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Hepatic Vagotomy Does Not Disrupt the Normal Satiation of NaCl Appetite

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FRANKMANN, S. P. AND G. P. SMITH. *Hepatic vagotomy does not disrupt the normal satiation of NaCl appetite.* PHYSIOL BEHAV **53**(2) 337–341, 1993.—The necessity of the hepatic branch of the vagus nerve for the normal satiation of NaCl intake under the condition of sodium depletion was tested. Sham- or hepatic-vagotomized male, Long–Evans rats were sodium depleted by injection with Lasix (furosemide, 10 mg) and were maintained overnight on sodium-deficient diet and water. The intake of 0.3 *M* NaCl during a 2-h salt appetite test was not significantly different between the sham- and hepatic-vagotomized groups. In a second group of sham- or hepatic-vagotomized rats, a load of 7.5 ml of 0.5 *M* NaCl was given by gavage at 15 or 90 min prior to a 1-h NaCl appetite test. The preload decreased NaCl intake equivalently in both groups. Gastric emptying of a preload of NaCl at 15 and 90 min was also the same for sham- and hepatic-vagotomized rats. Thus, the hepatic branch of the vagus nerve is not necessary for the normal short-term satiation of NaCl intake under the condition of acute sodium depletion produced by furosemide.

Sodium depletion Salt appetite Satiety Gastric emptying Vagal afferents

AFTER acute sodium depletion by furosemide, a natriuretic-diruetic drug, rats stop ingesting NaCl within 30 min (12). This behavioral satiety occurs before significant absorption and delivery of the ingested sodium to all of the tissues needing the sodium can have occurred (2). Although the mechanism of this short-term satiation is not known, the mechanism must be activated by postingestive NaCl because when the postingestive effects of NaCl are eliminated or minimized by sham drinking (13,18), short-term satiation is delayed or does not occur (9,16). Thus, the taste of sodium and other pregastric stimuli acting alone are not sufficient to elicit normal short-term satiation in the sodium-depleted rat.

The site of detection of sodium for the short-term satiation of NaCl appetite is unknown, but there is evidence that the liver or hepatic portal vein is involved. First, neurophysiological studies have shown that hepatic vagal fibers respond to changes in NaCl concentration of the portal blood (1,4). In accordance with this finding, infusion of hypertonic NaCl (0.6 *M*) into the hepatic portal, but not systemic, circulation of sodium-depleted rats reduces the intake of NaCl (15). Second, when the hepatic portal circulation is circumvented by portacaval shunts, intake of NaCl is increased, as would be expected if normal short-term satiety depends on detection by hepatic portal or hepatic mechanisms (7). These data suggest that the sensory fibers of the hepatic branch of the vagus could carry information about absorbed sodium that is necessary for normal short-term satiation of NaCl intake.

If the hepatic branch of the vagus nerve is necessary for normal short-term satiation of NaCl intake, then the hepatic-vagotomized rat that is depleted of sodium by furosemide should drink more NaCl during the first 30 min or it should drink NaCl for a longer time. Sectioning of the hepatic branch of the vagus nerve, however, has not produced significant increases of NaCl intake in response to sodium depletion by furosemide (15), extracellular fluid depletion by polyethylene glycol treatment (8), or prolonged dietary sodium deprivation (5).

There is one apparently positive result. Tordoff et al. (15) reported an attenuation of the suppressive effect of hepatic portal infusions of NaCl on depletion-induced NaCl intake in hepatic-vagotomized rats. Their data reveal, however, that infusion of hypertonic NaCl into the hepatic portal vein decreased NaCl intake to the same extent in hepatic-vagotomized rats as in intact rats. The apparent attenuation of NaCl intake after hepatic vagotomy was relative to an increased intake of NaCl in response to infusion of water into the portal vein. This suggests that while there may have been an abnormal ingestive compensation to the hypotonicity produced by portal infusions of water, the response to NaCl infusion was normal.

