Gastric distension induces c-Fos in medullary GLP-1/2-containing neurons

Niels Vrang,1 Curtis B. Phifer,2 Michele M. Corkern,3 and Hans-Rudolf Berthoud3

1Rheoscience, 2610 Rødovre, Denmark; 2Louisiana Scholars’College, Northwestern State University, Natchitoches; and 3Neurobiology of Nutrition Laboratory, Pennington Biomedical Research Center, Baton Rouge, Louisiana

Submitted 2 December 2002; accepted in final form 16 April 2003

Vrang, Niels, Curtis B. Phifer, Michele M. Corkern, and Hans-Rudolf Berthoud. Gastric distension induces c-Fos in medullary GLP-1/2-containing neurons. Am J Physiol Regul Integr Comp Physiol 285: R470–R478, 2003.—A group of neurons in the caudal nucleus of the solitary tract (NTS) processes preproglucagon to glucagon-like peptides (GLP)-1 and -2, peptides that inhibit food intake when administered intracerebroventricularly. The GLP-1/2-containing neural pathways have been suggested to play a role in taste aversion and nausea because LiCl activates these neurons, and LiCl-induced suppression of food intake can be blocked by the GLP-1 receptor antagonist exendin-9. As many gastrointestinal signals related to both satiety and nausea/illness travel via the vagus nerve to the caudal medulla, the present study assessed the capacity of different types of gastric distension (a purely mechanical stimulus) to activate GLP-1 neurons in the caudal NTS. Gastric balloon distension (1.4 ml/min first 5 min, 0.4 ml/min next 5 min, 9 ml total, held for 60 min) in nonanesthetized, freely moving rats produced 12- and 17-fold increases in c-Fos-expressing NTS neurons when distension was mainly in the fundus or corpus, respectively. Fundus and corpus distension increased the percentage of c-Fos-activated GLP-1 neurons to 21 ± 9% and 32 ± 5% compared with 1 ± 1% with sham distension (P < 0.01). Thus gastric distension that may be considered within the physiological range activates GLP-1/2-containing neurons, suggesting some role in normal satiety. The results support the view that the medullary GLP system is involved in appetite control and is activated by stimuli within the behavioral continuum, ranging from satiety to nausea.

Preproglucagon; fundus; corpus

Preproglucagon gene expression is limited to α-cells in the pancreas, L cells in the gut, and neurons in the brain stem nucleus of the solitary tract (NTS) (15). Whereas posttranslational processing of proglucagon in the pancreas leads to the formation of glucagon and the major proglucagon fragment, proteolytic cleavage in the L cells of the gut and in the NTS yields the peptides glicentin, oxyntomodulin, glucagon-like peptide (GLP)-1, and GLP-2 (2, 11, 18). GLP-1 is secreted from L cells in response to a meal (17) and plays an important role as an incretin (12, 23). Additionally, peripheral GLP-1 has been shown to delay gastric emptying (30), and several groups have demonstrated appetite-suppressive effects of peripherally administered GLP-1 or GLP-2 agonists in humans (7, 10, 47) and rats (19). L cell-derived GLP-2 was recently recognized to function as an intestinal epithelial growth factor (5).

In the central nervous system, GLP-1 and GLP-2 function as neurotransmitters in a discrete population of neurons located in the caudal part of the NTS. Immunohistochemical and neuroanatomical tract tracing methods have demonstrated dense GLP-1-containing projections to several hypothalamic areas involved in the regulation of food and water intake (14, 18, 22, 28), areas that also contain GLP-1 and GLP-2 receptors (22, 39). Consistent with these observations, intracerebroventricular administration of GLP-1 inhibits food and water intake in rats (38, 40, 44). Recently, GLP-2 (39) and oxyntomodulin (4) were shown to suppress food intake in rats when injected intracerebroventricularly. The precise function of GLP-1 in central appetite-regulating pathways has been intensely investigated over the last couple of years. Reports of GLP-1-induced taste aversion coupled with GLP-1-induced activation of central oxytocin-containing neurocircuits has led several groups to investigate the role of GLP-1 in aversive stimuli. Indeed, an increasing body of evidence points to a role of GLP-1 neurons in mediating anorexia associated with a variety of interoceptive stressors. Several chemical stimuli known to potently suppress food intake and produce conditioned taste aversion, such as intraperitoneal injections of LiCl or LPS as well as peripheral injections of cholecystokinin have been shown to induce c-Fos expression in brain stem GLP-1-immunoreactive (ir) neurons (28). Also, LiCl-induced anorexia and taste aversion can be significantly blunted by prior injection of GLP-1 receptor antagonists, further supporting a functional role of endogenous GLP-1-containing neural pathways as mediators of LiCl-induced aversive behavior (27, 35). Although these findings point to a role of GLP-1 neurons as important mediators of interoceptive stressors, the neurons are in an excellent position to respond also to

