Decreased orexigenic response to neuropeptide Y in rats with obstructive cholestasis

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Rioux, Kevin P., Tai Le, and Mark G. Swain. Decreased orexigenic response to neuropeptide Y in rats with obstructive cholestasis. Am J Physiol Gastrointest Liver Physiol 280: G449–G456, 2001.—Neuropeptide Y (NPY) is a key factor in the neurochemical control of food intake, and obstructive cholestasis can be associated with disturbances in food intake. Our aim in this study was to determine whether obstructive cholestasis in the rat is associated with defective central responsiveness to NPY. Cholestasis was induced in rats by surgical bile duct resection. Rats with obstructive cholestasis exhibited a 20% reduction in food intake 2 days after laparotomy (compared with sham-resected controls) that had resolved by 4 days after surgery. Responsiveness to the orexigenic action of NPY was tested by measuring food intake after intracerebroventricular injection of NPY. In sham-resected rats, NPY infusion strikingly increased food intake, whereas bile duct-resected (BDR) rats showed a consistently impaired feeding response to NPY at postlaparotomy days 2, 4, and 7. Separate experiments measured specific binding of [3H]NPY to hypothalamic receptors. Fos protein expression was measured in the hypothalamic paraventricular nucleus (PVN) as a marker of NPY-induced neuronal activation. The decreased orexigenic responsiveness to NPY was not caused by altered NPY binding at hypothalamic receptors or its ability to activate neurons in the PVN. Therefore, cholestatic rats demonstrate an attenuated NPY-induced orexigenic drive that occurs early after biliary obstruction, when cholestatic rats exhibit reduced food intake, and persists despite the return of food intake to normal levels and the presence of intact central NPY-related neuronal pathways.

cytokine; hypothalamus; anorexia; biliary obstruction

IN A VARIETY OF ACUTE and chronic illnesses, a generalized phenomenon termed “malaise” was once the best available explanation for anorexia. However, it is increasingly apparent that there are more fundamental and defined causes of anorexia based on the concept that central neural signals are important regulators of food intake (10, 17, 19, 33). Further to this concept, it is recognized that the dynamics of central regulation of food intake can be greatly affected by systemic factors such as stress hormones and inflammatory mediators (1, 13, 29, 30, 33, 39).

Disturbances in food intake are commonly encountered in patients with obstructive cholestasis, especially in those with malignant obstruction (3, 20). However, in patients with benign biliary obstruction alterations in food intake are less well characterized. Patients presenting with acute biliary obstruction due to cholecystolithiasis often experience anorexia and decreased food intake; however, with more prolonged nonmalignant biliary obstruction patients often exhibit little or no anorexia (12). Similar findings have been observed in rats with obstructive cholestasis due to bile duct ligation (15). Bile duct-ligated rats demonstrated decreased food intake in the days immediately after surgery compared with sham-resected controls; however, food intake returned to control levels with more prolonged biliary obstruction (15). These findings suggest that an adaptive process occurs over time in the central drive to feed in rats with obstructive cholestasis.

Neuropeptide Y (NPY) is a key neurochemical mediator involved in the central nervous system control of food intake. In both fasted and fed rats, central injection of NPY effectively stimulates food intake (10, 19, 34). NPY is produced endogenously by neurons whose cell bodies lie in the arcuate nucleus of the hypothalamus. These NPY-producing neurons have axonal projections that terminate in the paraventricular nucleus (PVN) and dorsomedial nucleus of the hypothalamus (2). During periods of fasting and prolonged starvation, synthesis of NPY is increased in neurons of the arcuate nucleus and there is a corresponding increase in NPY within the PVN (9, 17). This is the origin of an important physiological cue to feed.

Therefore, the aim of the present study was to determine whether experimental obstructive cholestasis is associated with alterations in NPY-induced feeding in the rat. To address this issue, rats with obstructive cholestasis due to surgical bile duct resection were compared with sham-resected controls with respect to daily food intake and orexigenic responsiveness to centrally infused NPY. In addition, hypothalamic NPY receptor binding and NPY-induced stimulation of the neuronal activation marker Fos were measured in cho-
lestatic and control rats to determine whether cholestatic is associated with defects in NPY receptor binding and receptor activation.

