Duodenal nutrient infusions differentially affect sham feeding and Fos expression in rat brain stem

Curtis B. Phifer and Hans-Rudolf Berthoud. Duodenal nutrient infusions differentially affect sham feeding and Fos expression in rat brain stem. Am. J. Physiol. 274 (Regulatory Integrative Comp. Physiol. 43): R1725–R1733, 1998.—Duodenal infusions of macronutrients inhibit sham and normal feeding. Neural substrates of this response were studied by infusing glucose, linoleic acid, an amino acid mixture, saline, or water into the duodenum of unanesthetized rats and then measuring sham feeding of 30% sucrose or Fos expression in the dorsal vagal complex. Linoleic acid and amino acids (both 1.5 kcal) and glucose (4.5 kcal) suppressed sham feeding relative to control infusions, and all three macronutrients triggered Fos expression in the nucleus of the solitary tract and area postrema. Although there were significant quantitative differences, the subnuclear distribution pattern of Fos-expressing neurons was not different for the three macronutrients and was largely localized to the medial, dorsomedial, and commissural subnuclei of the nucleus of the solitary tract and the area postrema. Linoleic acid suppressed intake and stimulated Fos expression similarly to glucose infusions of three times the caloric value. Amino acids strongly suppressed sham feeding but triggered relatively little Fos expression. These results indicate that the intake-suppressing potency of duodenal macronutrients is dependent on nutrient type, rather than simply caloric value, and that amino acids, although potent inducers of satiety, affect ingestion by processes different from those subserving lipids and carbohydrates. Furthermore, the similar patterns of neuronal activation after different duodenal infusions may indicate a large degree of convergence at the level of primary and second-order sensory neurons, whereas the distinctly different pattern obtained earlier with gastric distension indicates partially separate neural pathways for satiety signals generated by duodenal nutrients and gastric mechanoreceptors.

Area postrema; nucleus of the solitary tract; macronutrients; sham feeding; vagal afferents; dorsal vagal complex

Intestinal signals constitute an important subset of the postingestive cues that inhibit feeding behavior. Chronically implanted duodenal catheters allow infusions of nutrients directly into the intestine, thereby bypassing oral and gastric sensory receptors. Duodenal infusions of nutritionally complete liquid diet (8) or specific macronutrients (16, 31) suppress feeding in animals with open gastric fistulas (sham feeding) and in animals that are feeding normally (26).

Recent work has focused on the role of specific macronutrients in intestinal satiety, the location and nature of the receptors that detect intestinal nutrients, and contributions of the enteric nervous system. On the basis of results from several studies, individual fatty acids, mixtures of lipids, and some amino acids suppress feeding more than equicaloric infusions of monosaccharides or disaccharides (26, 31); therefore, caloric monitoring does not appear to be the primary mechanism for macronutrient detection. Receptors for lipid and glucose appear to be associated with the intestinal mucosa, inasmuch as duodenal infusions of various simple sugars (24) or fatty acids (5) suppress sham feeding but comparable infusions into the hepatic portal vein do not (6, 24). Also, different peripheral pathways appear to signal the presence of lipids or carbohydrates vs. amino acids, as total subdiaphragmatic vagotomy (31) completely blocks the intake-suppressing effects of lipid or carbohydrate but only partially attenuates the effect of amino acids.

We have begun to ask the question, How are these signals generated in the gastrointestinal tract used by the brain to initiate the satiation process? Our working hypothesis is that at least some signals from different gastrointestinal sites and/or sensors initially utilize different pathways but eventually converge at higher levels of the neuraxis to terminate an ongoing meal. To this end, we have shown that gastric balloon distension with two paradigms designed to preferentially stimulate different types of gastric mechanoreceptors produced similar topographical patterns of neuronal activation in the rat caudal brain stem, with a similar proportion of catecholaminergic neurons recruited (28). This finding suggested that different distension signals from within the stomach converge already at the caudal brain stem level. In the present study we wanted to test the hypothesis with respect to different small intestinal nutrient sensors and compare it with gastric distension. Although the induction of nuclear Fos as a measure of neuronal activation has important limitations (20), it is nevertheless the method of choice to obtain an initial in toto overview of the neural substrate underlying this particular sensory system, and its combination with transmitter and peptide immunocytochemistry will allow the identification of the neurochemical phenotype of activated neurons.