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.
more physiologically relevant gastrointestinal satiety signals in the course of a normal meal.

In the present experiment we sought to investigate the effect of a purely mechanical gastrointestinal stimulus (gastric balloon distension) on the activation of GLP-1/2 and catecholamine-synthesizing neurons in the caudal part of the NTS by the use of triple labeling immunohistochemistry for GLP-2, tyrosine hydroxylase (TH), and the immediate early gene c-Fos. The experiment was carried out in freely moving rats, and the rate and magnitude of distension were designed to mimic an average physiological meal in moderately food-deprived rats.

MATERIALS AND METHODS

Animals and surgical procedures. Eighteen male Sprague-Dawley rats (250–300 g) were obtained from Harlan Industries (Indianapolis, IN). The rats were housed individually in wire-mesh cages and maintained under standard laboratory conditions (23 ± 3°C, 12:12-h light-dark cycle with lights on at 0700). Food (Purina chow 5001) and water were available ad libitum, except during 15- to 17-h deprivation periods before infusion experiments. All experiments were carried out according to the guidelines of the local Animal Care and Use Committee and the National Institutes of Health. With rats under deep anesthesia (ketamine-xylazine-acepromazine, 80:4:1.6 mg/kg sc) and administration of atropine (0.5 mg/kg ip), a small stab wound was placed into the fundus of the stomach close to the glandular stomach at the greater curvature. The inner flange of a cylindrical stainless steel fistula was inserted through the hole and secured with a purse-string suture. The other end of the fistula was exteriorized through the peritoneal wall and skin, with a circular piece of Marlex mesh cemented to the core of the fistula, forming a tight connection with the inside of the peritoneal wall. The fistula was closed with a worm screw, using petroleum jelly as a sealant.

Distension experiments and perfusion. In several sessions, animals were adapted in special acrylic test cages to rinsing the stomach with warm saline and inserting a small latex balloon. On the experimental day, after 15–17 h of overnight food deprivation, the empty balloon was inserted through the fistula and placed in the proximal stomach. After animals rested for 1 h, the balloon was inflated with 9 ml of warm saline at a rate of 0.9 ml/min, and inflation was maintained for an additional 50 min. In control animals, the balloon was inserted but not inflated, and the external tubing to the balloon was cut to avoid mucosal stimulation by the empty balloon. One animal was excluded from the experiment because of a leaky balloon. One hour after the start of distension, animals were killed with an overdose of pentobarbital sodium and perfused transcardially with 150 ml of heparinized saline (20 U/ml) followed by 400 ml of cold 4% phosphate-buffered paraformaldehyde (pH 7.4) containing 0.3% picric acid. After the perfusion, the exact location of the inflated balloon within the stomach was determined by estimating the percentage of the total volume of the balloon in the fundus, corpus, and antrum, with the limiting ridge delineating the border between fundus and corpus. On the basis of this evaluation, two groups were formed: one group (n = 6; 1 animal excluded due to a leaky balloon) with >70% of the total balloon volume in the fundus, and one group (n = 7) with >60% of the balloon volume in the corpus and antrum. Brains were removed and postfixed in the same fixative at 4°C overnight. A block containing the caudal brain stem was placed overnight in 4% paraformaldehyde containing 25% sucrose and frozen, and coronal sections were cut in a cryostat in five series at 30-μm intervals. The sections were stored at −20°C in cryoprotectant solution (50% PBS, 20% glycerol, 30% ethylene glycol) for later processing.

