Duodenal loading with glucose induces Fos expression in rat brain: selective blockade by devazepide

Lixin Wang, Sylvain Cardin, Vicente Martínez, Yvette Taché, and K. C. Kent Lloyd

Center for Ulcer Research and Education/Digestive Disease Research Center, Veterans Affairs Medical Center, Department of Medicine and Brain Research Institute, University of California, Los Angeles 90073; 3School of Veterinary Medicine, University of California, Davis, California 95616; and 2Department of Molecular Physiology and Biophysics, Vanderbilt University Medical School, Nashville, Tennessee 37232

Wang, Lixin, Sylvain Cardin, Vicente Martínez, Yvette Taché, and K. C. Kent Lloyd. Duodenal loading with glucose induces Fos expression in rat brain: selective blockade by devazepide. Am. J. Physiol. 277 (Regulatory Integrative Comp. Physiol. 46): R667–R674, 1999.—The role of CCK in mediating neuronal activity in the brain in response to dietary carbohydrate was measured by detecting Fos immuno-reactivity in response to duodenal glucose load in rats after administration of the CCK-A receptor antagonist devazepide. In adult, male Sprague-Dawley rats, infusion for 30 min of 545 mg (2.18 kcal) dextrose through a duodenal cannula induced Fos expression in the nucleus of the solitary tract (NTS), area postrema (AP), lateral division of the central nucleus of the amygdala (CeAL), and the external subnucleus of the lateral parabrachial nucleus (LPBE). Devazepide treatment (1 mg/kg) attenuated Fos expression in the NTS and AP by 81 and 78%, respectively, but not in the CeAL or LPBE. These results indicate that central neuronal activation is elicited by dietary glucose in the intestinal lumen and that activation of neurons in the NTS and AP is mediated by CCK-A receptors.

cholecystokinin; nucleus of the solitary tract; area postrema; satiety; feeding

BECAUSE GLUCOSE is an essential metabolic fuel used preferentially by all tissues of the body, regulation of carbohydrate intake is important for maintaining homeostatic physiological functions. Although energy demand drives hunger and promotes food ingestion, the products of protein and lipid digestion elicit satiety and suppress further food intake. On the other hand, similar responses to carbohydrate digestion have been less well described. It was shown previously that the presence of glucose in the intestinal lumen causes a delay in gastric emptying (33) and suppression of food intake (29, 41). Regulation of nutrient homeostasis and food intake appears to involve, at least in part, the nucleus of the solitary tract (NTS) and the area postrema (AP) (11); suppression of feeding behavior in response to luminal glucose is coincident with Fos expression in both of these brain regions (29, 47). The remaining significant physiological question is the mechanism by which a luminal nutrient, such as glucose, influences feeding behavior through the central nervous system.

One mechanism by which the duodenal delivery of nutrients induces physiological responses mediated centrally is through CCK. Although CCK directly regulates pancreatic and gastric functions (22, 32), it also plays a role in maintaining glucose homeostasis (39) and can suppress sham feeding in rats (7) and carbohydrate intake in pigs (2). A centrally mediated role for CCK appears to be supported by studies in which Fos expression was stimulated in both the NTS and AP after exogenous administration of CCK, which increases circulating CCK levels (13, 40). However, in vivo immunoneutralization of circulating CCK using an anti-CCK monoclonal antibody had no effect on food intake behavior (34). Furthermore, there is no apparent correlation of plasma CCK levels, feeding behavior (4, 44), or brain stem Fos expression (47) with intestinal nutrient load. However, in at least one case, administration of a CCK-A receptor antagonist blocked satiety induced by duodenal infusion of glucose in the absence of an increase in plasma CCK levels (41). Whether this observation can be correlated with brain stem Fos expression has not been determined.

Therefore, in this study, we used immunohistochemical detection of Fos to identify specific brain regions activated in response to duodenal infusion of glucose in rats. Blood levels of glucose and insulin during and after infusion were monitored to ensure carbohydrate absorption and an appropriate insulin response. To determine to what extent CCK-A receptors are involved in this response, the CCK-A receptor antagonist devazepide was administered systemically before intraduodenal infusion of glucose to rats.

