

EFFECTS OF SOMATOSTATIN ON FOOD INTAKE IN RATS

G. Aponte^{1*}, P. Leung², D. Gross³, T. Yamada⁴

Departments of Animal Science and Food Intake Laboratory¹,
Physiological Sciences, School of Veterinary Medicine²,
Human Anatomy, School of Medicine³, University of California,
Davis 95616, and Division of Gastroenterology
University of Michigan Medical School Ann Arbor, Michigan 48109⁴

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Summary

We examined the possibility that somatostatin, a tetradecapeptide distributed in the gut and the central nervous system, may influence food intake and behavior in rats. Although intravenously infused somatostatin did not alter food intake in 8 hour fasted rats, intracerebroventricularly infused somatostatin resulted in a biphasic response, first increasing then decreasing food intake. We also observed that the effects of somatostatin vary depending upon whether animals are fed or fasted. In fed rats, food intake was decreased, while in fasted rats food intake was increased. These results suggest that somatostatin can act in the central nervous system to stimulate appetite; but that other factors, possibly related to gut motility or clearance, may inhibit further feeding once the stomach is full.

Somatostatin, a cyclic tetradecapeptide, was isolated from ovine hypothalami and named for its ability to inhibit growth hormone release from dispersed rat pituitary cells (1). Somatostatin-like immunoreactivity has since been localized to a variety of other tissues including pancreas (2,3) gastrointestinal tract (2) and central and peripheral nerves (4). Among its many actions, somatostatin has been proposed as a satiety factor because of its ability to inhibit food intake when administered intraperitoneally into rats and baboons, although somatostatin was unable to suppress food intake in animals after a prolonged fast (5,6).

The role of somatostatin in the control of food intake has been examined in the present experiments in which the route of infusion and the fed and fasted state of the animal were varied. The effect of intracerebroventricular infusion of somatostatin on meal fed, 8, or 24 hr food deprived rats was examined. Somatostatin was infused both pre and post-prandially in order to test the influence of pregastric loading on somatostatin induced changes in meal size and intermeal interval. Rats were also infused with somatostatin intravenously. We have observed that the action of this peptide in the central nervous system may result in an initial increase in meal size and decrease in intermeal interval which is followed by a decrease in meal size and increased intermeal intervals.

* Present address and for reprints: Center for Ulcer Research and Education, VA Wadsworth Hospital Center, Building 115, Room 217 Los Angeles, Calif. 90073

Methods

Food intake response of female Sprague-Dawley rats (150-200g) was studied in meal-fed and fasted rats infused with somatostatin either intracerebroventricularly or intravenously. For intracerebroventricular infusions, chronic cannulas were placed stereotaxically using the following landmarks: 1.5 mm lateral to the midsagittal sinus, 3.0 mm superior to the top of the dura, and 6 mm anterior to the interoral line. For intravenous infusion chronic cannulas were implanted with chronic cannulas in the jugular vein. All animals were kept on a dark-light cycle (lights off from 8 a.m. to 8 p.m.) and fed a purified 25% casein diet as shown in table 1. Water was available ad libitum. An episode of eating was considered a "meal" if it was followed by a 3 minute, or greater, period of non eating. The time between two meals was considered the intermeal interval.

Table 1: Composition of 25% Casein Diet

	% of Diet
casein+	25.00
L-methionine	0.30
vitamins++	1.00
salt mixture*	5.00
corn oil**	5.00
sucrose	21.20
starch§	42.40
choline chloride	0.10
<u>Total</u>	<u>100.00</u>

+ Vitamin free casein, ICN NUTRITIONAL BIOCHEMICALS, Cleveland, Ohio.
 ++Vitamin diet fortification mixture. ICN NUTRITIONAL BIOCHEMICALS, Cleveland, Ohio.

* Roger-Harper salt mixture, ICN NUTRITIONAL BIOCHEMICALS, Cleveland, Ohio.

**Mazola Corn Oil, Best Foods, Inglewood Cliffs, N.J.

§ Melojel food grade corn starch. National Starch and Chemical Corp., Bridgewater, N.J.

In experiment 1 rats (n=6) were trained to eat their entire daily rations of the purified diet (Table 1) during a 2 hour period starting at 9:00 AM. Somatostatin or 0.15 M saline was infused, on separate days, via the chronic indwelling intraventricular cannula at 9:00 AM and food intake monitored. Synthetic somatostatin (Peninsula Laboratories, Belmont, CA) in 0.15 M NaCl (1.6ug/ul) was infused at a rate of 0.5 ul/min for 20 minutes (until 9:20). After the infusions and feeding period, rats were killed by decapitation, and blood collected in Trasylol (500 IU/ml) and EDTA (1.5 mg/ml) for radioimmunoassay, as described previously (7). Plasma was stored at -70° C until assayed. The brains were removed and examined to confirm placement of the cannula. Differences between somatostatin and saline treated animals were compared using a paired t-test.

