Meal-related stimuli differentially induce c-Fos activation in the nucleus of the solitary tract

MICHAEL EMOND, GARY J. SCHWARTZ, AND TIMOTHY H. MORAN
Department of Psychiatry and Behavioral Sciences, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205

Received 26 April 2000; accepted in final form 12 December 2000

Meal-related stimuli differentially induce c-Fos activation in the nucleus of the solitary tract. Am J Physiol Regulatory Integrative Comp Physiol 280: R1315–R1321, 2001.—Feedback signals arising from the oral cavity and upper gastrointestinal tract contribute to the control of meal size. To assess how these signals are integrated at central sites involved in ingestive control, we compared levels of c-Fos activation in the nucleus of the solitary tract (NTS) and area postrema (AP) in response to meal ingestion or gastric and duodenal infusions in the rat. Ingestion of a liquid diet to satiety induced significant fos-like immunoreactivity (FLI) at multiple levels of the NTS and within the AP. The restriction of intake to one-half the normal ingestion of a rat did not result in significant FLI, although gastric infusion of this liquid diet volume did. Fast bolus infusion resulted in greater FLI than did the same volume infused at a rate to mimic that of normal ingestion. Prior experience with gastric infusions did not affect the amounts of FLI within the NTS or AP. In rats with pyloric cuffs blocking flow from the stomach to the intestine, combined gastric load and duodenal nutrient elicited significantly greater FLI than either gastric or duodenal infusions alone. These data demonstrate that neural activation arising from meal-related stimuli are integrated at the level of the NTS.

MATERIALS AND METHODS

Subjects. Male Sprague-Dawley rats weighing 350–400 g at the time of testing were housed individually in hanging wire mesh cages and maintained on a 12:12-h light-dark schedule (lights out at 1900) at 23°C. Rats had ad libitum access to tap water at all times and free access to rat chow pellets during the scheduled feeding times.

Brain fixation and Fos immunohistochemical staining. Ninety minutes after an experimental manipulation, rats were killed by a 1 ml/kg intraperitoneal injection of Euthosol. Rats were perfused transcardially via a 16-gauge needle placed in the left ventricle, with ~200 ml of 0.15 M NaCl followed by ~150 ml of 4% (wt/vol) paraformaldehyde in PBS. Brains were removed and stored overnight in 4% paraformaldehyde with 25% (wt/vol) sucrose.

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
Brains were frozen and sectioned at 40 μm on a cryostat through the rostrocaudal extent of the NTS at −19°C. Relevant sections of hindbrain were taken and placed in individual wells of a tissue culture plate (24 wells/plate). Three to four sections were placed in each well. Each well contained 2 ml of PBS (pH 7.3). The PBS was removed, and the sections were incubated in 0.3% H2O2 for 1 h, washed in PBS, and then incubated for 30 min at room temperature in 1.5% normal goat serum in PBS and 0.3% Triton X-100. Sections were then incubated for 39–44 h in 200 ml of Fos primary antibody (Oncogene, rabbit polyclonal, catalog No. PC38) diluted 1:1,000 in 2% (wt/vol) BSA in PBS. Sections were maintained at 4°C and gently agitated throughout the entire period.

The primary antibody was removed, and sections were washed three times (10 min/wash) with PBS-0.1% Triton X-100. They were then placed for 45 min in a 200-ml mixture of 0.5% (vol/vol) biotinylated secondary antibody, 1.5% (vol/vol) normal goat serum, and 0.3% (vol/vol) Triton X-100. Sections were then washed three times in PBS and incubated for 45 min in 200 ml of Vectastain ABC reagent (Dimension Laboratory). After three additional PBS washes, sections were incubated in a nickel diaminobenzidine tetrahydrochloride (Ni-DAB) chromagen for 8–10 min to stain Fos-like products black. The Ni-DAB reaction was halted with several PBS washes, and the sections were mounted on gelatin-coated slides. After being dried overnight, sections were cleared in an ascending series (70, 95, and 100%) of ethanol baths (10 min/bath) followed by a 45-min bath in xylene. Sections were mounted on gelatin-coated slides and coverslipped. To control for staining variability between immunohistochemistry runs, each immunohistochemistry run contained matched sections from all within experiment comparison groups.

