Stimulation of neurons in rat ARC inhibits gastric acid secretion via hypothalamic CRF1/2- and NPY-Y1 receptors

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Since the discovery of NPY in 1982, the most potent orexigenic neuropeptide known has been implicated in the control of many functions of the organisms by the brain. Among others, NPY plays a role in the CNS regulation of gastrointestinal function (12, 19, 40). NPY released in the PVN has been shown to inhibit gastric acid secretion. Recently, we have demonstrated that activation of ARC neurons inhibits gastric acid secretion via vagal pathways. To what extent NPY- and CRF-mediated mechanisms in the PVN contribute to the CNS modulation of gastric acid secretion is still an open question. In the present study, we performed consecutive bilateral microinjections of antagonists to NPY receptor subtypes Y1 and Y2 and to CRF1/2 receptors in the PVN and of the excitatory amino acid kainate in the ARC to assess the role of NPY- and CRF-mediated mechanisms in the kainate-induced effects on gastric acid secretion. Gastric acid secretion was measured at the basal condition and during pentagastrin (16 μg/kg body wt) stimulation. Microinjection of vehicle in the PVN and kainate in the ARC decreased gastric acid secretion. Microinjection of the specific NPY-Y1 receptor antagonist BIBP-3226 (200 pmol) in the PVN abolished the inhibitory effect of neuronal activation in the ARC by kainate on gastric acid secretion. The CRF antagonist astressin was more effective. Pretreatment with the NPY-Y2 receptor antagonist BIE-0246 (120 pmol) in the PVN had no significant effect. Our results indicate that activation of neurons in the ARC inhibits gastric acid secretion via CRF1/2 and NPY-Y1 receptor-mediated pathways in the PVN.

The central nervous system (CNS) control of the gastrointestinal tract has been investigated intensively in the last decade. Various experimental animal studies have shown that functions of the gastrointestinal tract, e.g., secretion and motility, are modulated by neuropeptides expressed in specific brain nuclei (10). Most of our knowledge as to which neuropeptides and neuropeptide receptors in the brain are involved in the CNS control of gastrointestinal function results from microinjection studies (13, 16, 25).

In particular, the paraventricular nucleus of the hypothalamus (PVN), playing a key role in the integration of efferent and afferent information in the complex interaction between the brain and the gut, has been shown to be involved in the CNS modulation of gastrointestinal function by neuropeptidergic mechanisms (1, 3, 28).

Neuroanatomical and electrophysiological studies suggest that the PVN is closely connected with the arcuate nucleus (ARC) by bidirectional monosynaptic neuronal projections (37). This so called ARC-PVN axis is involved in the neuroendocrine and autonomic control of various body functions (2, 20). PVN and ARC play a crucial role in the CNS regulation of, e.g., reproduction, food intake, and satiety, as well as energy homeostasis (2, 6). Neuropeptide Y (NPY)- and corticotropin-releasing factor (CRF)-expressing neurons, particularly abundant in the ARC and the PVN, appear to be involved in the physiological feedback control of these functions (2, 20, 22).

Since the discovery of NPY in 1982, the most potent orexigenic neuropeptide known has been implicated in the control of many functions of the organisms by the brain. Among others, NPY plays a role in the CNS regulation of gastrointestinal function (12, 19, 40). In particular, NPY released in the PVN has been proposed to exhibit bioactivity in the CNS control of gastrointestinal function (3, 16, 17, 28). NPY activates at least six receptor subtypes, NPY-Y1 to -Y6. NPY, NPY analogs, and other members of the pancreatic polypeptide family exhibit different affinities to these receptors. NPY binds preferentially to Y1 and Y2 receptors (with high affinity), and there is evidence suggesting...
that these two receptor subtypes are involved in CNS regulation of digestive function by NPY action in the ARC-PVN axis (5, 6, 9, 19, 21, 43).

There is further convincing evidence from several groups that endogenous CRF in the brain inhibits gastric acid secretion. Neuroanatomical and neurophysiological studies in rodents suggest a positive feedback loop mediated through CRF receptors in the PVN that is involved in the CNS regulation of gastrointestinal function (18, 30).

The vast majority of the NPY-positive neuronal fibers within the PVN originate from the ARC. Moreover, CRF-immunoreactive neurons in the PVN appear to be targets of NPY immunoreactive neuronal projections from the ARC. Conversely, there is discussion as to whether projections of CRF-immunoreactive neurons from the PVN affect activity of NPY-positive neurons in the ARC (3, 22). Recent morphological and physiological studies support comodulatory interactions between NPY and CRF in the CNS involving CRF1/2- and different NPY-receptor subtypes (12–14, 24).