In experiment 2, 4 rats fed ad libitum with the 25% casein diet were food deprived for 24 hours before being refed ad libitum. Infusion of somatostatin or 0.15 M saline, was started 2 hours after the onset of feeding via a chronic indwelling intracerebroventricular cannula. The total quantities of saline or synthetic somatostatin used, the duration of infusion, and the rate of flow were as described in experiment 1. Food intake was recorded during the 2 hour feeding period prior to the initiation of somatostatin infusion, and every 2 hours thereafter for an additional 4 hours. Each rat served as its own control, and the effect of somatostatin and saline infusion compared using a paired t-test.

In a third experiment 4 rats maintained on the 25% casein diet were food deprived for 8 hours before the initiation of intracerebroventricular infusion of somatostatin or saline, on separate days, as described above. Twenty minutes after commencing the infusion, animals were allowed to eat ad libitum and food intake was recorded every 2 hrs for 6 hrs. Differences between somatostatin and saline treated animals were compared using a paired t-test.

For experiment 4, a dose exceeding blood post-prandial concentrations of somatostatin monitored after intracerebroventricular infusion in experiment 1 was infused in 6 rats maintained on the 25% casein diet via chronic indwelling jugular vein cannulas. Rats were food deprived for 8 hours prior to the administration of saline or, on a separate day, somatostatin in normal saline (96mg/ml) at a flow rate of 0.5 ml/min. for 20 min. Animals were fed ad libitum after the infusion and food intake recorded every 2 hrs for 6 hrs. Food intake during somatostatin and saline infusion was compared using a paired t-test.

Results

The effect of intracerebroventricular somatostatin infusion on meal size in rats trained to eat one single meal per day is shown in fig. 1. Saline infused rats ate slightly more during the first hour after meal presentation than in the second hour. This response was significantly magnified following infusion of somatostatin ($P < 0.05$). Following the somatostatin infusion, but not the saline infusion, animals became agitated and demonstrated searching and frequent grooming behavior for 10 min. before eating. They then ate continuously for the remainder of the hour but virtually ceased eating ($P < 0.05$) thereafter.

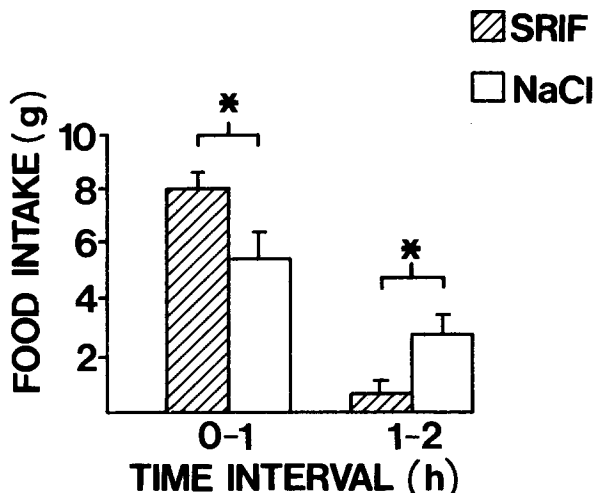


FIG.1

Effect of intracerebroventricular infusion of somatostatin on food intake of rats trained to eat their entire daily ration in 2 hours. Values are means, bar denotes SEM (n=6). Statistically significant differences are noted with an asterisk (* $P < 0.05$).

Food intake of animals which were food-deprived for 24 hours and then allowed free access to food for 2 hours before the intracerebroventricular infusion of somatostatin was virtually abolished ($P < 0.01$) after infusion (fig 2A). Food intake returned to control values during the 3rd and 4th hours following the somatostatin infusion.

The effect of intracerebroventricular somatostatin infusion on food intake in 8 hour fasted rats is shown in fig. 2B. Somatostatin infused at the same time as meal presentation significantly ($P<0.05$) enhanced food intake for the first two hours and then significantly ($P<0.001$) depressed food intake in the second 2 hour period. Food intake returned to control levels 4 to 6 hours after somatostatin infusion.

