Neuropeptide Y induces fasted pattern of duodenal motility via Y_2 receptors in conscious fed rats

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Fujiminya, Mineko, Etsuro Itoh, Naoki Kihara, Ikuo Yamamoto, Masaki Fujimura, and Akio Inui. Neuropeptide Y (NPY) induces fasted pattern of duodenal motility via Y_2 receptors in conscious fed rats. Am. J. Physiol. Gastrointest. Liver Physiol. 278: G32–G38, 2000.—Neuropeptide Y (NPY), a 36-amino acid peptide abundantly expressed in the brain, has been implicated in the regulation of feeding and visceral functions. The present study was designed to investigate whether or not NPY specifically regulates duodenal motility. The manometric method was used to measure duodenal motility in conscious, freely moving rats. The rat duodenum showed phasic contractions mimicking the migrating motor complex in the fasted state that were replaced by irregular contractions after the ingestion of food. NPY powerfully affected the contractile activity after intracerebroventricular (icv) administration, changing fed (postprandial) patterns into phasic contractions characterized as fasted (interdigestive) patterns. This effect was mediated via receptors with pharmacological profiles similar to that of Y_2 and Y_4 receptors, although neither Y_1 nor Y_5 agonists had any effects on motility despite potent feeding-stimulatory effects. Immunoneutralization with anti-NPY antiserum administered icv abolished fasted patterns and induced fed-like motor activities. An icv dose of peptide YY produced a different effect from NPY, with increase in the motor activities of both fed and fasted patterns. These results indicate that fasted and fed motor activities are regulated processes and that NPY induces fasted activity through Y_2 and possibly Y_4 receptors, which may represent an integrated mechanism linked to the onset of feeding behavior.

intracerebroventricular neuropeptide Y receptors; peptide YY; pancreatic polypeptide

It is widely accepted that gastrointestinal motor activity consists of two major contractile patterns, digestive (fed) and interdigestive (fasted) (13, 20). These contractile activities have been examined mostly in conscious dogs (13). Motilin, a gastrointestinal hormone, is considered to be an indispensable humoral agent to induce interdigestive phasic contractions in the stomach and duodenum (13). However, detailed studies on the control mechanism of digestive and interdigestive motor activities in rodents are somewhat hampered by the methodological difficulty in directly measuring the contractile activity and/or by the use of anesthesia. Furthermore, previous studies suggest that intracerebroventricular (icv) injection of motilin does not induce interdigestive phasic contractions in dogs (6) and rodents (1), despite anxiolytic and feeding-stimulatory effects of the peptide (1). Therefore, no candidates for the brain peptides regulating the interdigestive motor activity of intestine have been found.

Neuropeptide Y (NPY) is a potent feeding-stimulatory peptide that expresses in the arcuate nucleus of the hypothalamus and projects predominantly to the paraventricular nucleus (9, 18). There is evidence that elevation of NPY level in the paraventricular nucleus at the beginning of the dark period is associated with food intake in rodents (8). It is expected that feeding-stimulatory peptides may exert coordinated control of the gastrointestinal functions to prepare for the occurrence of digestion. In fact, it has been reported that gastric and pancreatic secretions were elevated by the icv injection of NPY (5). Therefore, it can be expected that feeding-stimulatory peptides such as NPY, peptide YY (PYY), and pancreatic polypeptide (PP) may induce the digestive pattern of intestinal motility.

NPY, PYY, and PP are structurally and functionally related peptides (9). NPY activates at least six receptor subtypes, Y_1 through Y_6, and NPY analogs and PYY and PP exhibit varying degrees of affinity to these Y receptors (9, 16). The Y_1 receptor binds NPY, PYY, [Leu^{31},Pro^{34}]NPY-NPY-(2—36), PYY-(3—36), NPY-(13—36), PP; Y_2 binds NPY, PYY, NPY-(2—36), PYY-(3—36), NPY-(13—36),PP;Y_5 binds NPY, PYY, NPY-(2—36), PYY-(3—36), human/bovine PP.-NPY-(13—36). The Y_6 receptor is absent in rats and is likely a pseudogene in primates. Except for the Y_3 receptor, molecular cloning of cDNAs encoding corresponding receptor proteins has been carried out.