**MATERIALS AND METHODS**

**Animals.** Male Sprague-Dawley rats (275–300 g; 90–100 days old) were obtained from Charles River Breeding Farms (Pointe Claire, QC, Canada). Animals had free access to food and water and were housed at 22°C in a light-controlled room with a 12-h day/night photoperiod. All experimental procedures were approved by the Animal Care Committee of the University of Calgary and were performed in accordance with the guidelines established by the Canadian Council on Animal Care.

**Experimental model of acute obstructive cholestasis.** Under halothane-induced general anesthesia, laparotomy was performed and the bile duct was isolated, double ligated, and resected between the ligatures as described by Cameron and Oakley (7). Sham resection consisted of laparotomy and bile duct identification and manipulation without ligation or resection. Cholestasis was confirmed by measuring serum total bilirubin levels with a commercially available kit (Sigma Chemical, St. Louis, MO).

Daily food and water intake. Rats were housed individually in hanging wire cages with both food and water available ad libitum. The rats were conditioned to eat ground rat chow from stainless steel containers with concave flanged lids designed to minimize spillage of food. Food and water intake was measured gravimetrically between 0830 and 0930 before laparotomy (baseline) and again for the 24-h periods from day 1 to day 2 (day 2), from day 3 to day 4 (day 4), and from day 6 to day 7 (day 7) after laparotomy. Any food spillage was collected on paper inserts beneath the cage and accounted for in the calculation of daily food intake.

**Surgical procedure for inserting intracerebroventricular cannulas.** Intracerebroventricular (icv) cannulas were inserted as previously described (36). Briefly, rats were anesthetized by intraperitoneal injection of pentobarbital sodium (65 mg/kg, Somnotol; MTC Pharmaceuticals, Cambridge, ON, Canada) and secured to a stereotactic positioning apparatus (Breslar’s screws and dental cement). Positioning of the cannula was confirmed by measuring serum total bilirubin levels with a commercially available kit (Sigma Chemical, St. Louis, MO).

**Characterization of specific NPY binding in rat hypothalamus.** Day 4 sham-resected and BDR rats were killed by decapitation, and the hypothalamus was immediately excised and placed in ice-cold 10 mM Tris-HCl containing 0.32 M sucrose (pH 7.4). For each sample, five hypothalami were pooled in 10 ml of the buffer and homogenized on ice (Brinkmann Polytron; 20 s, speed setting 4.5). Therefore, 30 BDR rats gave n = 6 in the BDR group, and 30 sham rats gave n = 6 in the sham group. All chemicals were purchased from Sigma Chemical unless otherwise noted.

**Gastric emptying.** To assess potential differences in gastric emptying in sham-resected and BDR rats, separate experiments were performed according to the method of McHugh et al. (25). Postlaparotomy days 2 and 4 sham-resected and BDR rats (n = 6/group) were fasted with free access to water for 24 h. All rats received a high-fat liquid test meal (10 ml Ensure; Abbott Laboratories, St. Laurent, QC, Canada) by gavage. One hour later, the rats were killed by lethal overdose of pentobarbital sodium and the stomach was ligated at the lower esophageal and pyloric sphincters. The stomach contents were collected in plastic weigh boats, dried for 24 h at 56°C, and weighed. Gastric emptying was expressed as the dry weight of the recovered test meal divided by the mean dry weight of three 10-ml meals dried separately.

**Subsequent centrifugation to obtain a synaptosome-enriched fraction was performed according to the method of Bergasa et al. (5). Homogenates were centrifuged at 1,000 g for 10 min at 4°C. The supernatant was then recovered and centrifuged at 20,000 g for 15 min. This pellet was subsequently resuspended in 5 ml of the buffer, incubated at room temperature for 15 min, and then centrifuged at 30,000 g for 10 min at 4°C. The pellet derived from this spin was resuspended in 5.0 ml of buffer and centrifuged at 30,000 g for 10 min at 4°C. The final pellet was frozen on powdered dry ice and stored at -70°C.

