Neuromedin U acts in the central nervous system to inhibit gastric acid secretion via CRH system

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Mondal, Muhtashan S., Yukari Date, Noboru Murakami, Koji Toshinai, Takuya Shimbara, Kenji Kangawa, and Masamitsu Nakazato. Neuromedin U (NMU) acts in the central nervous system to inhibit gastric acid secretion via CRH system. Am J Physiol Gastrointest Liver Physiol 284: G963–G969, 2003. First published February 12, 2003; 10.1152/ajpgi.00218.2002.—Neuromedin U (NMU) is a hypothalamic peptide involved in energy homeostasis and stress responses. NMU, when administered intracerebroventricularly, decreases food intake and body weight while increasing body temperature and heat production. In addition, NMU, acting via the corticotropin-releasing hormone (CRH) system, induces gross locomotor activity and stress responses. We studied the effect of intracerebroventricularly administered NMU (0.5–4 nmol) in the regulation of gastric functions in conscious rats. Intracerebroventricular administration of NMU significantly decreased gastric acid output to 30–60% and gastric emptying to 35–70% in a dose-dependent manner. Vagotomy did not abolish the inhibitory effect of NMU on pentagastrin-induced gastric acid secretion. Pretreatment with indomethacin (10 mg/kg), an inhibitor of prostaglandin synthesis, also did not affect NMU-induced acid inhibition. Pretreatment with anti-CRH IgG (1 μg/rat), however, completely blocked NMU-induced acid inhibition (P < 0.01). Administration of yohimbine (4 mg/kg), an α2-adrenergic receptor antagonist, also abolished NMU-induced acid inhibition (P < 0.01). These findings suggest that NMU is critical in the central regulation of gastric acid secretion via CRH.

hypothalamic peptide; gastric emptying; corticotropin-releasing hormone; central administration

NEUROMEDIN U (NMU), a 23-amino-acid peptide, was first purified from porcine spinal cord (28). This peptide was also later discovered in the brain, spinal cord, and intestine of other species (10, 31). NMU regulates blood pressure, ion transport in the gut, mesenteric blood flow, and adrenocortical function (5, 16, 27, 37). G protein-coupled receptors (GPCRs) without known cognate ligands, termed “orphan” receptors, have been used to identify novel signaling molecules, including neuropeptides. With the use of an intracellular calcium influx assay in cells expressing orphan GPCRs, FM-3 (also called GPR66) and FM-4 were identified as the endogenous receptors for NMU (15, 20, 24, 38). FM-3, designated NMU1R, is abundant in rat peripheral tissues, including the small intestine and lung with low expression in the brain (20). In contrast, FM-4, designated NMU2R, is restricted to the brain, including the hypothalamic paraventricular nucleus (PVN), the wall of the third ventricle in the hypothalamus, and the CA1 region of the hippocampus (20). Because NMU is ubiquitously expressed in the systemic tissues, these two receptors may mediate different functions of NMU (3). In the brain, NMU-producing neurons are restricted to the hypothalamus and caudal brain stem areas implicated in feeding regulation (3, 20). NMU suppresses dark (fasted)-phase food intake and fasting-induced feeding in rats when administered intracerebroventricularly (20, 24, 29). In addition, fasting downregulates NMU mRNA expression in the hypothalamic arcuate nucleus (20). These results indicate that NMU is a potent anorectic peptide. Hypothalamic peptides that influence feeding behavior, including CRH (39), TRH (41, 42), orexin-A (43), cocaine- and amphetamine-regulated transcript (CART) (33), and ghrelin (2, 9), contribute to the neural regulation of gastric acid secretion and gastric emptying. NMU implicated in feeding behavior thus may act to regulate gastric functions in the brain.

Here, we examined a role for NMU in the regulation of gastric function through measurements of gastric acid secretion and gastric emptying after intracerebroventricular administration of NMU to conscious rats. We also investigated the effect of vagotomy on the inhibition of gastric acid secretion by NMU. We examined the participation of the prostaglandin pathway in NMU-induced acid inhibition using indomethacin, an inhibitor of prostaglandin synthesis. We previously reported that NMU acts via CRH to induce stress responses, such as gross locomotor activity, face washing, and grooming (19). In rats, CRH regulates not only food intake and stress responses but also gastric secretion through the central adrenergic nervous system (4, 39). Finally, to investigate the functional relationship

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between NMU and CRH in the inhibition of gastric acid secretion, we examined the effect of NMU on acid secretion following pretreatment with anti-CRH IgG or α2-adrenergic receptor blocker yohimbine.