Immunohistochemistry. One series of sections from each rat was subjected to a triple immunohistochemical procedure to visualize c-Fos, GLP-2, and TH simultaneously. Because both the GLP-2 and TH antibodies were mouse monoclonal, it was necessary to visualize these antigens by the use of tyramide amplification technique as has been described by us and others previously (24, 36). Tyramide amplification allows the use of one of the primary antibodies in dilutions [monoclonal GLP-2 antibody (P1221) diluted 1:1,600] that are undetectable by a direct technique (fluorescent secondary antibodies). Therefore, once the tyramide signal amplification reaction has been terminated, the other primary mouse antibody can be used in a concentration suitable for direct visualization. This, combined with the fact that brain stem GLP-1 (which is costored with GLP-2)-immunopositive neurons are separate from TH-positive neurons (18), made the use of the common primary mouse antibody for TH and for c-Fos injections carried out on free-floating sections. First, sections were rinsed 3 × 10 min in phosphate-buffered saline containing 50 mM KCl (KPBS, pH 7.4). Next, sections were transferred to a 10 mM sodium citrate solution (pH 6.0) and heated in a water bath at 85°C for 90 min. Others (13) have described this method of unmasking antigens in formaldehyde and paraffin-embedded tissue in detail. Sections were allowed to cool for 20 min in the citrate buffer, then rinsed 2 × 5 min in KPBS followed by a 10-min incubation in 1 ml of 1% H2O2 in KPBS. After a 20-min incubation in 5% swine serum in KPBS containing 0.3% Triton X-100 (TX) and 1.0% BSA, the sections were incubated overnight at 4°C in rabbit anti-c-Fos [1:100,000; code 94012–4; (46)] and mouse anti-GLP-2 antibodies (1:1,600, code P1221, generously provided by Dr. Jes T. Clausen, NovoNordisk A/S) diluted in KPBS containing 0.3% TX and 1.0% BSA. The next day, the sections were washed with 3 × 10 min in KPBS with 0.1% TX (KPBS-T) before incubation for 60 min at room temperature in a biotinylated donkey anti-rabbit antibody (Jackson Immunoresearch Lab) diluted 1:200 in KPBS-T. After three rinses for 10 min in KPBS-T followed by 60 min in ABC-streptavidin-horseradish peroxidase (Vector Elite Kit; Vector Laboratories, Burlingame, CA) the sections were washed 3 × 10 min in KPBS-T. Sections were then reacted for 12 min in biotinylated tyramine [biotinylated according to the procedure described in Ref. 1 using tyramine-HCl (Sigma Aldrich) and sulfo-succinimidyl-6-(biotinimide) hexanoate (NHS-LC-biotin; Pierce, Rockford, IL)] diluted 1:100 in KPBS-T containing 0.005% H2O2. After the tyramide amplification, sections were rinsed 3 × 5 min in KPBS-T and incubated for 60 min in streptavidin-Alexa 546 (Molecular Probes, Eugene, OR) diluted 1:200 in KPBS-T. Three rinses for 5 min in KPBS-T preceded 10 min in KPBS-T + 1% H2O2, the latter to destroy remaining horseradish peroxidase activity. After 3 × 5 min in KPBS-T the sections were incubated for 60 min in a horseradish peroxidase-coupled donkey anti-mouse antibody (to label the mouse GLP-2 antibody), rinsed 3 × 5 min in KPBS-T, and subsequently incubated for 12 min in FITC-labeled tyramide diluted 1:100 in KPBS-T + 0.005% H2O2 (labeled using tyramine-HCl (Sigma Aldrich) and HS-LC-FITC (fluorescein, Pierce) in a procedure similar to the biotinylated tyramine described above). After this detection reaction, sections were rinsed 3 × 5 min in KPBS-T and incubated overnight at 4°C with a mouse monoclonal antibody to tyrosine hydroxylase (Sigma Aldrich, batch 020K4836) di-
luted 1:4,000 in KPBS + 0.3% TX + 1.0% BSA. On the third
day, sections were washed 3 × 10 min in KPBS-T, incubated
for 60 min in a Cy5-coupled donkey anti-mouse antibody
(Jackson Immunoresearch Laboratories) diluted 1:200 in
KPBS-T, then rinsed 2 × 10 min in KPBS-T and finally 10
min in distilled water before being mounted in a 0.5% gela-
tine solution. Slides were dehydrated through a graded series
of ethanol dilutions (30%, 60%, 80%, 96%, 99%, and xylene)
before being placed under a coverslip in Pertex.