**Membrane binding assays were performed according to the method of Martel et al. (24). On the day of the assay, each pellet was reconstituted in 1.8 ml of Krebs-Ringer assay buffer (Krebs-Ringer bicarbonate buffer, pH 7.4, containing 50 mM HEPES, 0.1% bovine serum albumin, 0.05% bacitracin, and 0.5 mM phenylmethylsulfonyl fluoride) to give a protein concentration of 10–12 mg/ml as measured by the method of Lowry et al. (22).**

The final volume of each NPY binding assay mixture was 500 μl, and all components were prepared in Krebs-Ringer assay buffer. The tracer ligand, N-[propionyl-3H]NPY (72 Ci/mmol stock; Amersham), was added to give final concentrations ranging from 0.22 to 44 nM. After addition of 100 μl of the membrane preparation, the assay mixture was incubated for 2 h at room temperature. Each incubation was stopped by 10.220.33.2 on March 31, 2017 http://ajpgi.physiology.org/ Downloaded from
terminated by rapid filtration through Whatman GF/C filters followed by two washes with ice-cold Krebs-Ringer bicarbonate buffer. To reduce binding of free tracer, the filters were pretreated by soaking overnight in 1% polyethylenimine in Krebs-Ringer bicarbonate buffer. Bound radioactive tracer was quantified by liquid scintillation counting. Specific binding was calculated as the difference between tracer ligand in the presence and absence of 1 μM [3H]NPY. At 1.0 nM [3H]NPY, specific binding was 80–85% of total binding.

Fos immunohistochemistry. Rats were implanted with stainless steel icv cannulas (as described in Surgical procedure for inserting intracerebroventricular cannulas), and 7 days later they underwent either bile duct resection or sham resection. Each rat was then handled for 2 min daily for 4 days before the experiment to acclimate them to such contact and thereby minimize handling stress-related effects on Fos expression. After an overnight fast with free access to water, sham-resected or BDR rats (postsurgery day 4) received either NPY (10 μg icv) or saline vehicle (at least 5 rats/group), after which food was still withheld. Two hours later, when Fos expression is maximal (21), the rats were deeply anesthetized with pentobarbital sodium (80 mg/kg ip) and perfused with 0.9% saline (100 ml) via transcardial catheter followed by 4% paraformaldehyde in phosphate buffer (~200 ml). The whole brain was excised and postfixed for 3 h in the paraformaldehyde fixative solution, after which the specimens were cryopreserved by transferring to 25% sucrose in phosphate buffer overnight.

Frozen coronal brain sections (40 μm) were cut and stained to identify cells expressing Fos protein using the avidin-biotin peroxidase method as described previously (36). Briefly, sections were incubated for 30 min in blocking solution (PBS containing 5% normal goat serum and 0.25% Triton X-100), followed by a 48-h incubation at 4°C with rabbit anti-rat c-Fos Ab-5 (Onogene Science, Uniondale, NY) diluted 1:20,000 in blocking solution. The secondary antibody (biotinylated goat anti-rabbit BA-1000; Dimension Laboratories, Mississauga, ON, Canada), was then applied 1:200 in PBS containing 1.5% normal goat serum for 30 min. This was followed by a 30-min incubation in avidin-biotin complex reagent (Vectastain Elite ABC Kit; Dimension Laboratories) and finally application of the diaminobenzene chromogen (Dimension Laboratories). Sections were washed for 10 min in PBS between each of the above steps. Cells showing Fos-like immunoreactivity in the PVN of the hypothalamus were visualized by video microscope. Fos-positive cells were counted on one side of the PVN, one slide per rat, after carefully matching of brain sections from different animals to match Fig. 25 in the rat brain atlas of Paxinos and Watson (28). Fos-positive cells were counted by two observers unaware of the identity of the specimen.

Statistical analyses. All data are expressed as means ± SE. For analyses of rat body weight, a Student’s t-test was used. For analyses of daily food and water intake, ANOVA was used. The Student’s t-test was used for comparisons between sham-resected and BDR rats at each dose. Gastric emptying and neuronal Fos expression data were analyzed by Student’s t-test. In studies of hypothalamic NPY binding, values of NPY binding affinity ($K_d$) and maximal binding ($B_{max}$) were determined by nonlinear regression using GraphPad Prism (GraphPad Software, San Diego, CA). Best fit was determined by comparing one- and two-site binding models by F-test. Student’s t-test was used to compare $K_d$ and $B_{max}$ in sham-resected and BDR rats. In all studies, groups of data were considered significantly different if $P < 0.05$.

RESULTS

BDR rats showed clinical evidence of cholestasis with jaundice, dark urine, and icteric plasma. Cholestasis was confirmed by a marked increase in serum total bilirubin in day 4 BDR rats (3.65 ± 0.21 mg/dl) compared with sham-resected controls (0.18 ± 0.03 mg/dl; $P < 0.01$).