c-Fos quantitative analysis. The IP Laboratory Imaging System (Scanalytics, Vienna, Virginia) image analysis software was used for quantitative analysis. Sections were magnified using a Zeiss microscope, and the image was relayed to the IP Laboratory Imaging System via a COHU high-performance charged coupled device video camera. c-Fos-positive cells were automatically counted by the imaging program by setting the same frame and maximum optical density level. These parameters were optimized by comparing software counts with sample visual counts and applying these settings to all experimental groups within an individual experiment.

Single coronal sections from four rostrocaudal levels of the NTS were analyzed per animal. These levels were differentiated on the basis of well-defined anatomic descriptions, particularly issues of cell size and density. The levels examined with coordinates from the interaural line corresponding to Paxinos and Watson (11a) were caudal, at the level of the obex, corresponding to the posterior edge of the area postrema (AP), 5.30 mm; medial, at the maximum extent of the AP, 4.68 mm; rostral, anterior to the AP, corresponding to the maximal extent of the gelatinous subnucleus of the NTS, 4.30 mm; extreme rostral, corresponding to 4.10 mm. c-Fos-positive cells with the AP were also quantified. This analysis provides a view for the rostral-caudal extent of c-Fos activation and did not allow differentiation of the pattern of c-Fos activation within NTS subnuclei.

Statistical analysis. Multiple ANOVA’s were used to analyze the c-Fos data with experimental group as a between factor and brain region a within or repeated factor. Newman-Keuls analyses corrected for multiple comparisons were used to identify differences between individual means.

Experiment 1: effect of meal size on NTS fos-like immunoreactivity. The first experiment was designed to contrast the neural activation elicited by a meal terminated spontaneously and a meal terminated prematurely at approximately one-half the food volume that the rats would normally ingest. Rats were given daily access to vanilla-flavored Ensure liquid diet (Ross Products Division, Abbott Laboratories, Columbus OH; 11% fat, 74% carbohydrate, 15% protein) in test cages for 30 min following a 6-h daytime food deprivation (0900–1500) for 1 wk. Their intake during these 30-min access periods was measured to be sure that intakes had stabilized and to calculate each rat’s normal meal size.

On the test day, following a 6-h food deprivation, rats were randomly assigned to one of three treatments. “Meal” rats (n = 8) were placed in the test cages and allowed unrestricted access to liquid diet for 30 min (mean intake 20.7 ml, range 16.2–28.2 ml). “Half-meal” rats (n = 7) were restricted to ingestion of only one-half of their individual normal meal size during the 30 min in the test cage (mean intake 8.0 ml, range 7.0–12.5 ml). Rats ingestion was monitored to determine when the consumption of limited volume was completed. The mean ingestion rate of the rats during the time the Ensure was being consumed as determined by the volume available and the time to completion was 1.1 ± 0.09 ml/min. “No meal” rats (n = 8) were simply placed in the test chambers for 30 min. Ninety minutes following the beginning of meal access or being placed in the test chambers, rats were killed and fos-like immunoreactivity (FLI) was assessed through the NTS.

Experiment 2: effect of gastric loads on patterns of FLI. The second experiment was designed to determine whether nutrient volumes that do not produce significant c-Fos expression when consumed were sufficient to elicit a response when given as gastric infusions. This experiment also assessed whether the rate of infusion affected the amount of FLI.