The effect of duodenal nutrient infusion on the expression of Fos in the rat brain has been investigated by Zittel et al. (33). Expression of Fos-like immunoreactivity (FLI) was seen in the nucleus of the solitary tract (NTS) after infusions of lipid emulsion (Intralipid, 2.7 kcal) or d-glucose (2.9 kcal), but not after infusions of glucose at 1.1 kcal or mannitol, hydrocholitic acid, or casein hydrolysate (1.2 kcal). However, the study did not address the question of possible different topographical distribution patterns of activated neurons, and the effects on sham feeding were not assessed in the same animals. McCaffery et al. (9), in a preliminary study,
found that the relative order of FLI induction by duodenal infusions of various lipids (linoleic acid, Intralipid, oleic acid, linolenic acid) was similar to the relative potency of the infusates for inducing satiety in separate studies. However, here again, the level of analysis did not allow detection of differences in FLI distribution patterns, and effects on behavior and neural activation were not directly compared. In addition, the Fos method was used in conjunction with a variety of other stimuli and treatments that increase or decrease food intake, including cholecystokinin (CCK) (13, 14, 18), dehydration (13), food ingestion or deprivation (13), hypoglycemia (13), and gastric distension (28). The fact that such treatments resulted in greatly different patterns of FLI in the caudal brain stem indicates that this method is a useful tool in probing the neural substrate underlying various aspects of ingestive behavior, including the central pathways of satiety signals.

One aim of the present study was, therefore, to compare the effects of duodenal infusions of different macronutrients on sham feeding and brain stem Fos expression by use of identical experimental parameters and, to some extent, the same animals for the behavioral and anatomic analyses. The second aim was to perform a fine-grained, subnuclear analysis of the neural FLI activation patterns achieved with the various infusions, allowing detection of possible quantitative and qualitative differences.

MATERIALS AND METHODS

Animals. Male Sprague-Dawley rats were obtained from Harlan; they weighed 250–300 g at the first surgery. They were housed individually and maintained on a 12:12-h light-dark cycle with lights on at 0700. Food (Purina Chow 5001) and water were available ad libitum, except during 18-h deprivation periods before infusion experiments.

Surgery. Gastric fistulas and duodenal infusion catheters were implanted at least 10 days before rats began adaptation to the testing conditions. After anesthesia with a mixture of ketamine (80 mg/kg), xylazine (4 mg/kg), and acepromazine (1.6 mg/kg) and treatment with atropine (0.5 mg/kg), rats received laparotomies along the linea alba. The stomach and duodenum were exteriorized, and a stab wound was made in the center of a purse string suture (6-0 monofilament suture) in the greater curvature of the nonglandular stomach. A silastic duodenal catheter (0.63 mm ID, 1.19 mm OD, 14 cm long) with an anchoring sleeve of slightly larger silastic (1.02 mm ID, 2.16 mm OD, 3 mm long; secured 1 cm from the internal end with cyanoacryl-based adhesive) was implanted through the stomach wall and secured in the duodenum (two 8-0 monofilament sutures and Marlex mesh) with the internal end 4 cm beyond the pylorus.

A stainless steel gastric fistula (10 mm long, 6 mm ID, with 14-mm-diameter outer and inner flanges; Marlex mesh secured around the fistula with dental acrylic) was implanted in the stomach and anchored with the purse string suture and by tissue adhesions that formed around the Marlex. The fistula exited through a stab wound in the left flank, and the laparotomy was closed with 3-0 absorbable Vicryl sutures in the musculature and 3-0 silk sutures in the skin. The external end of the duodenal catheter was secured by passing a loop through holes in the inner flange of the fistula and by attachment to a post on the inside of a fistula screw. Posturgical treatment included 0.2 mg of yohimbine (Yobine, Floyd Laboratories, Shenandoah, IA) to reverse effects of xylazine, 1.0 mg of gentamicin antibiotic (Schering-Plough, Kenilworth, NJ), and daily treatment of the area around the fistula with 3% hydrogen peroxide. Leakage of the fistula was prevented by coating the fistula screw threads with petroleum jelly.

Acclimation to testing conditions and environment. Ten of the animals were tested for the effects of duodenal nutrient infusions on sham feeding, with each rat receiving all nutrient and control infusions in randomized order. Subsequently, each of these animals received a single nutrient infusion just before it was killed and its brain was removed for c-Fos processing. The remaining animals were not used in sham-feeding experiments; rather, each animal was given only one nutrient or control infusion just before brain removal.

The testing chambers were clear Plexiglas cylinders (26 cm diameter, 35 cm tall) sitting on a stainless steel grid floor (parallel bars separated by 2 cm) suspended 20 cm above a waste-catch tray. In sham-feeding tests a 100-ml glass feeding tube was attached to the side of the testing chamber, with a siphon tube extending inside the chamber.

