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Peripheral Atropinization Does Not Change Meal Size Controlled By Prepyloric Mechanisms

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RAUHOFER, E. A., D. GREENBERG AND G. P. SMITH. *Peripheral atropinization does not change meal size controlled by prepyloric mechanisms.* PHYSIOL BEHAV **59**(2) 237–240, 1996.—To investigate the role of gastric contractility in the prepyloric control of meal size, we administered atropine methyl nitrate (3 mg/kg,ip) or saline prior to 30-min tests on 3 separate days in which 0.8 M sucrose was ingested by rats after 1 h of food deprivation. Ingested 0.8 M sucrose accumulated in the stomach and did not empty into the small intestine during the test because the pylorus of each rat was closed by inflation of a chronic pyloric cuff. Atropine methyl nitrate had no significant effect on 30-min intake or on the pattern of intake observed at 3-min intervals during the test on any of the three test days. Thus, neither acute nor repetitive experience with atropine methyl nitrate changed the pattern or volume of intake. These results suggest that gastric contractile responses elicited by the intragastric accumulation of 0.8M sucrose during ingestion are not necessary for the control of meal size by prepyloric mechanisms.

Gastric distention Gastric satiety Gastric motility Pyloric cuff Control of food intake Vagus nerve

INTRODUCTION

WHEN ingested food is prevented from emptying from the stomach by acute mechanical closure of the pylorus, meal size is not different from that observed in the same rats when ingested food empties from the stomach normally (5–7,10). The ability of pregastric and gastric stimuli acting without postpyloric satiating mechanisms to control meal size within normal limits has now been observed with a variety of diets including high-carbohydrate, low-fat diets, milk, sucrose, and corn oil, and after 0.5, 3, and 15 h of food deprivation. Furthermore, when the pylorus is closed, rats adjust meal size normally to changes in the concentration of sucrose and corn oil (12). They also decrease meal size when a volume of 0.15 M saline is infused during a meal (7), and increase meal size when ingested food is withdrawn from the stomach during a meal (2,7) or when ingested food drains out of a chronic gastric cannula (4).

Given the traditional emphasis on a distention-sensitive, vagal afferent mechanism for mediating the inhibitory control of meal size by gastric stimuli, it is surprising that meal size did not change when abdominal vagotomized rats ate during acute closure of the pylorus (7,8). In fact, the only significant effect of hepatic-spared abdominal vagotomy on intake in such rats is that it blocked the inhibitory effect of saline infusions during the meal (7). It is interesting that under the same conditions, vagotomy did not block the increased intake observed after withdrawal of 5 ml of stomach contents during a meal of concentrated or dilute milk (7).

Abdominal vagotomy produces complex effects that change over time. Abdominal vagotomy removes three types of extrinsic nerves to the stomach. These are vagal excitatory efferent fibers, vagal inhibitory efferent fibers, and vagal afferent fibers (13).

The loss of the vagal excitatory efferent fibers initially decreases the motility of the stomach, but after a period of weeks, motility returns to or near normal due to the action of the intrinsic neurons in the gastric wall (13). Despite this recovery of motility patterns, gastric emptying of solids remains abnormally slow while liquids empty abnormally fast (13).

Abdominal vagotomy also decreases intake. This effect evolves during the early postoperative period and tends to recover by the end of the second postoperative week (9). With appropriate postoperative care and a palatable liquid diet, the effects on intake can be minimized, but vagotomized rats usually eat significantly smaller meals than normal (1,16). The smaller meals are due to increased potency of postingestive negative feedback effects probably produced by the increased rate of emptying of the ingested liquid from the stomach (3).

In an attempt to investigate the role of the vagus in the gastric control of meal size more specifically, we administered atropine methyl nitrate intraperitoneally prior to test meals eaten with the pylorus closed. Atropine methyl nitrate, a peripherally acting

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antagonist of peripheral cholinergic receptors of the muscarinic-type (M_1 and M_2), decreases gastric motility because the major determinant of gastric muscle contraction is acetylcholine released from intrinsic neurons onto muscarinic synapses on gastric smooth muscle cells (13). Thus, atropine methyl nitrate reproduces one effect of abdominal vagotomy (i.e., the removal of the excitatory, efferent fibers that stimulate the intrinsic cholinergic neurons). Atropine methyl nitrate can produce a more complete decrease in motility than vagotomy because it also blocks the contractile effect of the intrinsic cholinergic neurons.

In addition to producing a more complete block of muscle contractility, atropine methyl nitrate has two advantages over abdominal vagotomy as a probe of the relationship between cholinergic control of gastric contractility and gastric mechanisms for the control of meal size: First, atropine methyl nitrate is more specific because it does not change the functions of efferent inhibitory fibers or afferent fibers (13,14). Second, because the effect of atropine methyl nitrate is acute and reversible, this precludes the adaptive changes that occur over time after abdominal vagotomy.

In this paper, we report that administration of atropine methyl nitrate prior to a meal ingested while the pylorus was closed did not change the size of the meal or the pattern of intake observed at 3-min intervals during the meal. A preliminary report has appeared (11).

