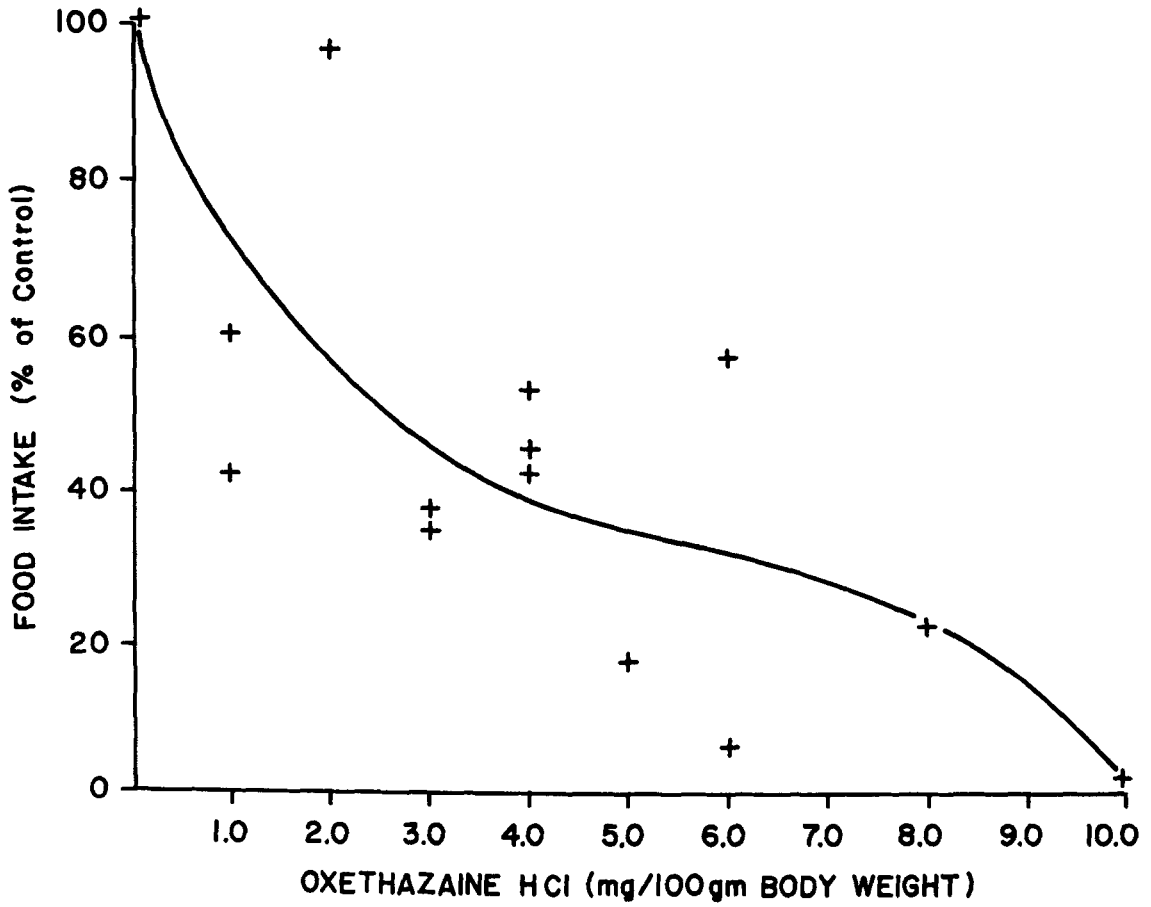

FIG. 4. Dose response curve for the effect of oxethazaine HCl on food intake. The oxethazaine HCl was administered in divided doses at 2-hour intervals. The curve is the best fit line calculated by polynomial regression.

TABLE 2

EFFECT OF OXETHAZAINE HCl (4.0 mg) ON FOOD INTAKE IN RATS PRELOADED WITH SUCROSE OR WATER. OXETHAZAINE GIVEN IN A DIVIDED DOSE AT 2-HOUR INTERVALS. SUCROSE SOLUTION WAS 1.5 g/100 g BODY WEIGHT IN A 75% SOLUTION

	g/Test Day	
	Water	Sucrose
Control	7.18 ± 0.58* (N = 17)	2.88 ± 0.37* (N = 17)
Oxethazaine	2.59 ± 0.42* (N = 17)	1.59 ± 0.42* (N = 17)

*Mean ± S.E.

TABLE 3

EFFECT OF OXETHAZAINE HCl (4.0 mg) ON WATER INTAKE IN RATS PRELOADED WITH SUCROSE OR WATER. OXETHAZAINE GIVEN IN A DIVIDED DOSE AT 2-HOUR INTERVALS. SUCROSE SOLUTION WAS 1.5 g/100 g BODY WEIGHT IN A 75% SOLUTION

	g/Test Day	
	Water	Sucrose
Control	9.82 ± 1.03* (N = 17)	8.47 ± 0.73* (N = 17)
Oxethazaine	6.05 ± 1.01* (N = 17)	8.41 ± 1.00* (N = 17)

*Mean ± S.E.

control animals. The failure of the oxethazaine HCl to affect water intake also indicated that the local anesthetic was not acting on the central nervous system. The reduced food intake was not produced by drowsiness in the animals. The results also suggest that the reduced food intake was not a function of nausea since nausea would decrease water as well as food intake.

The many explanations for the regulation of food intake have only recently included the role of the intestine. Both chemo- and mechanoreceptors are present in the intestine, but the extent to which they are involved in the control of food intake is not well established. Ehman *et al.* [4] demonstrated that intestinal distension will alter feeding. However, one cannot eliminate the potential influence of intestinal chemoreceptors on food intake, since fat produces a greater feeling of satiety than any other component of the meal [20]. Whether this is due to the decrease in gastric motility has not been shown. It is not known why the topical anesthetic produced results opposite to that expected from the work of Campbell and Davis [1], Ehman *et al.* [4] and Gibbs *et al.* [6]. However, the oxethazaine HCl was able to inhibit food intake. Whether the reduction of food intake was due to direct action of the drug on intestinal receptors is not known.

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