After recovery from surgery, animals were adapted to the test situation in two stages. First, rats were placed in the testing chambers on at least two separate days for 1 h/day. On two subsequent days, rats were food deprived, their fistulas were opened and duodenal catheters externalized, and a fistula extension tube (a threaded fistula coupling attached to Tygon tubing protected by a stainless steel wire coil) was attached to the open fistula. The extension tube passed freely through the floor grid and was attached to a 50-ml centrifuge tube for collection of stomach drainage. One-half hour adaptation periods were also used with the attached fistula extensions. A group of 10 animals were subsequently trained to sham feed. After 18 h of food deprivation, animals were allowed to ingest 30% sucrose from a feeding tube. A sham-feeding criterion of at least 56-ml consumption in 60 min (mean 73.9 ml), with stomach drainage equal to or greater than intake, was reached before animals began sham-feeding tests with accompanying duodenal infusions. Animals that were to receive duodenal infusions without previous sham-feeding experience had only the 4 days of adaptation described previously. Tests preceded by deprivation were separated by at least a 2-day interval.

Sham-feeding tests with duodenal infusions. After reaching the sham-feeding criterion described above, 10 rats were given sham-feeding tests while receiving duodenal infusions of specific macronutrients or control infusions (see below), with each rat receiving all treatments in random order (within-subjects design). Before sham-feeding tests the animals' fistulas were opened, and stomach contents were flushed with 6 ml of warm water. Then their duodenal catheters were attached to infusion leads running to 10-ml syringes on infusion pumps (model 210, KD Scientific). Infusion leads were constructed from an 80-cm length of polyethylene tubing (PE-100, 0.82 mm ID, 1.52 mm OD) attached to a 20-gauge needle for connection to an infusion syringe and joined to a 25-cm length of silicone tubing (1.02 mm ID, 2.16 mm OD) with 19-gauge tubing. A 2-cm length of 19-gauge tubing also connected the silicone tubing to the duodenal catheter. After a 60-min adaptation period without sham feeding, animals were presented with 30% sucrose in graduated feeding tubes. After 10 min of feeding without duodenal infusions, rats were given 30-min infusions followed by 20 min of sham feeding without infusion (total sham-feeding bout equalled 60 min). The volume consumed was determined at 5-min intervals through the sham-feeding test. Fistula drainage was ensured.
DUODENAL NUTRIENTS AND FOS EXPRESSION IN BRAIN STEM

Effects of macronutrient infusions on sham feeding. During the 10-min period of sham feeding before the infusions began, no significant differences in intake were seen between the different infusion conditions (Fig. 1). Rats sham fed rapidly (~2 ml/min) and with few interruptions. However, during the 30-min infusion period, a significant treatment effect was seen [Fig. 1; F(5,45) = 12.12, P < 0.001]. Infusions of 4.5 kcal of

by excluding animals when collected gastric drainage did not equal or exceed intake.

The total volume of the duodenal infusions was 4.5 ml, delivered over 30 min. Numerous studies (8) have shown that normal gastric emptying during and after a meal or gastric infusion begins with an unregulated rush of emptying that is subsequently brought under control by postprandial feedback. Also, Phillips et al. (15) showed that duodenal infusions of saline delivered at a rate of 1 ml/min caused duodenogastric reflux when total volume was >2.5 ml. To mimic normal gastric emptying and minimize duodenogastric reflux, the infusions in this experiment were given in two bouts and at two different rates. For the first 5 min the infusion rate was 0.4 ml/min, providing a total volume of 2.0 ml. For the following 25 min the rate was 0.1 ml/min. Both of these infusion rates were less than gastric-emptying rates measured previously in rats ingesting meals of a complete liquid diet (Vivonex High Nitrogen) or Intralipid (7). Although this infusion protocol minimized reflux, some animals still showed minor reflux (generally <10% of infusion). On the basis of appearance of dyed infusate in gastric fistula drainage, animals that showed reflux >20% of the infusion volume were excluded. (Refux between 10 and 20% of infusion occurred in >10% of infusions in this part of the study.)

Duodenal infusions of three different macronutrients and two control solutions were used. The macronutrients included equicaloric infusions (1.5 kcal/4.5 ml of total infusion volume) of linoleic acid (0.124 M, pH adjusted to 7.5), a mixture of amino acids (0.17 M L-arginine, 0.18 M L-leucine, and 0.18 M L-phenylalanine; pH adjusted to 7.5), and D-glucose (0.46 M). An additional dose of D-glucose (4.5 kcal/4.5 ml, 1.39 M) was also administered after pilot studies revealed that the lower glucose concentration had little effect on sham feeding. Control infusions were equal volumes of distilled water and isotonic saline (0.15 M).