Rats were implanted with chronic, indwelling gastric cannulas. Each cannula consisted of an 11-mm stainless steel tube with 8.5 mm outer diameter (OD), 7.9 mm inner diameter (ID), and flanged at both ends. Marlex mesh (2.5 × 2.5 cm) was secured to the middle of the cannula shaft with dental cement 24 h prior to surgery and helped stabilize the cannula once it was in situ. A set screw threaded into the cannula shaft allowed the gastric cannula to be occluded and, when in place, maintained the continuity of the gastrointestinal tract for real feeding. Animals were allowed a minimum of 14 days to recover from surgery and were maintained on ad libitum food during the first week. During the second week of their recovery period, rats had their cannulas opened and their stomachs flushed with warm water every third day to familiarize them with this type of handling. They were deprived of food for 6 h from 0900 to 1500 and then allowed to ingest an Ensure meal for 30 min. This gave them prior exposure to the test cages and Ensure before the test day.

On the test day rats were deprived of food at 0900 and had their stomachs flushed at 1430. Rats were then put into one of three groups: 1) fast infusion, 2) slow infusion, and 3) no infusion controls. Control rats (n = 3) received no gastric load; they simply had their cannula screw replaced with a 15-cm long delivery tube and placed into the test chambers. The test chamber was a Plexiglas cage that had a narrow strip cut out in the bottom to allow the delivery tube to hang through. Fast infusion rats (n = 10) had their cannula screws replaced with delivery tubes and were placed in the test chambers. In the chambers they received 10 ml of Ensure within 30 s delivered intragastrically through the tube via a syringe pump. Slow infusion rats (n = 10) underwent the same treatment as the fast infusion rats except the delivery
of the intragastric load was 1 ml/min, approximating the rate of the Ensure ingestion as demonstrated in experiment 1. Sixty minutes after being placed in the test chambers all rats had their screws replaced and were returned to their home cages. When the screws were replaced, an average of 2 ml of leakage occurred per rat. Thirty minutes later (90 min after beginning the gastric infusions) rats were killed and brains were taken for c-Fos labeling.

Experiment 3: effect of novelty of gastric load experience on patterns of FLI. This experiment was directed at the possibility that a c-Fos response to gastric loads was due to the novelty of the sensations rather than to the particular sensory feedback induced. Thus we compared the extent of c-Fos activation in rats that had multiple previous exposures to a gastric load and in rats that were experiencing a gastric load for the first time.

Rats were deprived of food overnight (1700–0900). At 0930 the following morning the rats were allowed access to an Ensure meal for 30 min, and their baseline intake of the Ensure meal was recorded. Thirty minutes later rats were allowed to eat rat chow ad libitum for the remainder of the day. After 10 days of this training, rats were split into two groups: experienced and inexperienced. Experienced rats were given a 10-ml intragastric load of Ensure every morning at 0900, following their overnight deprivation. The load was delivered as a bolus through an 8-French polyethylene feeding tube. Thirty minutes after the gastric load, all rats were allowed to ingest the Ensure meal, followed by their rat chow as usual. The rats were maintained on this schedule for 14 days, so that at the end of this 2-wk period the experienced rats would be familiarized with the experience of getting an intragastric load, whereas the inexperienced rats would have had no previous exposure with gastric loads.

On the test day rats were deprived of food overnight as usual. At 0900 subsets of rats from both the experienced (n = 10) and inexperienced (n = 9) groups received a 10-ml gastric load of Ensure by oral-gastric intubation. Control rats (n = 7), composed of the remaining inexperienced and experienced rats, did not receive any gastric infusion on the test day. Ninety minutes after gastric infusions, rats were killed and brains were taken for c-Fos labeling. Control rats were killed at the same clock time as rats that received gastric loads.