There are two possible explanations of the previous failures of hepatic vagotomy to produce increased NaCl intake after acute sodium depletion by furosemide. First, in the experiments of Tordoff et al. (15,16), the rats had been sodium depleted several times prior to the surgical vagotomies. Following recovery from a first sodium depletion, subsequent sodium depletions elicit

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larger NaCl intakes (12). This larger NaCl intake may have obscured an effect of hepatic vagotomy on NaCl intake. Second, although the hepatic branch of the vagus nerve is not known to influence gastric emptying (6,11), it is possible that its removal increased the rate of gastric emptying and thus resulted in a more rapid delivery of sodium to other postgastric sites for satiation. An increased rate of delivery of sodium to other, currently unknown, sites that provide satiating information by nonhepatic vagal mechanisms could compensate for the loss of information mediated by the hepatic branch of the vagus nerve. To investigate these possibilities, we measured the NaCl intakes of sham- and hepatic-vagotomized rats at a first sodium depletion with and without gastric preloads of NaCl. We also measured the gastric emptying of NaCl after hepatic vagotomy.

METHOD

Subjects

Male Long–Evans rats, weighing 200–250 g at the beginning of the experiment, were individually housed in a temperature-controlled room with a 12:12 D:L cycle. All subjects had free access to Purina chow (No. 5001), tap water, and 0.3 *M* NaCl, unless otherwise noted.

Surgery

Sham and hepatic vagotomies were performed using chloropent anesthesia (3 ml/kg, IP) in rats that were overnight food deprived. A ventral midline incision was made and the peritoneal cavity exposed. The stomach was retracted to expose the esophagus. Using an operating microscope, the right trunk of the abdominal vagus was located and followed distally to the hepatic branch. The hepatic vagal branch was isolated and ligated with two sutures placed 1 cm apart and then cut between the ligatures. The abdominal muscles were then sutured and the skin closed with wound clips. Sham vagotomy consisted of the same procedures except that the vagus was not ligated or cut. After the surgery the rats were maintained on Purina chow except when sodium depleted at 1 and 2 weeks postsurgery. Two weeks later, at the termination of the experiment, rats were anesthetized and the completeness of vagotomy was confirmed by locating the ligatures and looking for regrowth or any missed branches under 10–40× magnification. For further details of the surgical and verification techniques, see Smith and Jerome (14). Only data for those rats with confirmed vagotomies are reported here.

Sodium Depletion

Seven to 10 days after surgery, the rats were sodium depleted by combined injection of the diuretic-natriuretic drug, Lasix (furosemide), and removal of ambient sodium. Each rat received two SC injections of Lasix (5 mg/0.5 ml), separated by 2 h. At the time of the first injection, Purina pellets were replaced with a pelleted, sodium-deficient diet (BioServe AIN-76A). The 0.3 *M* NaCl, but not water, was removed. Thus, only sodium-deficient diet and water were available for overnight consumption. Overnight food intake, water intake, and body weight change were recorded, but no significant differences were observed. There were no differences between the sham- and hepatic-vagotomized rats in body weight at the time of the depletion.

Gastric Preload of NaCl

At 18–24 h after the first injection of furosemide and prior to the NaCl appetite test, rats were given a preload of 7.5 ml of 0.5 *M* NaCl (3.75 mEq Na^+) by gavage. The preload was chosen to be consistent with that of Tordoff et al. (16), which provides more than the amount of sodium lost during the overnight sodium depletion. An infant feeding tube was lowered down the esophagus to the stomach and the NaCl delivered as a bolus. All rats were mock gavaged several times prior to experimental treatment to accustom them to the procedures.

NaCl Appetite Test

Water and 0.3 *M* NaCl intakes were recorded to the nearest 0.1 ml at 5, 10, 15, 30, 45, and 60 min.

Statistical Analysis

All data were analyzed by ANOVA or *t*-test.

EXPERIMENT 1: EFFECT OF HEPATIC VAGOTOMY ON NaCl INTAKE AT A FIRST SODIUM DEPLETION

Following recovery from sham ($n = 6$) or hepatic vagotomy ($n = 6$), the rats were sodium depleted for the first time and their intakes of 0.3 *M* NaCl recorded for 120 min.