Final infusion test for Fos immunocytochemistry. In the second part of this study, 51 rats, including the 10 used in the sham-feeding study, received the duodenal infusions described above but were not allowed to sham feed. Thus eight or nine animals were assigned to each infusion group. Again, animals that showed duodenogastric reflux >20% of the infusion volume were excluded, and only 3 animals showed reflux between 10 and 20%.

After the 30-min infusions, rats were left in their test chambers for 60 min to allow c-Fos expression, and then they were killed by pentobarbital sodium overdose. Animals were then perfused transcardially with 150 ml of heparinized saline (20 U/ml) followed by 600 ml of 4% phosphate-buffered paraformaldehyde (pH 7.4) with 0.3% picric acid. Brains were then excised and postfixed in the same fixative overnight at 4°C. Fos immunocytochemistry. After post fixation, brains were cut into blocks (medulla, midbrain, posterior forebrain, anterior forebrain) for ease of sectioning. The medulla was then placed in 25% sucrose in 4% paraformaldehyde overnight before it was cut into 30-µm coronal sections. Sections were collected in PBS for subsequent free float processing. Some brains were stored at -20°C in cryoprotectant (50% PBS, 20% glycerol, 30% ethylene glycol) for several weeks with no apparent effect on c-Fos immunocytochemistry (27). Tissue sections from three to eight brains, spread across several different treatments, were processed at the same time. Sections were first removed from PBS and rinsed in 1% sodium borohydride in PBS for 30 min to reduce aldehyde cross linking of proteins, thereby enhancing antibody penetration. After they were rinsed in PBS, sections were pretreated for 20 min with 1.5% hydrogen peroxide, 20% methanol, and 0.1% Triton X-100 (TX) in PBS to reduce endogenous peroxidase.

Further rinsing in PBS alone continued until bubbling activity stopped. Sections were immersed in 5% normal goat serum, 1% BSA, and 0.5% TX for 30 min before incubation with an antibody to the protein product Fos of the c-fos gene (1:50,000 dilution in PBS with 0.5% TX, 0.1% gelatin, 0.05% sodium azide; Ab-5, Oncogene Sciences, Cambridge, MA; 48–60 h at 8°C). Sections were rinsed four times in PBS containing 0.1% gelatin and then incubated for 90 min in biotinylated goat anti-rabbit secondary antibody (1:1,000 dilution in PBS with TX; Biotin-SP IgG H + L, Jackson ImmunoResearch Labs, West Grove, PA). After three rinses in PBS alone, sections were immersed in an avidin-biotin complex (Vectastain ABC Elite Kit PK-6100, Vector Laboratories, Burlingame, CA) for 60 min. The Fos-antibody complex was then visualized by immersing sections for 4 min in a metal-enhanced diaminobenzidine substrate (1.9 dilution of diaminobenzidine in stable peroxide; Pierce Chemical, Rockford, IL). Cells labeled for FLI exhibited a dark blue-black nuclear stain. After three rinses in PBS, sections were immersed in 70% glycerol before they were mounted on slides with 100% glycerol.

Analysis. The volume of 30% sucrose sham fed during the 10 min before infusions began, the 30-min infusion period, and the 20-min period after the infusions was subjected to a randomized-block one-way ANOVA with infusion type as the main factor. When appropriate, differences between intakes after particular treatments were analyzed with Duncan’s post hoc tests.

Specific regions in the dorsal vagal complex and specific subnuclei of the NTS were analyzed for Fos labeling. Sections were viewed on a Zeiss Axiosvert 135 inverted microscope; images were captured using a videocamera and frame grabber and then analyzed using an automated image-analysis system (Kontron K5400). The cell-recognition characteristics (size and optical density criteria, object-separation parameters) utilized by the image-analysis system were first optimized using representative sections and then kept constant for analysis of all sections and for all treatments.

For sampling purposes, Fos-positive neurons were counted in every fifth brain section from caudal through rostral NTS, and the NTS was divided into three regions relative to the area postrema (AP) for analysis: the NTS caudal to the AP (1–3 sections/brain, mean 3.56), the NTS subjacent to the AP (1–3 sections/brain, mean 2.64), and the NTS rostral to the AP (2–4 sections/brain, mean 3.48). Fos-labeled neurons were also counted within the AP and dorsal motor nucleus of the vagus (dnmx; 1–3 sections/brain, mean 2.64). The average number of labeled neurons per section was calculated for each of these regions, and this average was used in subsequent statistical analyses. Subnuclei in the NTS were delineated using the atlas of Swanson (22) and the maps of Altschuler et al. (1) and Ter Horst et al. (23). Fos-labeled neurons found in specific subnuclei were counted in the single section from each brain that was closest to the cranial-caudal midpoint of the AP. One-way ANOVAs were used to determine main effects of the type of infusion on Fos expression, and post hoc comparisons were made with Duncan’s multiple-range test.