Experiment 4: comparison of patterns and extent of c-Fos activation produced by restricted gastric load, duodenal stimulation, or combined gastric and duodenal stimulation. To assess the particular contributions of gastric and duodenal signals to the patterns and extent of FLI produced by gastric nutrient infusions, we compared FLI in rats given 1) a non-nutritive gastric load that was confined to the stomach, 2) a duodenal nutrient infusion, or 3) both gastric load and duodenal nutrient. Rats were implanted with duodenal catheters and pyloric cuffs and allowed a 2-wk recovery time. Duodenal cannulas consisted of a 20-cm piece of 0.012 in ID × 0.025 in OD Silastic tubing attached to a 4-cm piece of 0.025 in ID × 0.047 in OD Silastic tubing. Two pieces of Marlex mesh were attached to the tubing as anchors. Duodenal cannulas were inserted through a ventral midline incision. A 22-gauge needle was used to perforate the duodenum 1 cm from the pyloric sphincter. The small end of the Silastic tubing assembly was inserted through this perforation up to the 1 × 1 cm piece of mesh such that the tip of the cannula extended ~3 cm into the duodenum. The mesh was sewn to the duodenal wall, anchoring the cannula to the duodenum. The other end of the cannula was run subcutaneously to the back of the neck, where it was exteriorized and the second piece of mesh sewn to the underlying muscles. The cannula was flushed daily with 1 ml of physiological saline, and the exit site was examined.

To temporarily isolate gastric contents, a pyloric cuff fashioned from a Silastic inflatable bag (4 mm wide × 5 cm long) attached to Silastic tubing was threaded around the pyloric sphincter region in two steps. First, the end of the cuff bag was threaded through a 5-mm space made at the top of the pylorus in between the antpyloric ligament and the distal antrum. The end of the cuff bag was then drawn through a 5-mm space made at the bottom of the pylorus between the pancreatic tissue and adjoining mesenteric blood supply. It was held in place by the gastropyloric ligament and was sewn so that it completely surrounded the pylorus. The cuff's tubing was drawn subcutaneously to the back of the neck where it was exteriorized. At the time of implantation, each cuff was individually tested to determine the volume necessary to inflate the cuff so that the pylorus was closed without inhibiting local blood flow. That volume for that animal was then used during the experiment. Rats are treated prophylactically with penicillin for 5 days. Rats also receive 0.2 ml intramuscular injections of Reglan (metoclopramide, a prokinetic agent) for 5 days.

After an overnight food deprivation, rats with closed pyloric cuffs were given one of four conditions: 1) no load, 2) gastric load (GL), 3) duodenal nutrient (DN) infusion, or 4) GL + DN. The no load group (n = 6) received no further treatment beyond the inflation of the pyloric cuffs. Gastric load rats (n = 7) had a bolus gastric load of 10 ml of saline delivered via an inserted feeding tube. DN rats (n = 7) had a duodenal nutrient infusion of Ensure (1 kcal/ml) delivered at a rate of 0.4 ml/min for 10 min. GL/DN rats (n = 6) received a gastric load immediately followed by a duodenal nutrient infusion. We also included a control group in which the pyloric cuff was not closed and no additional treatments were administered (n = 4). Ninety minutes after the beginning of the treatments, rats were killed and perfused and their brains were taken for c-Fos immunohistochemistry.

To compare the effects of combined gastric and duodenal stimulation to results from a gastric nutrient load in the absence of a pyloric cuff, we used data from the fast infusion group in experiment 2. The variability of FLI between experiments was controlled for by subtracting the mean cell counts from the appropriate control condition from the counts for each animal in the experimental groups.

RESULTS

Experiment 1. As shown in Fig. 1 the ingestion of a large meal over a 30-min period produced significant increases in the number of neurons expressing c-Fos above the control no meal condition within the AP and at a number of NTS levels. In contrast to this result, restricting rats to one-half their normal intake did not elicit significant c-Fos above that found in the no meal group within the AP or at any NTS level.

In the medial region of the NTS, where the AP is at its greatest cross-sectional extent, the meal condition resulted in significant increases in FLI over both the no meal and half-meal conditions (F2,20 = 12.8, P < 0.0005). Similar results were found in the rostral and extreme rostral levels of the NTS (F2,20 = 15.1, P < 0.0005; F2,20 = 7.5, P < 0.005). In the caudal region of the NTS, there were no significant differences between the three groups (F2,20 = 1.02, not significant [NS]). Within the AP there was a significant increase in the
number of c-Fos-positive nuclei in the meal rats compared with both the no meal and half-meal rats ($F_{2,20} = 6.8, P < 0.01$).