Because very few previous studies have examined the effects of centrally administered NPY-related peptides on intestinal motility, we investigated the role of NPY in the control of duodenal motility and its receptor mechanisms using a variety of NPY analogs. Conscious, freely moving rats were used to examine the...
effects on physiological fed and fasted patterns of motility.

MATERIALS AND METHODS

Male Wistar rats weighing 180–250 g at the initial period of the experiment were used. Care of animals was conducted in accordance with the Guide to the Care and Use of Experimental Animals (Shiga University of Medical Science). Rats were housed under controlled temperature (21–24°C) and light (lights on 0800–2000) conditions.

The icv injection of peptides. The rats were anesthetized with intraperitoneal injection of pentobarbital sodium (50 mg/kg Nembutal; Abbott Laboratories), placed in a stereotaxic apparatus, and implanted with a guide cannula (25-gauge, Eicom) that reached the right lateral ventricle. Stereotaxic coordination was 0.8 mm posterior to bregma, 2.0 mm right lateral to the midline, and 5.0 mm below the outer surface of the skull using a Kopf stereotaxic frame (Shiga University of Medical Science). Rats in accordance with the Guide to the Care and Use of Experimental Animals (Shiga University of Medical Science). Rats were used. Care of animals was conducted in accordance with the Guide to the Care and Use of Experimental Animals (Shiga University of Medical Science).

The following peptides were used: 1 µg and 10 µg human and rat NPY (Peptide Institute, Osaka, Japan), 1 µg and 10 µg porcine and rat PPY (Peptide Institute), 10 µg rat PP (Peninsula Laboratories), 10 µg bovine PP (Eli Lilly), 10 µg porcine [Leu31,Pro34]NPY (Peptide Institute), 7 µg porcine NPY-(13–36) (Peptide Institute), 10 µg porcine NPY-(2–36) (Peninsula Laboratories), and 10 µg human PYY-(3–36) (Peninsula Laboratories). Each chemical was dissolved with 10 µl saline and administered icv through the guide cannula anchored in the skull. Vehicle control was made by saline icv injection.

Measurement of duodenal motility. At 5–7 days after the brain operation, animals were fasted 18 h before operation. They were anesthetized with pentobarbital sodium (50 mg/kg), and the motility recording device was implanted as follows. A manometric catheter (3-Fr; ATOM, Tokyo, Japan) with a side hole was inserted through the gastric fistula, and the tip was placed 3 cm distal to the pylorus. The catheter was run subcutaneously to emerge at the crown of the neck. In some animals, a catheter (3-Fr; ATOM) was placed in the jugular vein instead of icv cannulation, run subcutaneously to emerge at the crown with the manometric catheter, and used for intravenous administration of peptides. During the first postoperative day, the animals were allowed water but no food, and the experiment was performed 1 wk after the operation. Animals were fasted 18 h before the experiment.

On the day of the experiment, the manometric catheter was connected to a pressure transducer (TP-400T; Nihon Kohden Kogyo, Tokyo, Japan), and the catheter was protected from biting by a flexible metal sheath and connected to the infusion swivel (dual type, 20-gauge; Instech Laboratories, Plymouth Meeting, PA) to allow free movement. The catheter was continuously infused with bubble-free distilled water at a rate of 1 ml/h by a low-compliance capillary infusion system using a heavy-duty pump (CFV-3100; Nihon Koden). The data were recorded on a polygraph (RM-6100; Nihon Koden) and simultaneously digitized and stored in a MacLab system (MacLab/Be, AD Instruments; Power Macintosh 7100/80AV and Power Book 5300CS, Apple Computer).

After the sequence of typical interdigestive patterns of motility was observed, rats were given two pieces of laboratory chow pellets (8 g). The duodenal motility was ascertained to change into the fed pattern. The icv injection of each of the above peptides was then performed. In some experiments, icv injection was performed in animals that showed the interdigestive pattern of motility.

Truncal vagotomy and mechanical sympathectomy. Four days before the measurement of duodenal motility, either truncal vagotomy or mechanical sympathectomy was performed as follows. After incision of the abdominal wall, the lower part of the esophagus was exposed and the anterior and posterior branches of the vagal nerve were incised. For mechanical sympathectomy, the roots of the celiac and superior mesenteric arteries were exposed and prevertebral ganglia between these arteries were completely removed.