RESULTS

Effects of macronutrient infusions on sham feeding. During the 10-min period of sham feeding before the infusions began, no significant differences in intake were seen between the different infusion conditions (Fig. 1). Rats sham fed rapidly (~2 ml/min) and with few interruptions. However, during the 30-min infusion period, a significant treatment effect was seen [Fig. 1; F(5,45) = 12.12, P < 0.001]. Infusions of 4.5 kcal of
Furthermore, animals consumed significantly more Fos labeling than after 4.5- and 1.5-kcal glucose infusions.

Fos expression in the dorsal vagal complex. As the NTS appears to be viscerotopically organized along its rostrocaudal axis (1), for purposes of analysis the NTS was subdivided into three regions relative to the AP. Across all three regions, animals that received saline, water, or 1.5-kcal glucose infusions showed very few Fos-positive neurons (Fig. 2), and no significant differences in Fos labeling were detected between animals receiving these infusion conditions in any portion of the NTS or AP. Animals that received 1.5-kcal infusions of amino acids or linoleic acid or 4.5-kcal infusions of glucose showed extensive expression of Fos-positive neurons (Fig. 2), so detailed comparisons were made only between animals that received these infusions and animals that received saline infusions.

In NTS regions caudal to the AP, different duodenal infusions produced different levels of Fos labeling [Fig. 3; F(5,42) = 11.03, P < 0.001]. Infusions of 4.5 kcal of glucose and 1.5 kcal of linoleic acid produced significantly more Fos labeling (P < 0.05) than all other infusions, including infusions of amino acids. No significant differences in Fos labeling were seen in post hoc comparisons between animals receiving other infusions and saline-infused animals. Also, infusions of 4.5 kcal of glucose and 1.5 kcal of linoleic acid did not trigger significantly different Fos labeling.

Different duodenal infusions also produced differential Fos labeling in NTS regions within the rostrocaudal limits of the AP [Figs. 2 and 3; F(5,42) = 28.07, P < 0.001]. As in the NTS caudal to the AP, infusions of 4.5 kcal of glucose and 1.5 kcal of linoleic acid produced significantly more Fos labeling (P < 0.05) than all other infusions, including amino acid infusions. However, in this middle region of the NTS, amino acid infusions produced significantly more labeling (P < 0.05) than saline infusions. Again, no significant difference was seen between labeling after infusions of 4.5 kcal of glucose and 1.5 kcal of linoleic acid.

The region of the NTS rostral to the AP revealed a pattern of Fos labeling that was similar to the NTS at the AP, but with fewer total neurons labeled (Fig. 3). Different duodenal infusions again produced differential Fos labeling [F(5,42) = 12.67, P < 0.001], and the 4.5-kcal glucose and 1.5-kcal linoleic acid infusions produced significantly more labeling (P < 0.05) than all other infusions. Here, as in the NTS at the AP, infusion of the amino acid mixture triggered significantly more labeling (P < 0.05) than saline infusions. No significant difference was seen between labeling after infusions of 4.5 kcal of glucose and 1.5 kcal of linoleic acid.

During the 20-min sham-feeding period after the duodenal infusions, a significant treatment effect was again found [Fig. 1; F(5,45) = 6.83, P < 0.001]. In this period a more dramatic rank ordering of effects appeared. At P < 0.05, post hoc tests on intake revealed that infusions of 4.5 kcal of glucose, 1.5 kcal of amino acids, or 1.5 kcal of linoleic acid suppressed intake relative to infusions of saline, but infusions of 1.5 kcal of glucose and saline did not have significantly different effects. Furthermore, animals consumed significantly more Fos labeling than after 4.5 kcal of glucose infusions, and animals consumed significantly less (P < 0.05) after linoleic acid infusions than after 1.5- or 4.5-kcal glucose infusions.

An analysis of the AP alone also showed differential Fos expression in response to different duodenal infusions [Fig. 3; F(5,42) = 33.86, P < 0.001]. The 4.5-kcal glucose and 1.5-kcal linoleic acid infusions produced significantly more labeling (P < 0.05) than all other infusions, and unlike the situation in all three rostrocaudal regions of the NTS, the 4.5-kcal glucose infusion produced significantly more labeling than the 1.5-kcal linoleic acid.