**MATERIALS AND METHODS**

**Animals**

Male Sprague-Dawley rats weighing 200–220 g (Charles River, Shiga, Japan) were housed in individual cages under controlled temperature (21–23°C) and light-dark (light on at 0700, off at 1900) conditions with ad libitum access to food and water. Before study, rats were implanted with intracerebroventricular cannulae in the lateral cerebral ventricle while under anesthesia by intraperitoneal injection of pentobarbital sodium (50 mg/kg) (21). Proper placement of the cannulae was verified at the end of the experiment by dye administration. Rats were sham injected before the study, then weighed and handled daily. Only animals demonstrating progressive weight gain after surgery were used for subsequent experiments. All procedures were performed in accordance with the Japanese Physiological Society and international guidelines for animal care.

**Measurement of Gastric Acid Secretion**

All experiments were performed using conscious rats, deprived of food for 24 h but given free access to water until the initiation of experimentation. Gastric acid secretion was measured using the pylorus-ligation method described previously (9). After light anesthesia with diethyl ether, rats were subjected to the occlusion of the pyloric portion of the stomach with a 4-0 silk ligature. Rat NMU (0.5, 1, and 2 nmol/10 μl saline) or saline was administered intracerebroventricularly to rats at 0900 (n = 10 per group). Either rat NMU (2 and 4 nmol/100 μl saline) or saline was administered intraperitoneally to additional groups of rats (n = 10 per group) at 0900. Rats were killed by an overdose of diethyl ether, their stomachs were quickly ligated at the esophagus-gastric junction and the pylorus and then removed. The stomachs and their contents were homogenized in 40 ml 0.1 N NaOH. The suspension was allowed to settle for 1 h at room temperature, and 5 ml of the supernatant were added to 0.5 ml of 20% trichloracetic acid and centrifuged at 3,000 rpm at 4°C for 20 min. The supernatant was mixed with 4 ml 0.5 N NaOH and the absorbance of the sample was measured at 560 nm. The absorbance of phenol red collected from stomachs (n = 8) immediately after the liquid meal intubation was used as standard (0% emptying). Gastric emptying (%) was calculated according to the following formula: (1 – absorbance of sample/absorbance of standard) × 100 (30).

**Substances and Chemicals**

Rat NMU was purchased from Peptide Institute (Osaka, Japan). Pentagastrin (Peninsula Laboratories, San Carlos, CA) was used to stimulate gastric acid output in vagotomized rats (32). Indomethacin (Sigma, St. Louis, MO) was used to suppress prostaglandin synthesis. Anti-CRH IgG was purified from anti-rat CRH rabbit antiserum (Peptide Institute) by Affi-Gel Protein A affinity chromatography (Bio-Rad, Hercules, CA), PD-10 gel permeation chromatography (Amersham Pharmacia, Uppsala, Sweden), and CNBr-Sepharose (Bio-Rad)-coupled CRH affinity chromatography. The amount of IgG purified was determined using a DC protein assay kit (Bio-Rad). Yohimbine hydrochloride (Sigma) was used to
block the central α2-adrenergic receptor. Other substances were purchased from Nacalai Tesque (Osaka, Japan).

**Statistical Analysis**

Data are expressed as means ± SE. Statistical analysis was performed using the unpaired Student’s t-test. Differences were considered significant when the P value was <0.05. The intracerebroventricular doses of NMU inhibiting gastric acid secretion and gastric emptying by 50% (ED50) were determined using nonlinear regression to sigmoidal equation with variable slope (StatView, Berkley, CA).