Image acquisition and estimation of cell counts. A Zeiss
confocal laser-scanning microscope (LSM 510) equipped with
two HeNe lasers and one argon laser was used to visualize
the three fluorophores simultaneously. Images used for the
semiquantitative analysis were acquired using a ×20 objective
and a pinhole size that yielded optical sections ~6 μm thick. One
typical section was acquired per section where all
three antigens were optimally stained. Images were acquired
from all sections containing the NTS caudal to the rostral tip
of the area postrema (10–12 sections per animal, each section
spaced 150 μm). In the commissural part of the NTS, one
image covered the entire NTS. At more rostral levels, two
images, covering each side of the NTS, were acquired. Cells
[c-Fos-ir nuclei, GLP-2-ir cells, TH-ir cells] were counted
automatically using Image Pro Plus 4.6. The program was set
to count the number of c-Fos-, GLP-2-, and TH-positive cells
based on size and color/intensity. The number of GLP-2- and
TH-positive cells colocalizing c-Fos was scored manually in
the same session by an observer blinded to the treatment.
To look for possible regional differences, the analyzed NTS sec-
tions were divided into rostral sections (sections of the NTS
underlying the area postrema), midsections (caudal to the
area postrema, rostral to the pyramidal decussation), and
caudal sections (at the level of the pyramidal decussation).
The fraction (percentage) of GLP-2-ir cells containing c-Fos
for the NTS as a whole was calculated as (total number of
GLP-2-positive cells with c-Fos)/(total number of GLP-2-pos-
itive cells)×100. The fraction of GLP-2/TH-ir cells containing
TH-positive cells based on size and color/intensity. The number of GLP-2- and
TH-positive cells colocalizing c-Fos was scored manually in
the same session by an observer blinded to the treatment.

RESULTS

The distribution of GLP-2-ir cells corresponds to the
distribution of GLP-1-ir cells reported previously (14,
18, 22, 28). Notably, double-labeling experiments be-
tween a rabbit anti-GLP-1 specific antibody [used pre-
viously in the rat (18)] and the mouse anti-GLP-2
antibody used in the present experiments showed
100% colocalization (data not shown). Gastric disten-
sion induced a pattern of c-Fos expression in the dorsal
cerebral complex identical to that reported previously (3,
45). In the NTS, the number of c-Fos-positive nuclei
increased dramatically on gastric distension. The
average number of immuno-positive cells per section is given
in Table 1. Whereas the same number of TH- and
GLP-2-ir cells was found throughout the caudal NTS
(except TH-ir cells at midlevels) in both control and
distended rats, significantly higher numbers of c-Fos-ir
were found at all levels (Table 1 and Fig. 3).

Table 1. Number of c-Fos-, GLP-2-, and TH-ir cells per section for the caudal NTS as a whole (Total)
and for the 3 subareas as defined in MATERIALS AND METHODS

<table>
<thead>
<tr>
<th></th>
<th>c-Fos Total</th>
<th>c-Fos Ros</th>
<th>c-Fos Mid</th>
<th>c-Fos Cau</th>
</tr>
</thead>
<tbody>
<tr>
<td>No-distension control</td>
<td>2.9 ± 1.6</td>
<td>2.8 ± 1.2</td>
<td>3.5 ± 2.4</td>
<td>2.0 ± 1.2</td>
</tr>
<tr>
<td>Fundus distension</td>
<td>36.9 ± 7.0*</td>
<td>74.7 ± 11.0*</td>
<td>31.1 ± 9.5*</td>
<td>13.0 ± 4.1*</td>
</tr>
<tr>
<td>Corpus distension</td>
<td>50.7 ± 5.9*</td>
<td>112.7 ± 10.8*</td>
<td>18.2 ± 6.9*</td>
<td>14.8 ± 2.2*</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>GLP-2 Total</th>
<th>GLP-2 Ros</th>
<th>GLP-2 Mid</th>
<th>GLP-2 Cau</th>
</tr>
</thead>
<tbody>
<tr>
<td>No-distension control</td>
<td>7.3 ± 1.3</td>
<td>3.6 ± 0.9</td>
<td>13.8 ± 2.4</td>
<td>2.7 ± 1.0</td>
</tr>
<tr>
<td>Fundus distension</td>
<td>9.0 ± 0.9</td>
<td>4.1 ± 1.5</td>
<td>17.7 ± 1.7</td>
<td>5.4 ± 0.9</td>
</tr>
<tr>
<td>Corpus distension</td>
<td>9.1 ± 0.5</td>
<td>5.4 ± 0.8</td>
<td>16.5 ± 0.9</td>
<td>5.0 ± 0.6</td>
</tr>
<tr>
<td>TH Total</td>
<td>TH Ros</td>
<td>TH Mid</td>
<td>TH Cau</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>TH Total</th>
<th>TH Ros</th>
<th>TH Mid</th>
<th>TH Cau</th>
</tr>
</thead>
<tbody>
<tr>
<td>No-distension control</td>
<td>25.0 ± 5.0</td>
<td>33.5 ± 11.3</td>
<td>23.4 ± 2.7</td>
<td>12.1 ± 1.9</td>
</tr>
<tr>
<td>Fundus distension</td>
<td>17.4 ± 1.6</td>
<td>19.1 ± 3.5</td>
<td>22.0 ± 2.4</td>
<td>11.7 ± 1.7</td>
</tr>
<tr>
<td>Corpus distension</td>
<td>25.8 ± 1.7</td>
<td>31.4 ± 4.1</td>
<td>30.4 ± 2.6‡</td>
<td>16.3 ± 1.6</td>
</tr>
</tbody>
</table>