Fig. 1. Effect of various nutrient and control infusions into duodenum on sham feeding of 30% sucrose. Suppression of sham feeding by infusion of water, 1.5 and 4.5 kcal of glucose, 1.5 kcal of an amino acid mixture, and 1.5 kcal of linoleic acid relative to saline infusion was calculated for each individual animal. Values are means ± SE of 10 rats. Amount sham fed was measured for periods of 10 min before infusion, 30 min during infusion, and 20 min after infusion. Water and 1.5 kcal of glucose did not significantly suppress sham feeding in any period. Higher dose of glucose, as well as amino acid mixture and linoleic acid, significantly suppressed sham feeding during and after infusion. *P < 0.05.
infusion. Fos labeling after amino acid infusions was not significantly different from control levels.

The dmX exhibited a notable absence of Fos labeling, with a mean of only one to two neurons showing the label in any infusion condition. Also, no significant differences between infusions were detected in dmX Fos expression \( F(5,42) = 2.18, P = \text{NS} \).

As the largest differences in Fos labeling associated with different macronutrient infusions occurred in the region of the NTS directly ventral to the AP, a more detailed analysis of NTS subnuclei in this region was conducted (Figs. 4 and 5). Brains of animals that received infusions of 1.5 kcal of glucose or H2O showed very few Fos-positive neurons, and no significant differences in Fos labeling were seen between these treatments in the larger analysis above; therefore, data for subnuclei are not shown for these treatments. Figure 5 shows data for only the saline control, 4.5-kcal glucose, and 1.5-kcal linoleic acid or amino acid infusions.

Figure 5 reveals that the great majority of Fos labeling occurred in more medial regions of the NTS, with very few labeled neurons in the lateral, interstitial, intermediate, or central subnuclei. Despite the absence of numerous Fos-positive neurons, significant treatment effects were found in the lateral subnucleus \( F(5,42) = 3.65, P < 0.01 \) and in the central subnucleus \( F(5,42) = 3.29, P < 0.05 \). In both of these subnuclei, infusions of 4.5 kcal of glucose produced significantly more Fos labeling \( (P < 0.05) \) than all other infusions, and in the central subnucleus, infusions of 1.5 kcal of linoleic acid also produced more labeling than control infusions. No significant differences in Fos expression were seen between any other infusion conditions.

In the more medial portion of the NTS, different infusions produced significant differences in Fos labeling in the medial \( F(5,42) = 10.14, P < 0.001 \), dorsomedial \( F(5,42) = 20.53, P < 0.001 \), and commissural subnuclei \( F(5,42) = 30.70, P < 0.001 \). Post hoc analy-
ysis of the medial subnucleus revealed significant differences between 4.5-kcal glucose infusions and all other treatments, including linoleic acid and amino acids. Infusions of linoleic acid also produced significantly more Fos activation than control infusions, but not more than amino acid infusions. The dorsomedial and commissural subnuclei showed very similar patterns of Fos activation in response to nutrient infusions. Infusions of 4.5 kcal of glucose and 1.5 kcal of linoleic acid produced significantly more labeling than any other infusion, including amino acids, but not different from one another. Amino acid infusions also produced more Fos labeling than control infusions.

**DISCUSSION**

Several conclusions can be drawn from the results of this study. 1) The intake-inhibiting effects of macronutrients involve more than simple caloric monitoring, because equicaloric duodenal infusions of different mac-
ronutrients produce different effects. 2) Different macronutrients trigger different levels of neural activation, as indicated by Fos expression. 3) The relative effects of different macronutrients on sham feeding and neural activation are not well correlated. 4) Although different macronutrients trigger different levels of neural activation, no differences in pattern of activation were detected in this study. 5) Qualitative differences in pattern do appear to exist between neural activity induced by duodenal infusions of macronutrients and gastric distension, two distinct but complementary inhibitors of ingestion.

Effects on sham feeding. Duodenal infusions of different macronutrients had dramatically different effects on intake during sham feeding. Linoleic acid and the amino acid mixture suppressed sham feeding more than equicaloric infusions of glucose and more than control infusions. These results agree with previous studies in which duodenal infusions of fatty acids or amino acids suppressed sham feeding (31) or normal feeding (26) similarly and both of these macronutrients suppressed feeding more than equicaloric infusions of glucose or maltose. In this study, linoleic acid suppressed feeding even more than glucose infusions containing three times as many calories. Although equicaloric infusions of linoleic acid tended to suppress feeding more than amino acids, this tendency did not reach significance.