**Experiment 2.** Examination of the extent of FLI in response to 10 ml of gastric nutrient infusions showed that gastric loads increased NTS FLI. As shown in Fig. 2 the extent of FLI depended on the rate that the gastric nutrient infusion was delivered. Within the caudal NTS, meal infusion resulted in a significant increase in FLI ($F_{2,20} = 9.64, P < 0.01$). Post hoc analyses indicated that the fast infusion increased FLI relative to both the slow and the control condition. Significant increases in FLI were also obtained in the medial ($F_{2,20} = 10.06, P < 0.01$) and in the rostral NTS ($F_{2,20} = 12.11, P < 0.01$). At both of these levels of the NTS, both infusion rates produced significant increases and delivery of the load over 30 s rather than over 10 min resulted in greater levels of FLI. No significant group differences in FLI expression were seen between any of the groups in the extreme rostral region of the NTS ($F_{2,20} = 0.279$, NS).

**Experiment 3.** Gastric load increased the number of Fos-positive cells in both experienced and inexperienced rats in the caudal ($F_{2,23} = 5.1, P < 0.05$), medial ($F_{2,23} = 4.7, P < 0.05$), and rostral ($F_{2,23} = 3.84, P < 0.05$) levels of the NTS and the AP ($F_{2,23} = 10.68, P < 0.01$) (Fig. 3). However, there were no significant differences among the degree of FLI expression between the experienced and inexperienced group at any NTS level nor within the AP.

**Experiment 4.** Individual or combined gastric and duodenal loads resulted in significant increases in the number of c-Fos-positive cells in the caudal ($F_{4,25} = 4.65, P < 0.01$), medial ($F_{4,25} = 8.32, P < 0.001$), and rostral ($F_{4,25} = 3.23, P < 0.05$) levels of the NTS (Fig. 4). Within the caudal NTS, combined gastric and duodenal stimulation significantly increased Fos activation relative to the untreated control condition and relative to the duodenal infusion alone. Closing the pyloric cuff alone or cuff closed with individual gastric stimulation did not differ from the control condition. Cuff closed with the duodenal load did result in more Fos activity than the control condition. In contrast, in the medial NTS, both the gastric load and duodenal nutrient infusion increased the number of c-Fos-positive cells relative to the control condition or the cuff closed condition. The combined gastric and duodenal loads produced greater FLI than either the control condition, the cuff closed, or individual gastric and duodenal loads. In the rostral NTS, gastric load, duodenal nutrient infusion, and the combined gastric and duodenal load produced significant increases in FLI relative to the control condition. The effect of combined stimulation was different from simply closing the pyloric cuff but was not different from either gastric or duodenal stimulation alone. Within the AP, there was again a significant treatment effect ($F_{4,25} = 6.104, P < 0.01$). Post hoc analyses indicated that duodenal infusion and combined gastric and duodenal infusion had
elevated levels of FLI compared with untreated controls. Combined duodenal and gastric load also had significantly increased FLI compared with the cuff closed group.

As shown in Fig. 5, the pattern of relative increase in FLI in the NTS in response to combined gastric and duodenal load with the cuff closed was similar to that found in response to a rapid gastric infusion allowing the infused contents access to both the stomach and the duodenum.

DISCUSSION

The results of these experiments confirm that the ingestion of a meal (4, 7–8, 13) or the gastrointestinal presence of meal-related stimuli can lead to the expression of c-Fos at particular levels of the NTS and within the AP (12, 18–20). The data further demonstrate that the degree and pattern of c-Fos activation depend on 1) the size of a meal, 2) whether it is consumed or delivered by intragastric gavage, 3) the rate of the intragastric infusion, and 4) whether the infused meal contacts the stomach, the duodenum, or both. Experience with intragastric infusions did not affect the degree of FLI produced by intragastric Ensure.