**RESULTS**

**Gastric Acid Secretion in Response to NMU Administration**

Intracerebroventricular administration of NMU to rats reduced the volume of gastric secretion and gastric acid output in a dose-dependent manner (Fig. 1). Gastric secretory volume and gastric acid output following treatment with NMU (0.5, 1, and 2 nmol/10 μl) were reduced to 79, 68, and 50% and to 60, 49, and 30% of normal levels, respectively. In contrast, NMU (2 or 4 nmol/100 μl) given intraperitoneally did not reduce either the volume of gastric secretion or gastric acid output (Table 1). The ED50 of NMU to suppress gastric acid output was 0.5 nmol.

**Effects of Pentagastrin, Vagotomy, Indomethacin, Anti-CRH IgG, and Yohimbine on NMU-Induced Inhibition of Gastric Acid Secretion**

The gastric acid output in the control group with administration of pentagastrin was increased 1.3-fold compared with that in the control group without administration of pentagastrin (P < 0.05; Fig. 2). NMU administration significantly reduced the gastric acid output in pentagastrin-treated rats without vagotomy (Fig. 2). The gastric acid secretion in vagotomized rats was greatly reduced from levels observed in sham-operated rats (Figs. 2 and 3). We thus could not observe reductions in gastric acid secretion by NMU administration in the vagotomized rats (Fig. 3). After pretreatment of vagotomized rats with pentagastrin to increase the basic gastric acid secretion, NMU administration significantly reduced gastric acid output (Fig. 3). NMU also significantly inhibited gastric acid output in rats pretreated with indomethacin (Fig. 4). NMU significantly reduced gastric acid output in rats pretreated with control IgG but could not influence gastric acid output in rats pretreated with anti-CRH IgG (Fig. 5). Yohimbine did not alter acid output by itself but blocked the acid inhibitory action of NMU (Fig. 6).

**Gastric Emptying**

We examined the effect of intracerebroventricular administration of NMU on gastric emptying. NMU significantly delayed the rate of gastric emptying in a dose-dependent manner compared with saline (Fig. 7). Gastric emptying following treatment with NMU (2 and 4 nmol/10 μl) was reduced to 76 and 35% of normal levels. The ED50 of NMU to suppress gastric emptying

**Table 1. Effect of intraperitoneal injection of NMU on gastric secretion volume or acid output in pylorus-ligated conscious rats**

<table>
<thead>
<tr>
<th></th>
<th>Volume, ml/1.5 h</th>
<th>Acid Output, mEq/1.5 h</th>
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<tbody>
<tr>
<td>Saline</td>
<td>4.3 ± 0.5</td>
<td>299 ± 37</td>
</tr>
<tr>
<td>NMU (2 nmol)</td>
<td>4.8 ± 0.6</td>
<td>231 ± 27</td>
</tr>
<tr>
<td>NMU (4 nmol)</td>
<td>5.4 ± 0.5</td>
<td>299 ± 37</td>
</tr>
</tbody>
</table>

Values are the means ± SE; n = 10 per group. NMU, neuromedin U.
was 2 nmol. NMU significantly delayed gastric emptying even in rats pretreated with anti-CRH IgG (data not shown).

**DISCUSSION**

This study demonstrated that centrally administered NMU inhibited gastric acid secretion and gastric emptying. Peripherally administered NMU did not affect gastric acid secretion, indicating that its regulation of acid secretion occurs in the central nervous system. Gastric acid secretion is regulated by both central and peripheral pathways, which involve endocrine, neuroendocrine, and paracrine pathways mediated by peptidergic, chemical, and mechanical factors. The central pathway is activated by sight, smell, taste, and thought of food (13, 22). The final integration of these central stimuli occurs in the dorsomotor nucleus of the vagus in the medulla, which supplies parasympathetic efferent fibers to the stomach (14, 18). TRH, orexin A, and ghrelin, which are appetite-regulating peptides produced in the hypothalamus, stimulate gastric acid secretion through the vagus system (9, 42, 43). To investigate the role of the vagus nerve in NMU-induced inhibition of gastric acid secretion, we examined the action of NMU in vagotomized rats. Because vagotomy itself inhibits gastric acid secretion, pentagastrin was administered to increase the baseline of acid secretion (26, 32). NMU significantly suppressed the pentagastrin-induced acid secretion in the vagotomized rats, suggesting that the vagus nerve is not involved in NMU-induced inhibition of gastric acid secretion.