A striking finding from this study was the apparent discrepancy between the relative behavioral and neural effects of amino acid infusions. Although 1.5 kcal of amino acids suppressed intake as much or more than the 4.5-kcal glucose infusions and almost as much as linoleic acid infusions, amino acid infusions invariably triggered significantly less Fos expression than linoleic acid or 4.5-kcal glucose infusions in the entire NTS and its subnuclei. This finding, along with results from several other studies, suggests that ingestion-inhibiting effects from duodenal infusions of carbohydrate, lipids, and amino acid may be dependent on different neural pathways. Blocking vagal afferent signals from the gastrointestinal tract by intraperitoneal (30) or fourth ventricular capsaicin administration (32) or by total subdiaphragmatic vagotomy (31) significantly attenuates the suppression of sham feeding caused by duodenal infusions of maltose or oleic acid but had little or no effect on suppression by L-phenylalanine infusions. Vagal projections from the intestine to second-order neurons in the NTS therefore appear necessary for suppression of sham feeding by intestinal carbohydrate and lipid, but different pathways may be responsible for suppression by amino acids. However, real feeding may present a different scenario, because selective celiac vagotomy equally attenuates intake suppression caused by duodenal infusions of glucose, oleic acid, or phenylalanine (26).

Effects on Fos expression. In the analyses of FLI, macronutrient infusions as a whole had their greatest effect on the region of the NTS immediately subjacent to the AP, with fewer cells expressing Fos in the NTS rostral to the AP and in the AP itself and even fewer cells showing Fos in the NTS caudal to the AP. This result was not surprising, because the NTS is larger and contains more neurons in the region of the AP than in its more rostral or caudal regions. Duodenal nutrient infusions had no detectable effect on Fos in the dmX. These results agree with work by Zittel et al. (33), where lipid and glucose infusions induced greater Fos labeling in the more rostral NTS (subjacent to the AP and the 4th ventricle) than in the caudal NTS (at obex). In an earlier study by Olson et al. (13), Fos expression was determined after treatments that inhibited food intake (e.g., CCK, food ingestion) or potentiated food intake (deprivation or insulin). Although the rostrocaudal distribution of Fos expression was not described, intake-inhibiting treatments were associated with substantial expression in the NTS and, to a lesser extent, in the AP, but no significant Fos expression appeared in the dmX. Conversely, intake-potentiating treatments triggered substantial Fos expression in the dmX but had little effect in the NTS or the AP. In the study of Olson et al. and in another study (28), gastric distension triggered substantial Fos expression in the NTS and the dmX.

Although we assume that most of the Fos induction was mediated by vagal afferent fibers with terminals in the small intestine, we cannot rule out 1) participation of vagal afferents innervating the stomach or liver, 2) spinal sensory pathways involving dorsal root afferents and the spinosolitary tract, or 3) humoral mediation. Because there was no gastric distension in our experiments, it is unlikely that potentiation of vagal gastric mechanosensor activity by CCK could be involved. It is still possible, however, that some vagal afferents have axon collaterals in the stomach and duodenum and may respond to gastric distension and intestinal nutrients. In the absence of evidence for gastric nutrient receptors, it is also unlikely that the small gastric reflux was responsible for the Fos expression. A role for portal hepatic nutrient sensors, particularly glucose-sensitive vagal afferents, is theoretically possible, but it would be difficult to reconcile the fact that increases in portal glucose concentration produce a decrease in hepatic vagal afferent firing (11) but Fos expression in second-order neurons is only induced by excitatory synaptic inputs. The long-chain linoleic acid is mainly absorbed through the lymph system and would not act on the liver within the time frame of our experiment. Regarding the possibility of nonvagal mediation, a recent study has found that perivagal capsaicin treatment blocks most of the Fos expression in the NTS, but only about one-half in the AP, as induced by duodenal lipid infusion (10). Because a CCK-B antagonist blocked the remaining Fos expression in the AP, the authors suggested partial mediation by increased levels of circulating CCK. Finally, because the high glucose infusion was hyperosmolar and we did not use a control infusion of mannitol or sodium chloride with the same osmolality, we cannot exclude the possibility that osmoreceptive mechanisms contributed to the neural and behavioral responses of high glucose.
The subnuclear distribution of Fos expression in the NTS, measured subjacent to the AP, showed the greatest number of labeled cells in the medial subnucleus, with similar expression in the dorsomedial and commissural nuclei. Few previous studies have included detailed analysis of NTS subnuclei in relation to food intake control. Transganglionic tracing with horseradish peroxidase injections into the subdiaphragmatic vagal branches and various gastrointestinal sites has been used to demonstrate the central termination sites of vagal afferent fibers (1, 12). Vagal afferents projecting through the celiac branches terminate in a large area, including most medial subnuclei, such as the medial, dorsomedial, and commissural (12), whereas gastric afferents terminate more heavily in the dorsomedial and commissural subnuclei, an area also referred to as the subnucleus gelatinosus (1). Because the proximal part of the duodenum is innervated mainly by the gastric and hepatic abdominal vagal branches and the more distal small intestine by the celiac branches, the central termination zones of afferent fibers traveling in all three branches could carry signals from small intestinal nutrient sensors. Therefore, the topographical distribution of Fos labeling in the present study is not inconsistent with results from retrograde tracing. It should be noted that the cell bodies of NTS neurons that receive direct vagal sensory synaptic input need not reside within the afferent termination zone, because some NTS neurons have extensive dendritic trees. Specific stimulation of small intestinal nutrient sensors has not yet been used in conjunction with electrophysiological recording from second-order neurons in the NTS.