METHODS

Animals

Adult male Sprague-Dawley rats (Harlan, San Diego, CA), weighing 290–310 g, were maintained under conditions of controlled temperature (21–23°C) and illumination (a 12:12-h dark-light cycle) with access to food and water ad libitum. Rats were deprived of food but not water for 16–18 h before experiments. Experiments were performed under the Veterans Affairs animal component of research protocol 96–080–08.

Surgery

Intraduodenal cannulation. One week before experiments, a cannula of polyethylene tubing (PE-90; ID 0.86 mm, OD...
DUODENAL GLUCOSE INDUCES BRAIN STEM FOS EXPRESSION

1.27 mm; Intramedic, Sparks, MD) was implanted into the duodenum—2.5 cm from the pylorus in rats anesthetized with a mixture of ketamine (75 mg/kg) and xylazine (25 mg/kg) intraperitoneally injected. The cannula was secured in place with a purse-string suture, and small pieces of nylon mesh and Silastic tube were positioned outside the PE-90 tubing between the duodenum and abdominal wall to prevent adhesion. The cannula then was exteriorized through the abdominal wall, tunneled subcutaneously to the back of the neck, fixed in place, and flushed, filled with saline, and capped.

Carotid catheter. In another group, in addition to the intraduodenal cannula, a catheter (PE-50; ID 0.58 mm, OD 0.965 mm; Intramedic) was inserted at the same time into the carotid artery, allowing for the withdrawal of blood. The catheter was tunneled subcutaneously to the back of the neck, fixed in the same place as the intraduodenal cannula, filled with a heparinized saline solution (200 U/ml), and capped.

After surgery, rats were housed individually and handled each day to accustom them to experimental manipulation.

Treatments

CCK-A receptor blockade. Devazepide (Merck Sharp and Dohme, Rahway, NJ) is a potent, nonpeptide, CCK-A receptor antagonist. Devazepide (1 mg/kg) was dissolved in 10% DMSO, 5% Tween 80, and 85% of 0.9% saline before the experiment and was injected intraperitoneally (1 ml/kg body wt) 40 min before duodenal infusion of glucose. Control rats received the solvent vehicle only.

Glucose loading. In rats fasted overnight, with or without treatment of devazepide, and unrestrained in their home cages, glucose (dextrose:D-glucose in H₂O; J. T. Baker, Phillipsburg, NJ) was infused into the duodenum (545 mg/rat, equal to 2.18 kcal) through the cannula using an infusion speed of 0.05 ml/min for 30 min, for a total of 1.5 ml. Control rats received the same volume of 0.33 M d-mannitol (Sigma) as isovolumic controls.

The amount of glucose (545 mg), volume of infusion (1.5 ml), and the postinfusion time period after duodenal infusion (1.5 h) selected in this study were based on findings from preliminary experiments in which the doses of glucose administered were either 136, 273, or 545 mg; the volumes of infusion were either 1.5 or 3 ml; and the postinfusion time period was compared between 0.5, 1.5, and 3 h (n = 1–3).

Fos Immunohistochemistry

Rats were anesthetized with pentobarbital sodium (Nembutal, 70 mg/kg) 1.5 h after intraduodenal glucose infusion and transcardially perfused with 50 ml of 0.9% saline followed by 500 ml of 4% paraformaldehyde and 14% saturated picric acid in 0.1 M phosphate buffer at pH 7.4. Brains were dissected, postfixed in the same fixative overnight, and then rinsed and cryoprotected in 20% sucrose for 1 day. Fos immunohistochemistry was performed on 30-µm-thick frozen sections using the avidin-biotin-peroxidase complex (ABC) method. Briefly, incubation with primary antisera, rabbit anti-human c-Fos protein residues 4–17 diluted at 1:10,000 (c-Fos Ab-S; Oncogene Science, Uniondale, NY), for 2 h at room temperature and then overnight at 4°C was followed by incubation with secondary antibody (biotinylated goat anti-rabbit IgG; 1:1,000; Jackson ImmunoResearch, West Grove, PA) and with ABC (1:100; Vector, Burlingame, CA) for 1 h at room temperature for each. Coloration of the conjugated compounds was elicited with 0.025% 3,3′-diaminobenzidine tetrachloride and 0.01% hydrogen peroxide for 5–7 min at room temperature. Sections were mounted, air dried, dehydrated, cleared, and placed under a coverslip.