Results

The NaCl intakes of the hepatic-vagotomized rats were not significantly different from the sham-vagotomized rats, $F(1, 10) = 0.52$, NS (Fig. 1). While the mean intake of the hepatic-vagotomized rats was less than the intact group from 15 to 60 min, the difference was not significant even at the 60-min time point, $F(1, 10) = 3.18$, $p = 0.11$.

Discussion

Hepatic-vagotomized rats did not drink more NaCl nor did they drink for longer than sham-vagotomized rats. Thus, even at a first sodium depletion, hepatic vagotomy did not increase NaCl intake. This is consistent with previous results in multi-depleted rats (15) and in rats depleted by other treatments (5,8). These results provide strong evidence that the hepatic branch of the vagus nerve is not necessary for the normal satiating effect of ingested NaCl in rats depleted of sodium by furosemide.

EXPERIMENT 2: EFFECT OF A NaCl PRELOAD GIVEN AT 15 OR 90 min PRIOR TO THE NaCl APPETITE TEST

To provide a more rigorous test of the necessity of the hepatic branch of the vagus nerve for the normal satiation of NaCl ap-

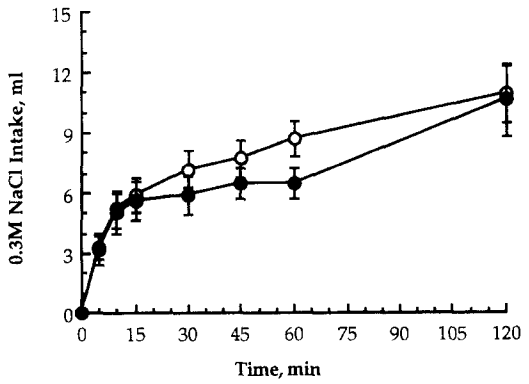

FIG. 1. Cumulative intakes (mean ± SEM) of 0.3 *M* NaCl for sham-vagotomized (open circles) and hepatic-vagotomized (filled circles) rats.

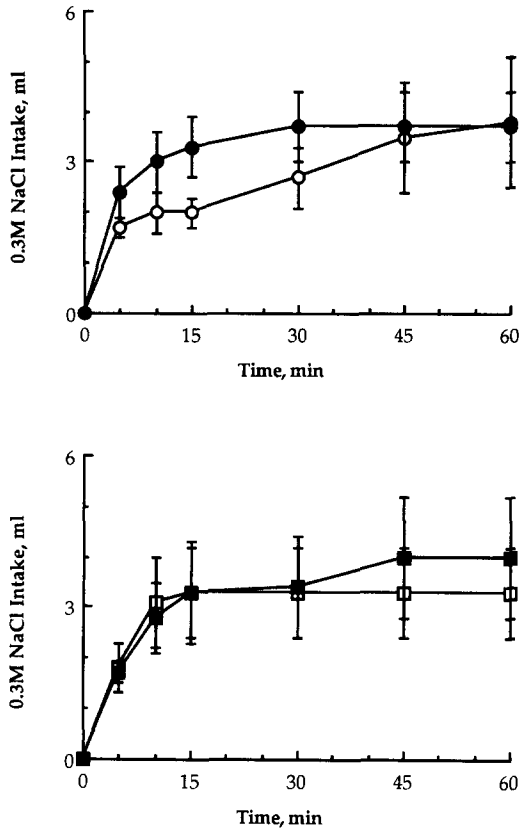

FIG. 2. Cumulative intakes (mean ± SEM) of 0.3 *M* NaCl for sham-vagotomized (open symbols) and hepatic-vagotomized (filled symbols) rats following a gastric preload of 7.5 ml of 0.5 *M* NaCl at 15 min (upper panel, circles) or 90 min (lower panel, squares) prior to the onset of the appetite test.

petite, rats were given a gastric preload of NaCl at 15 or 90 min prior to the sodium appetite test. The 90-min delay was chosen based on the previous work by Tordoff et al. (16). The 15-min delay was chosen based on the time course with which NaCl intake normally terminates or slows markedly (12). Further, the 15-min delay would be more likely to reveal a deficit in the early detection of sodium, a plausible function of a hepatic vagal mechanism.