Prostaglandin E2 reduces gastric acid secretion directly by inhibiting parietal cell secretion and indirectly by inhibiting gastrin release (36). Peripheral administration of indomethacin negates the inhibitory action of neuropeptides and cytokines, including dermorphin, calcitonin gene-related peptide, and interleukin-1, on gastric acid secretion (17). To clarify the relationship between NMU and prostaglandins, we examined the effect of NMU on gastric acid secretion in rats pretreated with indomethacin. The dosage of in-
domethacin (10 mg/kg) used in this experiment suppresses prostaglandin synthesis both in the brain and stomach (17). Intracerebroventricular administration of NMU significantly suppressed acid secretion in indomethacin-treated rats, indicating that NMU inhibits gastric acid secretion independent of the prostaglandin pathway.

CRH influences a variety of behaviors, including feeding and stress responses. CRH administration decreases food intake and increases sympathetic output (1, 6), thereby creating a state of negative energy balance. NMU neurons are primarily located in the arcuate nucleus of the hypothalamus, a critical region in the control of food intake. NMU-containing fibers project to the PVN, where NMU2R mRNA is highly expressed (3, 10, 20). Central NMU thus may play some roles through PVN neurons. Pretreatment with anti-CRH IgG or a CRH antagonist α-helical CRH suppressed NMU-induced stress responses (19). Furthermore, NMU did not induce locomotor activity in CRH knockout mice (19). These results imply that NMU interacts anatomically and/or functionally with the CRH pathway. We here examined the functional relationship between NMU and CRH by using anti-CRH IgG. NMU-induced inhibition of gastric acid secretion was blocked by pretreatment with anti-CRH IgG, suggesting that NMU-induced acid inhibition is mediated by CRH. The NMU-induced inhibition of acid does not depend on the vagal system. CRH inhibits gastric acid secretion by activation of the sympathetic, noradrenergic nervous system and not by vagal fibers (11). This finding may further support our speculation that CRH mediates the NMU-induced acid inhibition through a vagus-independent pathway.

Noradrenaline has been shown to act centrally to inhibit gastric acid secretion through the central α2-adrenergic and not α1- or β-adrenergic receptors (34). Blockade of α2-adrenergic receptors with selective antagonists such as yohimbine is known to stimulate sympathetic nervous activity (4). Yohimbine acts in the brain to block α2-adrenergic receptor-mediated inhibition of gastric acid output when administered peripherally (4). NMU-induced inhibition of gastric acid secretion was abolished by yohimbine, indicating that NMU may be involved in the regulation of acid secretion through the sympathetic nervous system.

Gastric emptying plays an important role in regulating food intake. Previous studies (12, 23) have shown that gastric distension acts as a satiety signal to inhibit food intake and rapid gastric emptying is closely related to overeating and obesity. Some anorectic peptides, including CRH, cholecystokinin, CART, and urocortin, have been shown to delay gastric emptying when they are administered to rats (7, 33, 35, 40). As expected, centrally administered NMU significantly decreased the gastric emptying rate in a dose-dependent manner. As mentioned earlier, NMU inhibits acid secretion through the CRH pathway. CRH has been known to influence gastric emptying through the parasympathetic nervous system (40), giving speculation that the delayed gastric emptying induced by NMU may be caused through the CRH pathway. However, and unexpectedly, centrally administered NMU also delayed gastric emptying in rats pretreated with anti-CRH IgG. This result suggests that NMU may delay gastric emptying independent of the CRH pathway. The ED50 of NMU to suppress food intake and gastric emptying were 1 and 2 nmol, respectively. Studying the mechanism of feeding behavior or delayed gastric emptying by NMU will lead to elucidation of the relationship between anorectic peptides and gastric function.

In summary, we showed that central NMU inhibited gastric acid secretion and gastric emptying in rats. Both NMU and CRH induce a state of negative energy balance that is partially a function of their anorectic effects on food intake (1, 20, 29) and partially a function of their activation of the autonomic nervous system (6, 8, 19, 25). NMU is also involved in the regulation of gastric functions via the CRH system. Further investigations under various physiological conditions will help elucidate new ways to clarify additional roles for NMU in the regulation of feeding behavior and energy homeostasis.

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