Food and water intake. Rats whose presurgical body weights were slightly but not significantly greater were assigned to the BDR group (day 0 body weights: BDR, 298.3 ± 3.9 g; sham-resected, 288.9 ± 3.8 g; $P = 0.1$; $n = 16$ and 15 rats/group, respectively). Assignment of somewhat heavier rats to the BDR group was important in that it ensured that sham-resected and BDR rats would both have matched body weights 4 days postsurgically, when the majority of the experiments were performed (day 4 body weights: BDR, 301.6 ± 7.8 g; sham-resected 299.3 ± 7.2 g; $n = 16$ and 15 rats/group, respectively). Baseline (day 0) food intake in sham-resected and BDR rats before laparotomy was not significantly different (Table 1). On postlaparotomy day 2, BDR rats displayed a 20% reduction in food intake compared with food intake in sham-resected rats 2 days after laparotomy (Table 1). However, food intake in BDR rats and food intake in corresponding sham-resected rats 2 days after laparotomy (Table 1). Therefore, food intake in BDR rats was similar at baseline and at all time points after laparotomy (data not shown).

NPY injection and feeding response. Considering the important role of NPY in central nervous system control of food intake, we assessed whether BDR rats responded normally to the potent orexigenic action of NPY. Although the 2-h feeding response (3.3 ± 0.8 g) to the 1-μg icv infusion of NPY in day 4 sham-resected rats did not quite reach statistical significance ($P < 0.06$), both the 5-μg and 10-μg doses caused striking feeding responses (8.8 ± 1.4 and 5.4 ± 1.1 g, respectively) compared with sham-resected rats that had received icv saline infusion (0.6 ± 0.2 g; $P < 0.01$ vs. both 5-μg and 10-μg doses; Fig. 1). In day 4 sham-resected rats, the feeding response to 10 μg of NPY was significantly less than that produced by the 5-μg infusion, suggesting that maximal orexigenic stimulation by NPY occurs at the 5-μg dose in sham-resected rats.

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Table 1. Twenty-four hour food intake in bile duct-resected and sham-resected rats

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Day 2</th>
<th>Day 4</th>
<th>Day 7</th>
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<tbody>
<tr>
<td>Sham</td>
<td>25.8 ± 1.3</td>
<td>22.5 ± 1.7</td>
<td>23.1 ± 1.2</td>
<td>22.0 ± 1.2</td>
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<tr>
<td>BDR</td>
<td>26.0 ± 0.9</td>
<td>18.0 ± 1.0*</td>
<td>21.3 ± 0.8</td>
<td>22.3 ± 1.3</td>
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Values (in g) are means ± SE (12–16 rats/group) for sham-resected (Sham) and bile duct-resected (BDR) rats at baseline and between days 1 and 2 (day 2), days 3 and 4 (day 4), and days 6 and 7 (day 7) after laparotomy. *$P < 0.05$ day 2 Sham vs. day 2 BDR.
In day 4 BDR rats, the 1-μg NPY dose also failed to significantly stimulate food intake (1 μg NPY, 0.67 ± 0.32 g; saline, 0.47 ± 0.24 g). The higher doses of NPY did produce small but significant increases in food intake in day 4 BDR rats (5 μg NPY, 3.9 ± 0.98 g; 10 μg NPY, 2.4 ± 0.56 g; both P < 0.05 vs. respective saline control; Fig. 1). Moreover, the feeding responses of day 4 BDR rats to all doses of NPY were significantly less than those observed for respective doses in sham-resected rats (Fig. 1).

In additional experiments, BDR and sham-resected rats were infused with NPY (5 μg/rat) or saline vehicle (as above) at postlaparotomy days 2 and 7 and 2-h food intake was determined. Saline-infused BDR and sham-resected rats had similar minimal 2-h food intakes at both day 2 and day 7 (Fig. 2). Moreover, similar to results obtained at postlaparotomy day 4, day 2 and day 7 BDR rats exhibited a significantly attenuated NPY-induced feeding response compared with respective sham-resected controls (Fig. 2). Two-hour food intake in saline-infused BDR and sham-resected rats was similar at postlaparotomy days 2 and 7. This finding is not surprising given that these rats were studied in the morning after the nocturnal feeding period.

Determination of gastric emptying. To determine whether differences in the degree of gastric distension after feeding might explain the markedly different responses to NPY observed in sham-resected and BDR rats, we assessed gastric emptying in these two groups. The degree of gastric emptying measured 1 h after gavage feeding of a high-fat liquid test meal was not significantly different in the day 2 (anorectic) and day 4 (nonanorectic) BDR rats compared with sham-operated controls (day 2: sham 39.8 ± 4.1% vs. BDR 42.1 ± 4.8%; day 4: sham 42.5 ± 3.1% vs. BDR 54.0 ± 5.9%; n = 6/group).