The specificity of the primary antibody was tested by preabsorption with peptide (c-Fos peptide-2; Oncogene) with the addition of 10 µg of the peptide to 1 ml of Fos antiserum at a titer of 1:10,000 (antigen:antibody of 100:1). The solution was applied to the sections used for control according to the procedures outlined above.

Sections were evaluated and photographed by bright-field microscopy. Fos-immunoreactive cells were counted in 10–15 sections for each nucleus. Data are expressed as means ± SE. Differences among groups of animals with different treatments were determined by Kruskal-Wallis nonparametric ANOVA and Dunn’s multiple comparison tests. Data were considered statistically significant when P < 0.05.

Plasma Glucose and Insulin Measurement

On each experimental day, rats were placed individually into plastic cages resembling their home cages. Thirty minutes later the arterial and duodenal catheters were uncapped, flushed, and connected to syringes installed on Harvard syringe infusion pumps (Harvard Apparatus, South Natick, MA). Once the rats were very calm and well accustomed to the catheter extensions (30–60 min later), the first blood sample (0.2 ml) was taken (t = 0 min). Blood samples for insulin measurements were taken every 15 min in the first hour after the beginning of glucose infusion and every 30 min in the second hour (t = 0–120 min). For glucose levels, blood samples were taken every 5 min during the first hour and every 10 min during the second hour. To avoid any possible complication derived from blood sampling, the red blood cells were resuspended in saline and reinfected. Plasma glucose levels were measured using a glucose oxidase method with a Beckman glucose analyzer (Beckman Instruments, Fullerton, CA). Immunoreactive insulin was detected by a double-antibody radioimmunoassay, with an interassay coefficient of variation of 8%. The antibodies and 125I tracer were purchased from Linco Research (St. Louis, MO).

RESULTS

Fos Expression in the Brain After Intraduodenal Carbohydrate Load

Fos immunoreactivity induced by duodenal infusion of glucose (545 mg·rat⁻¹·30 min⁻¹) was detected in the NTS, AP, lateral division of the central nucleus of the amygdala (CeA), and external part of the lateral parabrachial nucleus (LPBE) in comparison to the very low level of Fos immunoreactivity in rats infused with mannitol. Preabsorption of the primary antibody with Fos peptide abolished all Fos immunoreactivity. There was no increase in Fos expression observed in the hypothalamic nuclei and locus ceruleus compared with controls (Fig. 1). Pretreatment with vehicle for devazepide did not alter Fos immunoreactivity induced by intraduodenal glucose (Table 1). In preliminary studies, Fos expression was consistently increased using a glucose infusion dose of 545 mg/rat, whereas lower doses had no effect. However, there was no difference in the numbers of Fos-immunoreactive cells in rats killed 0.5, 1.5, or 3 h postinfusion nor when different isocaloric volumes (1.5 or 3 ml) of glucose were infused (data not shown). Therefore, all the data presented here are from rat brains fixed 1.5 h after infusion of 545 mg glucose in a volume of 1.5 ml.
Fos-expressing neurons in the NTS after intraduodenal glucose infusion were primarily found in the medial division (Fig. 2), including the medial, dorsomedial, commissural, parvicellular, and intermediate subnuclei, but not in the central subnucleus, as defined by Herbert and Saper (17); only a few immunoreactive neurons could be found in the lateral division. The number of Fos-immunoreactive neurons per section was increased by 7.4- to 10.4-fold compared with that in control rats (Table 1, Figs. 2 and 3). In the AP, immunoreactive neurons were distributed in the entire caudal section, whereas, rostrally, they were distributed only in the ventral lateral zone neighboring the NTS. The number of Fos immunoreactive neurons per section was increased by 3.4- to 4.6-fold compared with that in control rats (Table 1, Figs. 2 and 3). In the LPBE, numerous Fos-positive neurons were found in the external subnucleus, whereas they were scattered and found inconsistently within other subnuclei; the numbers per section were increased by 6.8- to 14.4-fold (Table 1, Figs. 3 and 4). Fos-positive neurons in the CeA were more numerous only in the lateral part where the cell density was greater (Fig. 5). The number of Fos-positive neurons per section was increased by 8- to 8.4-fold compared with that in control rats (Table 1, Fig. 3).