Values are means ± SE. GLP, glucagon-like peptide; TH, tyrosine hydroxylase; NTS, nucleus of the solitary tract; Ros, rostral; Cau, caudal.
*Significant difference (P < 0.05) from control; ‡significant difference from fundus distension.
was observed in the caudal part of the NTS and declined rostrally (Fig. 2). Because the highest numbers of GLP-2-ir cells were located in the midregion, the cell counts most closely match the total counts (compare Fig. 1 and Fig. 2). Only a few TH-ir cells were found to colocalize c-Fos (Fig. 2B). Due to the presence of one rat in the control group with an exceptionally high degree of TH/c-Fos colocalization (see rat PG-46, Table 2), no statistically significant degree of c-Fos induction in TH neurons was found. However, the fraction of TH neurons activated in the two gastric distension groups corresponds to our previous findings (45). Figure 3 contains examples of tricolor confocal images from one

Table 2. Number of TH and GLP-ir cells containing c-Fos (expressed as % total TH/GLP-ir cells) from individual animals

<table>
<thead>
<tr>
<th></th>
<th>TH with c-Fos Total</th>
<th>TH with c-Fos Cau</th>
<th>TH with c-Fos Mid</th>
<th>TH with c-Fos Ros</th>
<th>GLP-2 with c-Fos Total</th>
<th>GLP-2 with c-Fos Cau</th>
<th>GLP-2 with c-Fos Mid</th>
<th>GLP-2 with c-Fos Ros</th>
</tr>
</thead>
<tbody>
<tr>
<td>No-distension control</td>
<td>PG-45 0 0 0 0 0 0 0 0</td>
<td>PG-46 17 31 20 7 4 10 4 0</td>
<td>PG-55 0 0 0 0 0 0 0 0</td>
<td>PG-58 0 0 0 0 0 0 0 0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fundus distension</td>
<td>PG-18 14 19 12 11 5 11 4 0</td>
<td>PG-43b 6 11 2 11 25 29 26 8</td>
<td>PG-50 3 8 10 8 18 27 19 11</td>
<td>PG-52 1 0 1 0 8 11 8 0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>PG-64 2 7 0 0 7 7 13 0 0</td>
<td>PG-83 7 0 7 13 66 78 64 33 0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corpus distension</td>
<td>PG-44b 25 23 9 38 45 42 47 44 0</td>
<td>PG-51 8 3 9 12 43 39 44 57 0</td>
<td>PG-59 8 19 5 2 20 33 44 33 0</td>
<td>PG-61 7 8 8 5 26 33 27 17 0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>PG-62 15 25 16 10 44 68 43 30 0</td>
<td>PG-65 5 4 5 0 38 50 41 11 0</td>
<td>PG-65 5 4 5 0 38 50 41 11 0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>PG-62 15 25 16 10 44 68 43 30 0</td>
<td>PG-65 5 4 5 0 38 50 41 11 0</td>
<td>PG-65 5 4 5 0 38 50 41 11 0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>PG-62 15 25 16 10 44 68 43 30 0</td>
<td>PG-65 5 4 5 0 38 50 41 11 0</td>
<td>PG-65 5 4 5 0 38 50 41 11 0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note the presence of one outlier in each group (PG-46, PG-83, and PG-82).