Immuneutralization of intrinsic NPY. After the sequence of interdigestive patterns was observed, 5 µl of anti-NPY antiserum or normal rabbit serum plus 5 µl of saline was injected icv and the change in duodenal motility was observed. The specificity of the antiserum has been described in our previous study (10). Cross-reactivities of the antibody were tested against growth hormone-releasing factor, vasoactive intestinal peptide, peptide histidine isoleucine, luteinising hormone-releasing hormone, somatostatin, human PP, and bovine PP (10).

Analysis of motor activity and statistical analysis. To examine the motor activity of conscious rat duodenum induced by icv or intravenous drug infusion, more than three animals were used for each experiment and one animal was used for each treatment group. The frequency of interdigestive activity was obtained from the average of onset of phase III-like activities per hour (see Fig. 2A) and that of postprandial activity was obtained from the average of onset of spike waves per minute (see Fig. 2B). Because amplitude of pressure waves changes considerably between animals, the drug effects were evaluated by changes in the motor index before and after drug administration. Motor index (cmH2O/min) was defined as the summation of amplitude of contractions per minute (26), and mean motor index for 30 min before and after drug infusion was compared in each experiment. Results were expressed as means ± SD. Comparisons were made with the Student’s t-test. A value of P < 0.05 was considered statistically significant.

RESULTS

In the fasted state, the cyclic changes of pressure waves were detected in the duodenum by a manometric method, including the quiescence period during which relatively low amplitude contractions occur (phase I-like contraction), followed by a grouping of strong contractions (phase III-like contractions). Frequency of the onset of phase III-like activity was 5.6 ± 1.3/h (n = 14), which is in accordance with the migrating motor complex (20). After food intake, the fasted (interdigestive) motor pattern was disrupted and replaced by a fed (postprandial) motor pattern with irregular contractions of high frequency (1.8 ± 0.6/min, n = 10) (Fig. 1 and Fig. 2B). Such a fed pattern of motility continued for 119.0 ± 30.9 min (n = 5) after the ingestion of 8 g of chow and then changed into the interdigestive pattern (Fig. 1).
The icv injection of NPY (1–10 µg), given in the fed state, immediately changed the postprandial motility into pressure waves characterized as an interdigestive pattern consisting of consecutive phase I- and phase III-like activities (Fig. 3A) with frequencies of 5.1 ± 1.6/h (10 µg NPY, n = 3) and 5.6 ± 0.6/h (1 µg NPY, n = 3). In contrast, when icv injection of NPY was given in the fasted state, the interdigestive pattern of motility (5.8 ± 1.6/h, n = 3) observed before drug infusion was hardly affected by the drug infusion (6.5 ± 1.7/h, n = 3) (Fig. 3B). When saline was infused icv in the fed or fasted state, no change was observed in the motility pattern; the postprandial or interdigestive pattern persisted. To examine the receptor subtypes mediating this NPY action, the following analogs were used: [Leu31,Pro34]NPY as a Y1/Y5 agonist, NPY-(13—36) as a Y2 agonist, rat PP as a Y4 agonist, NPY-(2—36) and PYY-(3—36) as Y5 agonists, bovine PP as a Y5 agonist, and PYY as a Y1/Y2/Y4/Y5 agonist (9, 16). When NPY-(13—36) (Fig. 4B), rat PP (Fig. 4C), NPY-(2—36) (Fig. 4D) and PYY-(3—36) (data not shown) were injected icv, the postprandial pattern of motility was replaced by an interdigestive pattern of motility with frequencies of 5.8 ± 1.7/h [NPY-(13—36), n = 3], 4.8 ± 0.2/h (rat PP, n = 3), and 5.7 ± 0.9/h [NPY-(2—36), n = 3], respectively. However, neither [Leu31,Pro34]NPY (Fig. 4A) nor bovine PP (Fig. 4E) altered the postprandial motility.