Effect of Devazepide

The increase in the number of Fos-positive cells in the NTS and AP induced by duodenal infusion of glucose was attenuated 81 and 78%, respectively, after devazepide treatment, and there was no subnuclear difference observed (Figs. 2 and 3). However, there was no change in numbers of Fos-immunoreactive cells in the LPBE or lateral CeA after devazepide treatment (Figs. 3–5).

Plasma Levels of Glucose and Insulin

Plasma levels of glucose and insulin were increased during and after intraduodenal glucose infusion (545 mg·rat$^{-1}$·30 min$^{-1}$), but remained unchanged during and after mannitol infusion (Fig. 6). The average basal glucose level was 106.9 ± 2.6 mg/dl in fasted freely moving rats. By the end of duodenal infusion of glucose, circulating glucose was elevated to a peak of 221.6 ± 8.4 mg/dl.

---

Table 1. Number of Fos immunoreactive cells in NTS, AP, LPBE, and CeAL in rats infused intraduodenally with glucose with or without treatment with vehicle or devazepide

<table>
<thead>
<tr>
<th></th>
<th>Without Treatment</th>
<th>Vehicle for Devazepide</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mannitol</td>
<td>Glucose</td>
</tr>
<tr>
<td></td>
<td>Mannitol</td>
<td>Glucose</td>
</tr>
<tr>
<td>NTS</td>
<td>14 ± 1</td>
<td>104 ± 4*</td>
</tr>
<tr>
<td></td>
<td>17 ± 1</td>
<td>130 ± 3*</td>
</tr>
<tr>
<td>AP</td>
<td>34 ± 2</td>
<td>116 ± 5*</td>
</tr>
<tr>
<td></td>
<td>27 ± 2</td>
<td>125 ± 6*</td>
</tr>
<tr>
<td>LPBE</td>
<td>15 ± 2</td>
<td>102 ± 5*</td>
</tr>
<tr>
<td></td>
<td>7 ± 1</td>
<td>101 ± 5*</td>
</tr>
<tr>
<td>CeAL</td>
<td>27 ± 3</td>
<td>216 ± 6*</td>
</tr>
<tr>
<td></td>
<td>30 ± 2</td>
<td>221 ± 6*</td>
</tr>
</tbody>
</table>

Data are presented as means ± SE of cells per section (n = 4 in each group). *P < 0.05 vs. respective mannitol infusion control. NTS, nucleus of the solitary tract; AP, area postrema; LPBE, external subnucleus of the lateral parabrachial nucleus; CeAL, central nucleus of the amygdala.
0.8 mg/dl at 30 min. Thereafter, values declined over time to a level ~123.8 ± 1.3 mg/dl at 120 min. Insulin levels were low in fasted rats (0.18 ± 0.02 ng/ml) and reached a peak (6.48 ± 0.75 ng/ml) by the end of the glucose infusion. Insulin levels decreased thereafter and returned to preinfusion levels (0.21 ± 0.06) at 90 min.

**DISCUSSION**

As demonstrated using immunohistochemistry, an intestinal glucose load in conscious rats induces Fos expression in brain stem neurons including those in the NTS, AP, external part of the LPB and lateral division of the CeA. Consistently, all of these areas participate in regulating feeding behavior and glucose homeostasis. Lesions of the NTS and AP have been shown to increase the intake of a liquid diet and have attenuated the feeding response to glucoprivation and hypoglycemia (9, 11). Furthermore, our results are consistent with recent reports of brain stem Fos expression in response to duodenal nutrient infusions in rats (29, 47).