Fig. 1. Percentage of glucagon-like peptide-2 (GLP-2)-immunoreactive (ir) cells containing c-Fos in rats with fundic distension (n = 7), corpus/antrum distension (n = 7), or sham distension (n = 4). Data are means ± SE. *Significant difference from control group as determined by 1-way ANOVA followed by Fisher’s post hoc test. No significant differences were found between fundus- and corpus-distended rats.

Fig. 2. Percentage of GLP-2-ir (A) and tyrosine hydroxylase (TH)-ir cells (B) containing c-Fos at 3 different rostrocaudal levels of the caudal nucleus of the solitary tract (NTS; caudal, the commissural part of the NTS; rostral, the subpostremal NTS; mid, sections in between). Open bars, control groups; shaded, fundus; filled, corpus-distended groups. Although corpus distension tended to increase the number of TH cells containing c-Fos at all levels examined, this induction failed to reach statistical significance. *Significant difference from control group as determined by 1-way ANOVA followed by Fisher’s post hoc test.
no-distention control animal (Fig. 3, A, C, and E) and from one corpus-distended rat (B, D, F, G, and H) showing double-labeled neurons. Distribution maps of immunoreactive neurons within the dorsal vagal complex are shown in Fig. 4 for one no-distention control and one corpus-distended rat.

**DISCUSSION**

The present study points to a role of central preproglucagon neurons as mediators of meal-related stimuli emanating from the gut. We show that by selectively engaging vagal mechanoreceptors located predominantly in the proximal and distal stomach, c-Fos expression is induced in NTS neurons, including a significant percentage of preproglucagon (GLP-2 positive) neurons. Additionally, the data show gastric distension-induced c-Fos induction in a small fraction of TH-ir neurons in the NTS. The degree of activation of TH-containing neurons is in line with previous work (45), but due to one outlier in the control group, this activation failed to reach statistical significance.

The aim of our study was to elucidate the specific role of the gastric phase of meal ingestion on activity of medullary GLP-1/2 neurons. Whereas normal ingestion of a meal activates many additional neural and humoral sensory systems, including signals from the oral cavity, the small intestine, and postabsorptive sites, balloon distension is a method to selectively activate gastric mechanoreceptors. Notably, to reflect the gastric distension associated with the ingestion of a large meal, the balloon was inflated to mimic the normal fill pattern of rats given a liquid diet after an overnight fast (9). Several studies have examined the pattern of c-Fos expression in the NTS after gastric...
and it has been shown that c-Fos expression increases with both volume and the rate of distension (pressure) (6, 43, 45). This is in line with electrophysiological recordings from the afferent vagus nerve, showing a dose-dependent relationship between firing rate and gastric volume (31, 32). Additionally, it has been demonstrated that the load-sensitive gastric vagal afferents encode volume rather than nutrients (20), in line with conclusions from behavioral experiments examining appetite-suppressive feedback signals arising from the stomach (26).

The importance of vagal mechanoreceptors in short-term satiety was recently examined in neurotrophin-4 (NT-4)-deficient mice (8). NT-4 knockout mice display a
selective loss of one class of vagal mechanoreceptor, the intraganglionic laminar ending (IGLE), whereas the other class of mechanoreceptor, the intramuscular array, remains intact (8). IGLE-deficient mice have longer and larger meals, suggesting a role for IGLEs in short-term satiety, probably by conveying distension feedback to the NTS (8).

Interestingly, the GLP-1 receptor-null mutant mouse also displays mild disturbances in short-term satiety (34). Initial characterization of the GLP-1 receptor knockout mouse questioned the role of GLP-1 in appetite regulation due to the lack of an obese phenotype (33). However, closer examination of the data presented by Scrocchi et al. (34), shows that indeed the GLP-1 receptor knockout mice display a disturbed satiety sequence by terminating their initial feeding period of the dark phase significantly later than that of wild-type controls, resulting in an increase in food intake in the first hours of the dark phase (34). Coupled with our observation of GLP-1/2 neuron activation by gastric distension, it is tempting to speculate that these neurons play a role in mechanoreceptor-mediated short-term satiation. It would be highly interesting to examine gastric distension-induced activation of GLP-1/2 neurons in NT-4-deficient mice.