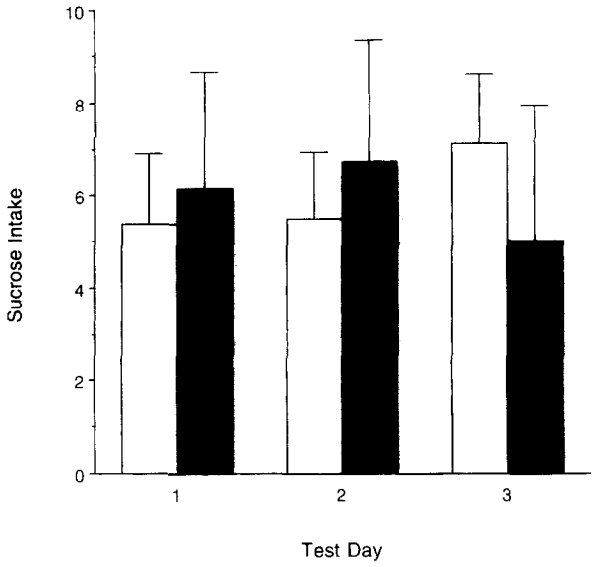

FIG. 1. Data are mean ± SE for 30-min intakes of 0.8M sucrose. Filled bars represent the atropine group ($n = 7$). Open bars represent the control group ($n = 8$). There are no significant differences.

METHODS

Subjects

Male Sprague–Dawley rats, Taconic Farms (Germantown, NY), weighing 300–450 g at the start of the experiment were housed individually in wire-bottom, hanging cages and maintained on a 12:12 light/dark cycle. Except for the testing period and one hour prior to the test, rats had access to powdered chow (Purina Rat Chow #5001) and tap water ad lib.

Surgery

Rats were surgically implanted with a modified pyloric cuff that could be reversibly inflated to prevent gastric emptying (17) according to the technique described below. Each pyloric cuff was constructed using a 20 × 40 mm piece of PharmElast sheeting (SF Medical #20-05) 0.005″ thick, a 5 × 40 mm piece of PharmElast sheeting (SF Medical #50-10) 0.010″ thick and a 20-cm piece of 0.030″ i.d. × 0.065″ o.d. Silastic tubing (Technical Products,Inc. #TPI 602-175). The edges of the 20 × 40 mm piece were covered with silicone medical adhesive (Dow Corning #891). The tubing was placed in the center of the 20 × 40 mm piece. The 5 × 20 mm piece was placed directly over the tube and lightly pressed into the glued edges of the underlying larger sheet. Each edge of the larger sheet was folded on top of the smaller piece, pulling the edge of the top sheet over as far as possible to make an air-tight pocket.

Surgery was performed under surgical anesthesia produced by a mixture of chloral hydrate and pentobarbital (3 ml/kg, IP). An incision was made at the xiphoid process and extended 5 cm caudally. The stomach was located and the pylorus was identified. A curved hemostat was used to separate the pylorus from the underlying tissue. The hemostat was then used to pull the pyloric cuff under the pylorus. The ends of the pyloric cuff were sutured together lateral to the pyloric cuff tube. The cuff was placed between the serosal surface of the pylorus and the overlying blood vessels and nerves, so that when the cuff was inflated, it did not compress the vessels or nerves. Saline (0.15M NaCl) was infused through the end of the pyloric cuff tube to determine the volume (< 0.5 ml) needed to close the pylorus completely. The end of the pyloric cuff tube was fitted onto a 19-gauge trochar and pulled through an incision made on the dorsal surface of the neck. The incisions were closed using 3-0 silk sutures (Ethicon 3-0 taper needle with silk suture #K843H).

TABLE 1
INTAKE IN 3-MIN INTERVALS AFTER ATROPINE METHYL NITRATE OR VEHICLE TREATMENT

	Interval Intakes of 0.8M Sucrose									
	0–3	3–6	6–9	9–12	12–15	15–18	18–21	21–24	24–27	27–30
Day 1										
Atropine	1.4 ± 0.5	1.6 ± 0.5	1.0 ± 0.5	0.9 ± 0.7	0.4 ± 0.3	0.1 ± 0.1	0.4 ± 0.3	0.1 ± 0.1	0	0
Vehicle	2.5 ± 0.7	1.4 ± 0.6	0.3 ± 0.3	0.3 ± 0.2	0.6 ± 0.3	0.3 ± 0.3	0.1 ± 0.1	0	0	0
Day 2										
Atropine	2.1 ± 0.6	1.0 ± 0.6	0.9 ± 0.5	0.3 ± 0.2	0.4 ± 0.3	0.9 ± 0.4	0.3 ± 0.3	0.1 ± 0.1	0.3 ± 0.3	0.6 ± 0.4
Vehicle	2.3 ± 0.6	1.5 ± 0.5	0.9 ± 0.5	0.6 ± 0.3	0.1 ± 0.1	0.1 ± 0.1	0	0.1 ± 0.1	0	0
Day 3										
Atropine	1.6 ± 0.7	0.9 ± 0.4	1.0 ± 0.7	0.1 ± 0.1	0.3 ± 0.3	0.4 ± 0.3	0.3 ± 0.3	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1
Vehicle	3.3 ± 0.8	1.5 ± 0.5	0.3 ± 0.3	0.6 ± 0.5	0.5 ± 0.3	0.5 ± 0.4	0.1 ± 0.1	0	0.3 ± 0.3	0.1 ± 0.1

Data are mean ± SE intakes of 0.8M sucrose. Atropine methyl nitrate (3 mg/kg) or vehicle (0.15M NaCl) was injected ip 30 min before the intake test on 3 test days.