Twenty-four depletion-naive rats were either sham (n = 12) or hepatic vagotomized (n = 12). Following overnight sodium depletion, half of each surgical group was given a preload of 7.5 ml of 0.5 *M* NaCl at 15 or 90 min prior to the appetite test. Thus, there were four groups:

1. Sham-vagotomized/15-min preload,
2. Sham-vagotomized/90-min preload,
3. Hepatic-vagotomized/15-min preload, and
4. Hepatic-vagotomized/90-min preload.

Water was available following the 90-min delay preload, but not following the 15-min delay preload.

Results

The preload was equally effective in the sham- and hepatic-vagotomized rats following the 15-min delay, $F(1, 10) = 0.96$, NS (Fig. 2 upper panel), and following the 90-min delay, $F(1, 10) = 0.02$, NS (Fig. 2 lower panel). Although the mean intakes of the hepatic-vagotomized group were larger than the sham-vagotomized group following the load given with a 15-min delay, the difference was not significant even at the at 15-min time point, $F(1, 10) = 3.61$, $p = 0.09$. There was no difference in the effectiveness of the preload given at 15 min or 90 min prior to the appetite test, $F(1, 20) = 0.06$, NS.

The water intakes during the 90 min following the preload did not differ between the two groups, $F(1, 10) = 1.22$, NS (Fig. 3), and are similar to those reported by Tordoff et al. (16). Note that the intakes at 15 min were only 1 ml; thus, withholding water following the 15-min preload was unlikely to produce any difference in the results.

Discussion

The finding that the hepatic-vagotomized rat compensates normally for a gastric preload of NaCl given 15 min or 90 min prior to NaCl access is further evidence against the hepatic branch of the vagus being necessary for the normal satiation of NaCl intake.

The two preload intervals (15 and 90 min) were not different in their suppressive effects on NaCl intake, suggesting that detection mechanisms that exert their actions soon after the preload were sufficient to produce the maximal suppression of NaCl intake. Thus, a longer time for the absorption and distribution of NaCl did not produce a larger suppression of NaCl intake. Wolf et al. made a similar observation under different conditions (17).

Although our results did not reveal a loss of the satiating effect of NaCl after hepatic vagotomy, it is possible that the hepatic vagotomy did remove a significant detector mechanism for satiation, but that this was obscured because hepatic vagotomy also increased the rate of gastric emptying. This could result in a more rapid distribution of NaCl to other postabsorptive satiating sites that would obscure the effect of removal of a hepatic vagal mechanism. While the hepatic branch of the vagus nerve has not been shown to have a major effect on gastric emptying (6,11), its role in gastric emptying during sodium depletion has not been tested.

EXPERIMENT 3: GASTRIC EMPTYING OF A NaCl PRELOAD

To address this question, the rats from Experiment 2 were again sodium depleted, and the amount of sodium remaining

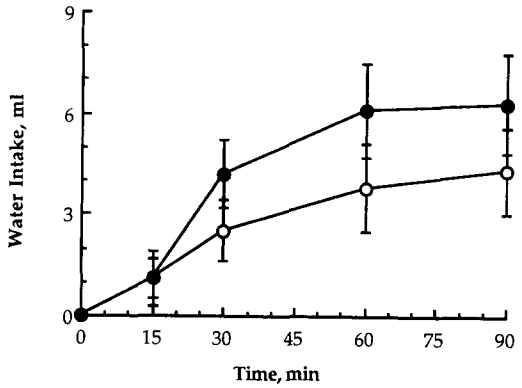

FIG. 3. Cumulative intakes (mean ± SEM) of water for sham-vagotomized (open circles) and hepatic-vagotomized (filled circles) rats during the 90-min interval following the gastric preload of 7.5 ml of 0.5 *M* NaCl.