The induction of c-Fos in GLP-containing neurons by gastric distension demonstrated in the present experiments contrasts with recent data from Rinaman (28), who examined c-Fos expression in GLP-1/ir neurons after the consumption of a large meal. Whereas ingestion of a large liquid meal was unable to induce c-Fos expression in NTS preproglucagon neurons, Rinaman showed that up to 70% of these cells contained c-Fos after the administration of interoceptive stressors (LPS, LiCl, CCK in high doses) (28). Although it is possible that the differences could have been influenced by the immunohistochemical methods used, the completely different nature of the gastric stimulation used in the two studies clearly makes direct comparison difficult. These differences are emphasized by data from a recent study by Emond et al. (6). Using c-Fos immunocytochemistry, Emond et al. (6) showed that a large degree of signal convergence and integration of different meal-related stimuli occur at the level of the NTS. Notably, the same gastric load resulted in a greater induction of c-Fos in the NTS when present alone than when present together with other meal-related stimuli (6). This clear example of integration at the level of the NTS could explain why selective stimulation of mechanoreceptors (present study) and ingestion of a large meal (28) lead to differential activation of GLP-1/2-containing neurons.

Our finding of distension-induced activation of GLP-1/2-containing neurons raises a number of questions regarding the functional role of these neurons in the regulation of food intake. To date, preproglucagon-derived peptides, GLP-1, GLP-2, and oxyntomodulin have all been shown to inhibit food intake when administered intracerebroventricularly to rats (4, 38, 39, 41, 44). Although the specificity of GLP-1-induced anorexia has been questioned by reports showing taste aversion after centrally administered GLP-1 (16, 41, 42), specific (that is nonaversive) appetite suppression by GLP-1 can be elicited by direct injections into the hypothalamic paraventricular nucleus (21) or into the fourth ventricle (16). Furthermore, in monosodium glutamate-treated rats, central GLP-1-induced appetite suppression is eliminated, whereas GLP-1-induced taste aversion is intact (40), suggesting that indeed GLP-1 acts specifically on central appetite-regulating neurocircuits. A firm body of evidence now supports a role of NTS preproglucagon neurons as mediators of the anorexia elicited by visceral malaise. Rinaman (28) has shown that three potent interoceptive stressors (CCK, LiCl, and LPS) all induce c-Fos expression in a large proportion of GLP-1/ir neurons in the NTS, and LiCl-induced suppression of food intake can be partly blocked by prior central administration of GLP-1 receptor antagonists (27, 35), pointing to a functional role of central GLP-1 neurons in LiCl-induced anorexia. In line with these observations, it was recently found that GLP-1 neurons are activated by central oxytocin administration and that GLP-1 receptor blockade diminishes anorectic responses to oxytocin (29). As central blockade of oxytocinergic neural systems have also been implicated in LiCl anorexia (25), these data suggest that GLP-1 neurons are activated by nauseating stimuli tapping into central oxytocinergic neural pathways (29). In light of the literature supporting a role of GLP-1 in mediating the anorexia associated with gastric malaise, it would be interesting to investigate the ability of gastric balloon distension to produce a conditioned taste aversion.

In conclusion, we showed that moderate gastric distension can activate hindbrain GLP-1/2-containing neurons, consistent with a role of central preproglucagon-derived peptides as inhibitors food intake. The tendency for corpus distension to activate more GLP-2-positive neurons than fundus distension further suggests that, even among gastric distension sensors, the capacity to signal to the brain stem may be different.

We are grateful to S. Jensen for excellent technical assistance.

DISCLOSURES

The study was supported by grants from the National Institute for Diabetes and Digestive and Kidney Disease (DK-47348), The Danish Diabetes Association, The Novo Nordisk Foundation, and Fonden til Lægevidenskabens Fremme.

REFERENCES


29. Mueller IC, Moller M, Lar森 P, and Vrang N. Light-induced c-Fos expression in supraspinalcamic nuclear neurons targeting the paraventricular nucleus of the hamster hypothala-