Our results are consistent in many aspects with earlier studies that identified glucose-sensitive neurons after intestinal infusion (12, 29, 47). Neurons in the NTS also have been shown to respond to glucose applied electrophoretically in vitro (25), infused into the portal vein (1), or administered to induce hyperglycemia or hypoglycemia (42, 43). In monkeys, glucose-sensitive neurons in the CeA have been shown to be involved in the control of food acquisition behavior (27). Neurons in the AP also have been shown to be responsive to intestinal glucose (1, 29). However, to our knowledge, this is the first study to indicate Fos expression in response to intestinal glucose in neurons of the LPBE and CeA.

Several neuronal populations in the hypothalamus have been implicated as glucose sensors responsive to circulating levels of glucose. However, we did not observe any significant changes in Fos expression in the hypothalamus in response to intraduodenal glucose infusion. Responses of hypothalamic nuclei to homeostatic adjustments in glucose levels appear to be highly variable. For example, intra-arterial infusion of glucose in rats increased Fos immunoreactivity in the paraventricular nucleus (PVN) and ventromedial hypothalamic nucleus in only about half of the animals tested (10). In contrast, hypoglycemia induced by infusion of 2-deoxy-D-glucose, a nonmetabolized analog of glucose, induced Fos-like immunoreactivity consistently in the PVN, supraoptic nucleus, and locus ceruleus (37). Therefore, Fos expression may be more inclined to increase in hypothalamic nuclei in response to changes in circulating glucose levels or glucose metabolism greater than those measured in our study.

Our study showed that CCK-A receptors mediate intestinal glucose signaling in the brain stem, because Fos expression in the NTS and AP, but not the LPBE or CeA, was attenuated by pretreatment with the CCK-A receptor-selective antagonist devazepide. The NTS and AP are known to express CCK-A receptors with a high affinity for CCK (26). Furthermore, because the blood-brain barrier is permeable to the dose of devazepide we administered systemically (31), it is possible that the Fos expression we detected was mediated by activation of central CCK-A receptors. Alternatively, peripheral CCK-A receptors such as those in the pancreaticoduodenal arterial bed (8) may signal through an afferent neural pathway and stimulate brain stem Fos expression. Whether CCK-A receptors mediating Fos expression are expressed centrally or peripherally cannot be determined definitively by this study.

We also observed that devazepide failed to attenuate Fos expression in the LPBE and CeA as it was in the NTS and AP. One possible explanation for this is that the LPBE and CeA have not been shown to express CCK-A receptors and therefore would not be expected to respond to attenuation by devazepide (26). Another possibility is that brain stem neuronal activity as...
reflected by Fos expression depends on the dose of glucose loaded into the intestine. In an earlier study (41), devazepide appeared to completely reverse suppression of food intake behavior by a low dose, but not by higher doses, of intestinal glucose (9.2 mmol·kg⁻¹·h⁻¹). Although brain stem Fos expression was not evaluated in that study, perhaps the continued suppression of feeding behavior was due to persistent neuronal activity in the LPBE and CeA that was not attenuated by devazepide. This also would be consistent with why devazepide had no effect on Fos expression in the LPBE or CeA in our study. Further studies to measure the effect of devazepide on Fos expression in the LPBE and CeA in response to smaller glucose loads would be required to confirm this hypothesis.

Although our study clearly demonstrates that duodenal glucose-dependent Fos expression in the NTS and AP is mediated by CCK, it is not likely due to an increase in plasma CCK levels. Although we did not measure circulating CCK concentrations, other studies (21, 24) indicated that glucose infusion does not increase plasma CCK levels. Furthermore, there is no apparent correlation between circulating levels of CCK and either brain stem Fos expression (47) or feeding behavior (4, 44). Therefore, our results are consistent with the hypothesis that CCK released locally in the intestinal mucosa in response to duodenal glucose may activate a signaling mechanism to stimulatebrain stem Fos expression (36). In this case, one would not expect to measure an increase in plasma CCK in...
response to a duodenal glucose load. Sources of CCK in the intestinal epithelium include intestinal “I” cells and afferent neurons (15), which may be stimulated by CCK (3). This hypothesis is further supported by reports showing that both vagotomy (46) and capsaicin (45) attenuate the suppression of feeding behavior induced by intestinal nutrient loads.