The results of the first experiment, which compared the degree of Fos activation in rats that consumed a scheduled Ensure meal to satiety or were limited to half their normal intake, demonstrated that significant levels of FLI were only attained with ingestion of a large meal to satiety. Ingestion of a meal restricted to one-half the size of the expected meal produced low levels of c-Fos expression not different from those found in control rats. This finding is consistent with prior results of Rinaman et al. (13) who demonstrated low hindbrain c-Fos levels in rats who were given access to food but not allowed to eat to satiety.

The fact that a meal restricted to one-half what is normally consumed produced no significant increase in NTS or AP Fos expression may simply be a function of the smaller magnitude or duration of stimulation or may indicate that there is something unique about the neural representation of a meal that has been terminated due to satiation. The gastric load experiments began to address these alternatives. A gastric load of comparable size to the volume consumed in the half-meal condition elicited significant amounts of Fos expression in the NTS, suggesting that the volume consumed in the half-meal condition should have been sufficient to elicit a significant Fos response.

Results from the gastric load experiments are consistent with work by Fraser et al. (8), Willing and Berthoud (19), and Traub et al. (17) who showed that gastric distension, produced by inflating a gastric balloon, elicited Fos expression in the NTS. The present experiment showed that a nutrient load delivered directly into the stomach, thus eliminating the oral stimulation elicited by food ingestion, leads to the expres-
sion of c-Fos in the NTS and AP. These data also show that the rate of delivery can have a strong effect on the amount of c-Fos elicited by the load, with bolus loads eliciting significantly more Fos than infusions that approximate the normal liquid diet ingestion rates in rats. This pattern of results may reflect that a greater intragastric volume is obtained with the bolus than with the slower infusion rate. Electrophysiological activity of mechanoreceptive vagal afferent fibers (11) and single NTS neurons (2) increases in proportion to intragastric volume. Thus greater afferent input into the NTS may account for the greater number of c-Fos-positive cells in response to the bolus infusion. It is also possible that the slower infusion permits gastrointestinal relaxation reflexes to take effect, reducing acute distension or gastric tone relative to the rapid bolus infusion and concomitantly reducing distension-sensitive vagal afferent neurophysiological activity relative to that induced by bolus infusions.

We examined whether the greater degree of FLI produced by gastric loads compared with meal ingestion was due to the novelty of the intragastric load experience. We compared the patterns and extent of FLI in two groups of animals, one which had been familiarized with the experimental treatment of a gastric load and one that had no prior experience with either the intubation or the intragastric load. The results demonstrated that a gastric load did not produce a greater degree of Fos expression in animals that were experiencing it for the first time as opposed to the fourteenth time.

Having eliminated the possibility that the FLI observed after a slow gastric infusion was due to a novelty effect we are left with the conclusion that the same gastric load, in the context of other meal-related stimuli, results in lower Fos expression than when it is presented in isolation. This result of combined oral and gastrointestinal stimulation resulting in less FLI than the gastrointestinal stimulation alone is similar to previous findings of Emond and Weingarten (4) that demonstrated that sham ingestion of 1 M sucrose produced more NTS FLI than when the same volume was consumed in a real feeding situation. Those results indicated that the simultaneous gastric stimulation decreased the degree of FLI produced by the oral activation alone. It may be that oral activation also reduces the degree of FLI produced by gastric stimulation alone. Thus data from this and the prior work by Emond and Weingarten (4) suggest that either individual oral or gastric stimulation results in more NTS FLI than combined oral and gastric nutrient exposure.

Although combining oral and gastric exposure reduced the overall degree of FLI compared with either alone, combining gastric and duodenal stimulation enhanced FLI within the NTS. The combination of gastric distension and duodenal nutritive infusion produced far greater c-Fos activation within the caudal and medial NTS than either stimulus in isolation. These data suggest a degree of signal convergence and integration within these levels of the NTS. This may represent the outcome of activated vagal afferents converging on single cells within the NTS resulting in sufficient neural activation to produce a Fos response. Alternatively, this response may represent differential activity within gastric vagal afferents in response to duodenal nutrient stimulation. We have demonstrated previously that intestinal nutrient activates gastric vagal afferent fibers. This may be the outcome of vagovagal reflexes changing gastric motor activity or may be the result of duodenal nutrient-induced peptide release and subsequent peptidergic activation of gastric afferent fibers (16). The c-Fos method does not allow us to differentiate among these possibilities.