When PYY (1–10 µg) was administered icv in the fed state, a different pattern of motility changes was observed compared with that of NPY. A 10-µg dose of PYY changed postprandial motility into irregular contraction waves with a high frequency (31.1 ± 3.4/min, n = 5) (Fig. 5A). The baseline of the pressure waves was elevated for 50.0 ± 15.5 min (n = 5) and returned to the basal level, and then the fasted pattern appeared (Fig. 5A) as seen in normal animals (Fig. 1). The motor index was significantly increased (647.6 ± 199.6 cmH2O/min, n = 5) compared with that before PYY injection (186.9 ± 83.7 cmH2O/min, n = 5). A 1-µg icv dose of PYY produced similar stimulation of the motor index (504.5 ± 200.5 cmH2O/min, n = 3) compared with that before drug administration (158.6 ± 76.8 cmH2O/min, n = 3) (Fig. 5A). When PYY was given icv in the fasted state, the motility pattern remained an interdigestive one (Fig. 5B); however, the motor index was significantly increased (761.9 ± 292.2 cmH2O/min, n = 3) compared with that before drug administration (285.3 ± 105.0 cmH2O/min, n = 3). To rule out the possible leakage of icv-injected peptides into the systemic circulation, duodenal motility was examined after intravenous injection of NPY (Fig. 6A) and PYY (Fig. 6B). Both NPY and PYY at the 10-µg intravenous dose elevated the baseline of the pressure waves for ~30 min with a higher frequency but did not induce a fasted pattern of motility in fed rats (Fig. 6).

We examined the involvement of the autonomic nervous system using rats that had received truncal vagotomy or sympathectomy. Because no postprandial pattern of motility was observed in vagotomized animals, we could not determine whether NPY effects are vagally mediated or not. However, vagotomy abolished PYY-induced changes in interdigestive motor activity shown in Fig. 5B, but no change was detected in the motor index before (309.8 ± 58.1 cmH2O/min, n = 5) or after (297.3 ± 110.4 cmH2O/min, n = 5) the drug administration (Fig. 7A). In contrast, sympathectomy failed to alter fasted and fed motor patterns, as well as NPY- and PYY-induced changes in duodenal motor activities. In sympathectomized animals, icv injection of PYY given in the fed state caused similar effects (Fig. 7B) to those seen in animals with intact autonomic nerves (Fig. 5A).

Finally, we examined the effect of passive immunoneutralization with NPY antiserum on the duodenal motor...
activity in fasted rats. The icv-administered antiserum against NPY (5 µl) abolished fasted patterns and induced fed-like motor activities (Fig. 8). This response occurred rapidly after injection of antiserum and continued for about 1 h, after which the fasted pattern appeared again. The icv injection of normal rabbit serum did not alter the pattern of duodenal motility (data not shown).

Fig. 3. A: effects of intracerebroventricular (icv) injection of 10 µg and 1 µg NPY on postprandial motility. Postprandial pattern is changed into interdigestive pattern immediately after NPY injection. B: effect of icv injection of 10 µg NPY on interdigestive pattern of motility. Pattern is not affected by NPY.

Fig. 4. Effects of icv injection of different analogs of NPY on postprandial motility. After injection of NPY-(13—36) (B), rat PP (C), and NPY-(2—36) (D), postprandial pattern is replaced by interdigestive pattern. However, [Leu^{31}, Pro^{34}]NPY (A) and bovine PP (E) do not alter postprandial pattern.
DISCUSSION

In the present in vivo conscious rat model using a manometric method, spontaneously occurring pressure waves were detected in the duodenum; such contraction waves changed from an interdigestive to a postprandial pattern after the ingestion of food. Those motility patterns were in accordance with the myoelectric activity recorded in the conscious rat small intestine (19). The icv injection of NPY induced the change of pressure waves from a postprandial to an interdigestive pattern, which was observed in the same dose range as in stimulating food intake in this species (9, 21). This effect was mediated via receptors with a pharmacological profile similar to the rat Y2 receptor, a major NPY receptor in the brain, and Y4 receptor, but neither Y1 nor Y5 agonists had any effect on motility despite the potent feeding-stimulatory effects (9). The effects of icv-injected peptides might be mediated by brain mechanisms but not the peripherally originated effects, because different effects were elicited from intravenous injection of peptides. Results from intravenous injection of NPY and PYY were quite consistent with the previous reports in anesthetized rat duodenum with intravenous injection of NPY-related peptides (23, 24). The present results indicate that the vagal pathway may mediate the effects of central administration of peptides on duodenal motility, which is consistent with the previous findings that gastric motility and secretion affected by icv injection of NPY may require intact vagal pathways (3–5).