Hypothalamic NPY receptor binding. Characterization of specific NPY binding in hypothalamic membrane preparations from day 4 animals did not reveal any significant difference in $K_d$ or $B_{max}$ between BDR and sham-resected rats (Fig. 3).

NPY-induced hypothalamic Fos expression. We used immunohistochemical methods to measure cellular expression of Fos protein as a marker of neuronal activation (36). In sham-resected rats, there was a striking increase in Fos-like immunoreactivity in the PVN after administration of NPY, compared with saline-treated controls, which showed very little Fos expression (Fig. 4). In BDR rats, the number of cell bodies in the PVN expressing Fos-like immunoreactivity after NPY infu-
that were not significantly different between the 2 groups.

K yielded values for binding affinity ($K_d$) and maximal binding ($B_{max}$) that were not significantly different between the 2 groups.

DISCUSSION

We have demonstrated that rats with biliary obstruction exhibit significantly diminished responsiveness to the orexigenic action of NPY. Furthermore, this defect in NPY-induced feeding occurs early after biliary obstruction (when BDR rats exhibit a reduction in food intake compared with sham-resected animals) and persists until at least day 7 after the onset of biliary obstruction, a time when BDR and sham-resected rats have similar daily food intake. This resolution of the decreased food intake in BDR rats occurs despite the persistence of a consistent attenuation in NPY-induced feeding in the BDR animals. This defect in the NPY-related orexigenic drive may contribute to the decreased food intake in BDR rats early after biliary obstruction, but it appears to be compensated for at later time points after bile duct obstruction via some as yet unidentified pathway(s). Moreover, despite the presence of attenuated NPY-induced feeding in BDR rats, central NPY-related neuronal pathways appear to be intact.

Unresponsiveness to central NPY infusion has been described in other experimental models of disease that are associated with anorexia. For example, in tumor-bearing rats, decreased feeding responses to intrahypothalamic injection of NPY were noted to precede and correspond with the development of anorexia (8). Turner et al. (40) described a state of unresponsiveness to the feeding effects of NPY in a model of profound anorexia induced by cobalt protoporphyrin, a synthetic heme analog. In this model, the observation that hypothalamic NPY mRNA levels are significantly increased also suggests unresponsiveness to an appropriate endogenous drive to feed (14). Together, these observations suggest that decreased food intake in disease states, despite the underlying cause, may be caused by a critical disruption of the neuronal pathways by which NPY normally stimulates feeding.

Dramatic changes in membrane fluidity have been reported in obstructive cholestasis that may contribute to impaired function of receptors that are sensitive to compositional changes in the plasma membrane (18, 23). If such alterations extend to neurotransmitter receptor function in the central nervous system, it seems plausible that impaired NPY receptor function caused by altered membrane properties might explain the decreased responsiveness of cholestatic rats to the feeding effect of NPY. However, our measurements of specific NPY binding at hypothalamic receptors suggest that there were no significant changes in NPY receptor affinity or density in BDR rats.

We next examined whether some impairment of NPY signaling occurs distal to synaptic activation of NPY receptors, that is, in the second messenger signaling systems that couple NPY receptor occupancy to neuronal activation. Immunohistochemical identification of Fos protein expression is a valid and reproducible marker of neuronal activation (31, 36). This method has been used to trace the neuronal pathways activated by icv infusion of NPY. In agreement with microinjection experiments, Fos immunohistochemistry identified the hypothalamic PVN as a critical target of NPY in its effect on feeding (21). Our experiments demonstrated robust Fos-like immunoreactivity in the PVN in response to central administration of NPY, whereas saline infusion evoked essentially no Fos-like immunoreactivity. Fos expression after NPY infusion encompassed both the parvo- and magnocellular divisions of the PVN. These experiments were performed in fasted animals. Although fasting increases endogenous release of NPY, fasting has been shown by others not to affect Fos expression in the PVN in response to exogenous NPY administration (21). Of key importance was the finding that NPY-induced Fos protein expression in BDR rats was equivalent to that in sham-operated control rats. These data offer further evidence that hypothalamic NPY receptor function is intact in BDR rats and, in particular, that it is intact in the hypothalamic region that is critically important in mediating the orexigenic effect of NPY (9). Moreover, these studies suggest that second messenger systems coupled to the NPY receptor are functional in anorectic BDR rats as well as subsequent cellular events involved in neuronal activation in response to NPY.