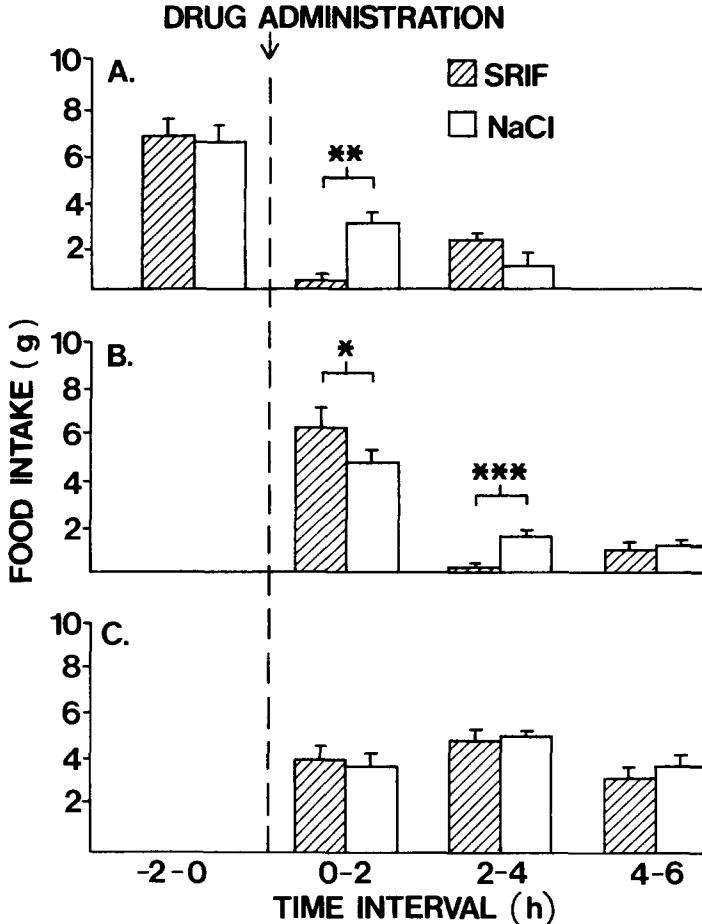


FIG.2

Food intake response of rats to somatostatin infusion. A, intracerebroventricular infusion of 24 hour food deprived rats ($n=4$); B, intracerebroventricular infusion of 8 hour food deprived rats ($n=4$); C, intravenous infusion of 8 hour food-deprived rats ($n=6$). Values are means, bar denotes SEM. Statistically significant differences are noted with asterisks. (* $P<0.05$, ** $P<0.01$, *** $P<0.001$).

Intracerebroventricular infusion of somatostatin resulted in an increase in plasma somatostatin concentration from control values of 40 ± 3 fmol/ml to 1.1 ± 0.1 pmol/ml ($n=6$). Intravenous infusion resulted in a plasma somatostatin concentration of 2.9 ± 0.2 pmol/ml which was approximately 3 times the value observed following intracerebroventricular administration of somatostatin. Animals infused intravenously with somatostatin did not exhibit behavioral changes, and food intake was not significantly different from that of the saline treated controls (fig 2C).

Discussion

Numerous studies have suggested a role for somatostatin in nutrient homeostasis. Schusdziarra has proposed that post-prandial rise in plasma somatostatin-like immunoreactivity regulates the rate of nutrient entry into the gut (8,9). Others have shown that somatostatin administered intracerebroventricularly decreases hepatic glucose output either directly (10), or indirectly, through alterations of pancreatic insulin and glucagon output, or pituitary growth hormone secretion (11,12). It is possible that somatostatin may regulate food intake indirectly by altering nutrient assimilation or metabolism. Lotter et al (5) have examined the potential role of somatostatin in regulating food intake more directly. In contrast to our studies, they observed a decrease in food intake in rats and baboons given a bolus injection of somatostatin intraperitoneally, although no change in food intake was observed when somatostatin was administered intracerebroventricularly. Levine and Morley reported that an observed decrease in spontaneous feeding in rats given intraperitoneal administration of somatostatin was inhibited by vagotomy, thus suggesting that somatostatin's action is vagally mediated (6). The differences between their results and our observations may be explained by study design. As indicated in our studies, the effects of intraventricularly administered somatostatin depends on the feeding status of the animal. Furthermore, the effects of somatostatin are time dependent, observed over a period of several hours, but not seen after 6 h. In this light it is interesting to note that Vijayan and McCann (13) observed a decrease in food intake between the first and six hours following intracerebroventricularly injected somatostatin. If we had not noted increased food intake in the the first hour following somatostatin infusion, it would have appeared that food intake decreased between the first and sixth hours in our studies as well. Recent studies suggest that intraventricularly administered peptides appear rapidly in the circulation (14). We observed that following intraventricular infusion of somatostatin there was a significant increase in plasma somatostatin. Peripheral changes in somatostatin concentrations, however, did not affect feeding response. The mechanism by which somatostatin alters food intake is uncertain. The biphasic nature of somatostatin's effects may be explained by a combination of centrally mediated hunger or appetite stimulation, coupled with inhibition of gastric emptying and intestinal motility.

Previous studies have suggested that the duration and frequency of eating is directly related to the size of intragastric preloading (15,16) and that gastric distension can induce acute satiety (17); thus, any stimulatory effect of somatostatin on food intake administered to fasted rats 2 h after refeeding may have been masked by limited gastric volume. Indeed, the second group of rats food-deprived for only 8 h demonstrated enhanced food intake when infused with somatostatin at the onset of a meal. It remains to be determined if endogenous somatostatin has the same actions; however, recent evidence that patients with anorexia nervosa have diminished concentrations of somatostatin-like immunoreactivity in their cerebrospinal fluid (18) suggests that intracerebral somatostatin may be of physiological importance in the regulation of food intake.

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