NPY and its family peptides, PYY and PP, are emerging as potent central regulators of gastrointestinal functions (2, 3, 15, 23). However, the information is limited to the gastric functions, such as motility or acid secretion, and very few previous studies have examined the intestinal motility affected by the centrally administered NPY family. In previous studies, there has been a considerable debate concerning the mechanisms and even the direction of the effects mediated by these peptides. Both stimulatory and inhibitory effects of

Fig. 5. A: effects of icv injection of 10 µg and 1 µg PYY on postprandial motility. Postprandial pattern is changed into irregular contraction with high frequency and, with 10-µg dose, baseline of pressure waves is elevated. B: effect of icv injection of 10 µg PYY on interdigestive pattern of motility. PYY induces pressure waves of high frequency and amplitude with basic pattern remaining an interdigestive one.

Fig. 6. Effects of intravenous injection of NPY and PYY on postprandial motility. Both NPY (A, 10 µg) and PYY (B, 10 µg) induce elevation of baseline pressure waves.
NPY on gastric motility were observed when NPY was injected into the paraventricular nucleus of the hypothalamus (4) or the dorsal vagal complex (DVC) (3). Recently, on the basis of the use of [Leu31,Pro34]NPY and NPY-(13—36) microinjected into the DVC, the Y1 and Y2 receptors were proposed to induce a stimulating and an inhibitory effect, respectively, on gastric motility in anesthetized rats (3). The central action of NPY on gastric functions may thus vary with the brain sites of injection, receptor subtypes involved, gastric parameters, and experimental conditions investigated.

Because NPY is a feeding-stimulatory peptide, it might be expected that it would induce a digestive pattern of duodenal motility. However, contrary to the expectation, centrally administered NPY elicited the interdigestive pattern of motility. The physiological significance of interdigestive phasic contractions in the gastrointestinal tract has been considered to be a mechanical and chemical cleansing of the empty stomach and intestine in preparation for the next meal (13) and therefore possibly linked to hunger sensations. From this point of view, it is conceivable that interdigestive patterns induced by icv injection of NPY might be tightly correlated with the onset of feeding and lead to a vicious cycle of hyperphagia. This hypothesis is supported by the previous studies demonstrating that cholecystokinin (17) and bombesin (7), potent feeding-inhibitory peptides, induce a digestive pattern of motility after icv injection in fasted dogs.

Because galanin and other feeding-stimulatory peptides do not have such a definite effect on gastrointestinal motor activities (unpublished observations), NPY in the brain may be one of the key brain peptides regulating interdigestive motor activity of intestine, which may represent an integrated mechanism linking the onset of feeding behavior. This was confirmed by the present immunoneutralization experiment, indicating that shortly after infusion of NPY antiserum the interdigestive pattern was replaced by the digestive pattern. The dose of the antibody was justified in our preliminary study, which demonstrated that the same dose of antibody completely blocks the hyperphagia induced by icv-injected NPY. Furthermore, it has been shown that the antibody level in the systemic circulation after 30 min of icv infusion is 2.4% of the corresponding antibody level of intravenous injection (22). Because the response in the present study emerged within 10 min after icv injection, the antibody might inhibit neuropeptide signaling in the brain but not in the peripheral tissue. Therefore, the results suggest that the endogenous NPY may be involved in the regulation of interdigestive motility of small intestine.

Of particular interest is the fact that PYY produced a qualitatively and quantitatively different pattern of motor effects from NPY. It was recently demonstrated that the stimulating effect of PYY on gastric acid secretion, when microinjected into the DVC of rats, may involve a PYY-preferring receptor subtype that is yet to be cloned (25). The present finding that PYY-(3—36) does not mimic the PYY effect indicates that NH2-terminal amino acids may be essential for recognizing the PYY-preferring receptor in the brain, as previously suggested (25).

Elevated levels of NPY and PYY in cerebrospinal fluid were reported in patients with anorexia nervosa and bulimia nervosa, respectively (14), both of which are frequently associated with gastrointestinal motor dysfunction (11). Assessment of the relative contributions of NPY receptor subtypes to the feeding behavioral and gastrointestinal pathology in these patients could lead to a novel therapeutic development.
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REFERENCES