Testing

Rats were tested during the light phase from 1030 to 1200 for 5 days with the pyloric cuff inflated to prevent gastric emptying during the test. Each rat was given an intraperitoneal injection of saline or atropine methyl nitrate (Sigma, St. Louis, MO) 30 min prior to the test. (Atropine methyl nitrate will be referred to as atropine throughout the rest of this paper.) The atropine group ($n = 7$) received a saline injection on days 1 and 5, and atropine methyl nitrate (3mg/kg) on days 2, 3, and 4. The control group ($n = 8$) received a saline injection on all 5 test days. We did repetitive tests to examine the reproducibility of any effect of atropine and to attempt to detect any change that was based on experience (e.g., extinction of conditioned satiety or acquisition of conditioned aversion).

Testing began after rats were adapted to licking 0.8 M sucrose (Fisher reagent grade, Fair Lawn, NJ) from drinking tubes in testing cages. Five minutes prior to the tests, rats were removed from their home cages and pyloric cuffs were inflated by infusing the volume of 0.15 M saline that had been demonstrated during surgery to close the pylorus. Then rats were placed in the testing cages. Each test lasted 30 min and intakes were measured at 3-min intervals.

At the end of the test, the pyloric cuffs were deflated by withdrawing the 0.15M saline. Rats were returned to their home cages and 30 min later their maintenance diet and water were returned.

At the end of the experiments, the ability of the saline infusion to close the pylorus completely was confirmed under surgical anesthesia.

Statistical Analysis

Because the intakes of both groups after saline injections on the first and fifth test days did not differ significantly (atropine group = 8.9 ± 2.0 ml, control group = 8.1 ± 0.8 ml), we analyzed the 30-min intakes on the three test days in which one group received atropine (ip) and the other received 0.15 M saline by a 2-way ANOVA (SAS Statistical programs Cary, NC) with group and test day as main factors.

Because the two largest differences in intake of the atropine and control groups in a 3-min interval were not significantly different by *t*-test, we did no further statistical analysis of the 3-min interval intakes.

RESULTS

Atropine had no significant effect on 30-min intakes on the 3 test days compared to control (Fig. 1). Atropine not only did not change total intake, it also did not produce a significant difference in the pattern of intake measured at 3-min intervals during the tests (Table 1).

DISCUSSION

Acute blockade of muscarinic synapses on gastric smooth muscle by atropine did not change the size of the meal (Fig. 1) or the pattern of intake observed at 3-min intervals (Table 1) during a meal ingested while the pylorus was closed. Thus, when ingested liquid food accumulates in the stomach that cannot empty its contents into the duodenum, gastric contractile reflexes elicited by chemical or mechanical intragastric stimuli and mediated through intrinsic cholinergic neurons and excitatory efferent fibers of the vagus are not necessary for the gastric control of meal size under these conditions.

This is consistent with previous reports that abdominal vagotomy did not change meal size when the pylorus was closed (7,8). Our results extend this work by demonstrating that reversible blockade of the function of only one type of vagal fibers to the stomach had no effect. Note, however, that there is no available data to decide whether the gastric mechanisms that control meal size after abdominal vagotomy are the same as those that operate after atropine.

The lack of effect of atropine in three consecutive tests (Fig. 1) indicates that test experience did not change the result. Thus, neither extinction of a conditioned satiety effect nor acquisition of a conditioned aversion effect occurred in these experiments.

Our results are also consistent with the neurophysiological analysis of vagal afferent fiber activity produced by 2 ml of 0.15 M saline infused into the stomach of anesthetized rats for 30 s (14). Under these conditions, intraarterial injections of atropine sulfate (15 mg/rat) did not change the increased afferent activity produced by the intragastric load.

Our interpretation of these experiments assumes that the dose of atropine used prevented all gastric contractions. Because this was not measured, our interpretation is an inference from the relatively large dose used; this dose is 12 times larger than the dose of atropine sulfate that essentially abolishes gastric emptying and is 30 times larger than the dose that abolishes motility recorded by a gastric balloon in unanesthetized rats (15).

Another caveat is that actions of atropine in sites other than the stomach may have contributed to our results in unidentified ways.

With these interpretive limitations in mind, we conclude that gastric contractile reflexes mediated by cholinergic muscarinic receptors are not necessary for the control of meal size when gastric emptying is prevented by acute mechanical closure of the pylorus.

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