TABLE 1

GASTRIC SODIUM CONTENT AFTER A GASTRIC PRELOAD OF 3.75 mEq OF SODIUM

	Sodium Remaining in Stomach	
Surgical Group	15 min	90 min
Sham	0.67 ± 0.03	0.12 ± 0.04
Hepatic vagotomy	0.56 ± 0.14	0.10 ± 0.04

Data are mean ± SEM mEq of sodium. There were six rats in each treatment group.

in the stomach following the gastric preload was measured at 15 or 90 min (the onset of the NaCl appetite test).

To control for a possible effect of being multidepleted on the rate of gastric emptying of the preload, the amount of sodium remaining in the stomach following a gastric preload was measured in depletion-naive rats. They were divided into two groups, sham ($n = 6$) and hepatic vagotomized ($n = 6$). The first sodium depletion and the first preload (with a 15-min delay) were given at 1 week following surgery.

The rats were sodium depleted as described previously. The following day, all rats were given a gastric load of 7.5 ml of 0.5 *M* NaCl, and 85 or 10 min later (as appropriate to their first depletion delay) they were anesthetized with chloropent (3 ml/kg, IP). Water was not available following the gastric load. The stomach contents were collected by a procedure similar to that described by Moran and McHugh (10). The stomach was clamped at the esophageal and pyloric sphincters, and the esophagus and intestine cut just distal to the clamps, to allow removal of the stomach. An incision was made along the greater curvature of the stomach, and the contents flushed out with 10 ml of deionized water and collected. Two additional 10-ml rinses were made and each was collected separately for analysis of sodium concentration (flame photometry, IL-943). Pilot work indicated that by the third rinse the sodium content was minimal, indicating that all sodium had been flushed from the stomach. The time from injection of chloropent to the actual collection of the stomach contents was approximately 5 min. The sodium content of the stomach was calculated by determining the sodium in each flush [flush volume (deionized water plus stomach contents) multiplied by the sodium concentration] and adding the three together. By subtracting this value from the amount given in the preload, the amount of sodium that emptied from the stomach could be estimated. Comparisons between groups were made by *t*-test. Microscopic verification of the vagotomy was done after the removal of the stomach (14).

Results

At 90 min after the preload, the gastric sodium contents of the sham- and the hepatic-vagotomized groups were not different, $t(12) = 0.75$, NS (Table 1). In contrast to earlier work by Adolph (3), only 2–3% of the sodium load of 3.75 mEq remained in the stomach at this time. Thus, while most of the sodium preload, which exceeds the amount required to restore sodium balance by at least 1 mEq, had emptied from the stomach by this time, this was insufficient to abolish the NaCl appetite elicited by sodium depletion (see Fig. 2).

The gastric sodium content at 15 min was also not different between the sham- and hepatic-vagotomized rats, $t(10) = 0.82$, NS (Table 1). Although more sodium remained in the stomach at 15 min than at 90 min, $F(1, 20) = 12.63$, $p < 0.01$, more than 80% of the sodium load had left the stomach between 0 and 15 min in both sham- and hepatic-vagotomized rats.

In depletion-naive rats there was also no difference in the amount of sodium remaining in the stomach at 15 min for the sham-vagotomized (1.97 ± 0.23 mEq) and hepatic-vagotomized (2.14 ± 0.32 mEq) groups, $t(10) = 1.23$, NS. Thus, a history of sodium depletion did not differentially affect the rate of stomach emptying of gastric loads of sodium for sham- and hepatic-vagotomized rats.

Discussion

The removal of the hepatic branch of the vagus nerve did not change the rate of emptying of a gastric preload of NaCl. Taken together with the observation that the satiation of NaCl appetite was normal in the hepatic-vagotomized rats (Fig. 1), the data are not consistent with the suggestion that an increased rate of gastric emptying obscured a defect in satiation of NaCl appetite in the hepatic-vagotomized rat.

GENERAL DISCUSSION

On the basis of our results and those of others (5,8,15), we conclude that the hepatic branch of the vagus nerve is not necessary for the normal short-term satiation of NaCl appetite. Thus, the site(s) and neural or endocrine mechanism(s) that mediate the potent, short-term, satiating effect of NaCl in the sodium-depleted rat remain to be determined.

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