All these medial NTS areas, in particular the medial subnucleus, have been shown to project directly to various nuclei of the hypothalamus and other forebrain sites that are believed to have important roles in the control of food intake (17).

Convergence vs. separate pathways of potential satiety signals. Although several NTS subnuclei exhibited significantly greater Fos expression after nutrient infusions than after control treatments, the rank ordering of effects from different nutrients was similar across all affected subnuclei. The greatest degree of labeling was always triggered by the 4.5-kcal glucose and 1.5-kcal linoleic acid infusions, followed by amino acid infusions. Only in the medial subnucleus did 4.5 kcal of glucose trigger significantly more Fos expression than linoleic acid. Although these similar distribution patterns of activated neurons across subnuclei strongly indicate a high degree of convergence of sensory signals, additional experiments are necessary to substantiate this explanation. It is possible that the different sensory stimuli activate different populations of neurons in the same general areas. Such populations may differ in their neurochemical phenotype and or in their projection patterns (see Perspectives). Because the Fos method does not allow testing for more than one stimulus for a given animal, individual neurons cannot be compared across subjects. To detect true convergence of two stimuli on a given second-order neuron, electrophysiological recording will be necessary.

We recently compared the effects of continuous vs. phasic gastric distension on Fos expression in the dorsal vagal complex in an attempt to preferentially activate slowly adapting tension receptors vs. rapidly adapting mucosal touch receptors. The relative distribution pattern of FLI across subnuclei was not affected by the magnitude or type of distension. In addition to the similar distribution, we found that a similar, albeit low, proportion of catecholaminergic neurons was activated. These results strongly suggested a high degree of convergence of different gastric distension signals.

Convergence and separation of sensory signals from the extended alimentary canal have been reported in studies using electrophysiological recording from primary vagal afferents (21) or second-order neurons in the NTS (2–4). The degree of convergence was typically larger between stimuli originating in the same viscus (4, 21).

Although we have not directly compared the subnuclear distribution patterns with gastric distension and duodenal nutrient stimuli within the same experiment, an across-study analysis clearly showed that such potential satiety signals emanating from the stomach and small intestine produce strikingly different patterns of FLI (cf. Fig. 1 in Ref. 28 with Fig. 5 of this study). In contrast to the relatively high level of Fos-positive neurons seen in the dorsomedial and commissural subnuclei after duodenal infusions of macronutrients, gastric distension induced relatively little Fos expression in these subnuclei. Also of note, gastric distension induced relatively little Fos expression in the AP, but duodenal infusions of linoleic acid or a high dose of glucose induced substantial Fos expression in the AP. Furthermore, although gastric distension induced considerable neuronal activation in the dorsal motor nucleus, none of the duodenal nutrient infusions did. The sites of Fos induction in our study can also be compared with the sites of responsive neurons in electrophysiological recording studies. If gastric afferents are stimulated, changes in neuronal firing rates are predominantly found in the medial subnucleus and in the dorsal motor nucleus, although the sampling strategy in these studies was not always comprehensive (2–4, 19). Thus gastric distension and duodenal macronutrients, both potent inhibitors of ingestion, appear to stimulate separate populations of second-order neurons in the dorsal vagal complex.

**Perspectives**

The difference between the relative effects of carbohydrate, lipid, and amino acid infusions on sham feeding and Fos expression in the NTS might be explained by different populations of brain stem neurons subserving processing of signals induced by different nutrients. These putatively different neuronal populations may produce different neurotransmitters or have different receptor types or subtypes. The hypothesis that different neurotransmitters mediate effects of different nutrients is supported by the finding that CCK-receptor...
antagonists attenuate suppression of sham feeding by duodenal infusions of maltose or oleic acid but not by L-phenylalanine (29). Different neuronal populations may also project to different midbrain and forebrain regions involved in feeding behavior control. Double labeling of the NTS for fos expression and neurotransmitter precursors or receptors, Fos-labeling of third-order cells in the midbrain and perhaps forebrain, and retrograde tracing from forebrain sites to Fos-expressing neurons should help identify which of these explanations is more probable.

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