Various cytokines are known to produce anorexia in experimental animals, and much work has been done to discover the specific effects of such cytokines on central mechanisms that normally drive feeding (29). Because immunological activation is a feature of a wide variety of acute and chronic diseases (e.g., neoplastic, infectious, inflammatory), central action of cytokines may underlie the anorexia that occurs in such conditions. Focusing on obstructive cholestasis, increased levels of endotoxin and various proinflammatory cytokines were demonstrated in peripheral blood from patients with biliary obstruction (38) as well as in animal
models (4, 16, 27). As a consequence of systemic endotoxemia in obstructive cholestasis, cytokines such as tumor necrosis factor-α (TNF-α) and interleukin (IL)-6 (4, 27) are released by reticuloendothelial cells and appear in increased amounts in the systemic circulation. Moreover, in cholestasis macrophages appear to be inherently more responsive to endotoxin-induced release of TNF-α (27.38). In our model of obstructive cholestasis, TNF-α levels are known to be increased by approximately twofold in day 4 BDR rats compared with sham-resected rats (37), whereas plasma IL-1β levels are not significantly increased in BDR rats at this time point (M. G. Swain, unpublished observations). TNF-α profoundly suppresses food intake at the level of the central nervous system (13), although it is presently unclear what specific effect it has on the hypothalamic NPY system. Interestingly, anorexia observed in endotoxemic sheep also appears to be related to a downregulation of the NPY-mediated feeding response, although the mechanism for this has not been elucidated (26). One possible mechanism could involve an endotoxin-related reduction in hypothalamic NPY levels. This does not appear to occur in cholestatic rats because BDR and sham rats have similar hypothalamic NPY levels (M. G. Swain, unpublished observations). It is reasonable to suggest that increased serum endotoxin levels in obstructive cholestasis, acting through secondary mediators such as TNF-α, may play an important role in the anorexia observed in this condition, and this warrants further investigation. However, the fact that administration of neutralizing anti-TNF-α antibodies only partially reverses experimentally induced anorexia (32) suggests that TNF-α is not the only systemic factor that suppresses food intake during disease.

Circulating levels of endogenous glucocorticoids are elevated in rats with obstructive cholestasis (35). Moreover, endogenous glucocorticoids can modulate NPY gene expression (30) and NPY-induced feeding (1, 39). However, it appears that in the rat endogenous glucocorticoids potentiate NPY-induced feeding (1, 39). Therefore, elevated circulating glucocorticoid levels in BDR rats do not appear to be responsible for the observed defect in NPY-induced feeding.

As discussed above, anorexia may be caused by diminished drive to feed, but equal consideration must be
paid to increased satiety signals as a cause of anorexia. We were unable to demonstrate any defect in the response to NPY at the neuronal level in hypothalamic sites key to its effects on food intake. Therefore, it was important in our studies to address the possibility that simple delay of gastric emptying in BDR rats might have served as a signal of peripheral satiety that inhibited an otherwise normal feeding response to NPY. Although our experiments showed that gastric emptying in BDR rats demonstrating decreased food intake (day 2) and those exhibiting normal food intake (day 4) is similar to that in sham-resected controls, the possibility remains that an equivalent degree of gastric distension in BDR rats may produce a greater satiety signal than in sham-resected rats. That is, the decreased food intake in obstructive cholestasis may be caused by enhancement of peripheral satiety signals generated by gastric distension. Cholecystokinin mediates an important satiety signal generated in response to gastric distension (33). The potential role of peripheral satiety signals in decreased food intake in obstructive cholestasis must be the focus of future studies.

In summary, we have shown decreased responsiveness to the orexigenic effect of centrally administered NPY in BDR rats. This does not appear to be caused by impaired neuronal signaling of NPY’s effects at the level of the PVN. Because the known elements of the central NPY-induced feeding drive appear to be intact, this finding implies the existence of pathways within or outside the central nervous system that actively inhibit the NPY-induced drive to feed in rats with obstructive cholestasis. Given that NPY is one of the most powerful orexigenic signals yet identified, this inhibition of NPY-induced feeding likely contributes to the decreased food intake observed in rats with early biliary obstruction, but adaptations appear to occur that compensate for the NPY-related orexigenic defect in the setting of biliary obstruction of longer duration.

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