We compared the patterns of FLI produced by combined isolated gastric and duodenal stimulation with that produced by the fast intragastric infusion of Ensure in which the infused nutrients would both fill the stomach and, through gastric emptying, contact the duodenum. The overall extent of FLI produced by the two treatments was quite similar, suggesting that the experimental compartmentalization of stimulation through use of pyloric cuff did not produce artificially high or altered extents of FLI. The gastric stimulation in isolated gastric load situation was produced with intragastric saline rather than Ensure as in the fast intragastric infusion situation. The overall degree of c-Fos activation was similar despite this difference suggesting that the c-Fos activation is produced by load rather than gastric contents as we have demonstrated for gastric vagal afferent activity (11).

The present results suggest that normal ingestion of a large meal activates FLI through the medial, rostral, and extreme rostral levels NTS as the ingested meal contacts the oral cavity, the esophagus, the stomach, and the proximal intestine. In contrast, gastric and duodenal stimulation activates FLI throughout the caudal, medial, and rostral NTS. This rostral caudal distribution of activation in response to gastric and
duodenal stimulation is similar to what has been demonstrated previously (12, 18–20). Consistent with our findings, Phifer and Berthoud (12) have identified significant increases in Fos expression in response to duodenal nutrient within areas of the NTS corresponding to our medial and rostral levels. They also noted small but significant increases at more caudal levels. Our use of an inflated pyloric cuff may have prevented our ability to see an increase at this more caudal site, as simply inflating the cuff resulted in significant FLI. Cuff inflation may have caused mechanical stimulation of vagal afferents innervating the distal antrum, the pylorus, and the proximal duodenum.

**Perspectives**

The results of these experiments support the view that there is a neural representation of meal-related stimuli at the level of the NTS and suggest that this representation is more than a simple additive of the stimulation of individual sites along the alimentary canal. Consumption of a half-meal results in significantly less FOS than the intragastric infusion of a similar volume of nutrient given at a given rate. Some aspects of active ingestion result in a diminution of Fli at levels of the NTS that receive inputs from visceral afferents. This result is similar to previous data demonstrating that the gastric and intestinal accumulation of consumed nutrients that result from normal ingestion reduce the NTS stimulation produced by sham feeding in which consumed nutrients contact the oral cavity and esophagus but then drain from the stomach, resulting in neither gastric distention nor intestinal nutrient stimulation (4). The activation arising from gastric and intestinal stimulation combine in a different way. Both alone are sufficient to result in significant FOS in the aspects of the NTS that receive visceral inputs, but in combination the degree of stimulation is greatly increased. At caudal and medial NTS sites the degree of Fli in response to combined gastric and duodenal infusions is greater than either alone. Together, these results suggest that the context in which stimulation occurs is critical for how that stimulation will result in activation at hindbrain sites. Although the determination of the results of isolated stimulation is informative, the patterns and extent of Fli produced by isolated stimulation do not predict how such stimulation will affect neural activity when other parts of the alimentary tract are also stimulated. Meal-related signals occur within an integrated context, and the context determines how those signals will affect neural activity and the central representation of a meal.

This work was supported by the National Institute of Diabetes and Digestive and Kidney Diseases Grant DK-19302.

Present addresses: M. Emond, Dept. of Psychology, Univ. of Regina, 3737 Wascana Parkway, Regina, Saskatchewan S4S 0A2 CA; G. Schwartz, Dept. of Psychiatry, Weill Medical College, Cornell Univ., Bourne Laboratory, 21 Bloomingdale Road, White Plains, NY 10605.

**REFERENCES**