We measured an increase in circulating glucose and insulin responses to intraluminal glucose infusion, confirming these expected physiological responses in our model. Although we did not perform this experiment in devazepide-treated animals, it is unlikely that carbohydrate absorption or insulin responses are any different between devazepide and vehicle pretreatments (30). Several published reports indicate that carbohydrate and insulin responses are similar to control after devazepide treatment in rats (38) as well as other species (18, 19, 23, 28).

In an earlier report, Fos expression in the NTS and AP after oral feeding of an isocaloric meal was not reversed by devazepide, which at first glance appears to contradict our findings. However, we directly infused the duodenum with glucose to study the role of CCK in mediating Fos expression in response to an intestinal glucose load. Therefore, these results suggest that CCK mediates brain stem Fos expression in response to duodenal delivery of glucose, whereas CCK-indepen-

dent mechanisms mediate Fos expression in response to cues arising from mechanical distension and chemical stimulation of the stomach during oral feeding.

Our experiments ruled out the possibility that induction of Fos expression was due to distension of the intestinal lumen. Our preliminary studies showed that an equivalent number of Fos-positive cells were measured after infusion of 3.0 ml of 1 M glucose or 1.5 ml of 2 M glucose, whereas significantly fewer Fos-positive cells were seen in control animals infused with identical volumes of mannitol. Moreover, the amount of loaded glucose under these conditions was within a range of daily consumption of carbohydrate in rats (33, 47).

Because we did not use a control infusion with the same osmolality as the glucose solution, the contribution of osmoreceptive mechanisms contributing to central Fos expression cannot be entirely excluded. Osmotic loads can be as effective as glucose loads in causing satiety (20), and Fos expression is increased in response to intragastric loading or intraperitoneal injection of hypertonic solutions in osmoreceptors situated in the PVN or supraoptic nucleus (6, 16). Although we used smaller volumes of hypertonic glucose, Fos expression was unchanged in either the PVN and supraoptic nucleus after glucose loading. In addition, intestinal chemosensory mechanisms have been shown to be dependent on the amount of carbohydrate loaded rather than on its concentration (33, 35).

In summary, intestinal glucose infusion in conscious rats induces Fos expression in specific brain nuclei. Fos expression in the NTS and AP, but not in the external LPB and lateral CeA, is mediated by CCK-A receptors, indicating a role for CCK in central signaling of dietary nutrients present in the intestine after a meal.

Perspectives

To better understand nutrient signaling from the intestine to the brain, it would be necessary to precisely locate receptors responding to nutrients and to determine the neuroendocrine pathways driving nutrient-related information from peripheral tissues. In particular, to understand intestinal glucose-mediated responses in the central nervous system, it would be advantageous to 1) identify and map cells acting as glucosensors and identify the transduction mechanisms used to detect and differentiate from other nutrients the presence of carbohydrates in the intestinal lumen, 2) determine the peripheral or central mechanism of CCK-A receptor-dependent communication between the gut and brain in the absence of changes in plasma CCK levels, and 3) characterize the pathways and mediators used to transduce the presence of intestinal glucose centrally to the brain. Furthermore, it would be necessary to integrate these observations with those changes observed after the administration of other simple nutrients and after the administration of a complete meal.

This work was supported in part by National Institute of Diabetes and Digestive and Kidney Grant DK-45752 (to K. C. K. Lloyd), Animal Models Core of Center Grant DK-41303, and National Institutes of Mental Health Grant MH-00663 (to Y. Taché).
REFERENCES


36. Verspohl, E. J., C. Zoli, M. A. Wahl, and H. P. Ammon. The role of cholecystokinin (CCK8) on glucose production and elmina-