In previous studies, we and others (6, 20, 36) have shown that apart from the PVN the ARC is involved in the autonomic regulation of gastrointestinal function. We have proposed that NPY-positive neurons projecting from the ARC to the PVN mediate the effects of ARC neuronal activation-induced inhibition of gastric acid secretion (36). However, it is still unknown whether NPY and CRF in the ARC-PVN axis are involved in the CNS control of digestive function, besides regulation of satiety and energy homeostasis.

Therefore, in the present study, the role of the ARC-PVN axis and of hypothalamic NPY and CRF pathways in CNS regulation of gastric acid secretion were investigated in more detail.

It has been shown before that low-dose kainate microinjected in brain nuclei exhibits an excitatory (not a cytotoxic) effect on neurons, inducing neuropeptide release in other brain areas via neuronal projections (38). Kainate receptors are located in many brain nuclei, particularly in hypothalamic brain areas and, among others, in the ARC (38, 39).

Thus we performed experiments in an established in vivo animal model, where endogenous neuropeptide release in the brain was induced by microinjection of the excitatory amino acid (EAA) kainate in the ARC (36). The role of hypothalamic NPY and CRF pathways concerning CNS modulation of the ARC neuronal activation-induced inhibition of gastric acid secretion was investigated using antagonists to NPY-Y1, -Y2, and CRF1/2 receptors microinjected in the PVN.

Taken together, the aim of the present study was to investigate if CRF and NPY pathways in the ARC-PVN axis are involved in the modulation of gastric function by activation of ARC neurons.

MATERIALS AND METHODS

Animals

All experimental procedures were performed in accordance with the requirements of the German law for the protection of animals and were licensed and supervised by the appropriate government institutions.

Male Sprague-Dawley (SD) rats (strain of Deutsche tierversuchsanstalt, Hannover, Germany; distributed by Harlan Winkelmann, Borchen, Germany) with a mean body weight of 400 ± 50 g were maintained on a 12:12-h photoperiod, 30–35% humidity, and constant temperature (22 ± 2°C) for at least 7 days before the surgical procedure. Animals were housed in group cages with free access to food (standard rat diet; Altromin, Lage, Germany) and tap water. The animals were food but not water deprived 18 h before experiments.

Drugs

All drugs were dissolved immediately before use in sterile 0.15 M saline (Braun-Melsungen, Melsungen, Germany) or double-distilled water (assistenz; Baxter, Unterschleißheim, Germany) and then separated into aliquots and frozen (−30°C). The NPY-Y1 receptor antagonist BIBP-3226 (200 pmol; Sigma-RBI, Natick, MA; see Ref. 32), the NPY-Y2 receptor antagonist BIIE-0246 (120 pmol; Boehringer-Ingelheim, Biberach, Germany; see Ref. 8), and the CRF1/2 receptor antagonist astressin (30 pmol; Polypeptide Laboratories, Wolfenbüttel, Germany) were used in similar equipotent picomolar concentrations. The EAA kainate (Sigma, St. Louis, MO) was used in a concentration of 60 pmol/100 nl.

The control groups were treated with the respective vehicle. Fresh aliquots were thawed on each experimental day before injections.

Preparation of Gastric Fistula

In adult SD rats, a double-lumen polyethylene catheter was implanted acutely in the nonglandular part of the stomach under deep urethane anesthesia (1.25 g/kg; Sigma), as described previously (29, 36). The pylorus was closed permanently by means of a ligature. The gastric surgery was followed by a 30-min postoperative rest period before the start of the experiment.

Hypothalamic Microinjections

Hypothalamic microinjections were performed as previously described (29, 36). Briefly, at 50 min after the start of the experiment, the animals were positioned in a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA), the head was fixed in a nose-down position (~3 mm), and the skull was exposed. Next, two trepanations of the skullcap were performed according to microinjection coordinates obtained from Paxinos and Watson (31; mm from bregma: PVN, anterior-posterior = −1.8; lateral = ±0.2; dorsoventral = −8.0; ARC, anterior-posterior = −2.80; lateral = ±0.1; dorsoventral = −10.0) to facilitate subsequent microinjections in the PVN and ARC. Distilled water, 0.15 M sterile saline, BIBP-3226 (200 pmol/100 nl 0.15 M saline), BIIE-0246 (120 pmol/100 nl 0.15 M saline), or astressin (30 pmol/100 nl double-distilled water) was microinjected bilaterally in the PVN. At 15 min after microinjection of the receptor antagonists or vehicle in the PVN, kainate (60 pmol/100 nl of 0.15 M saline) or vehicle was microinjected bilaterally in the ARC. Bilateral microinjections were performed slowly over a 60-s period utilizing a single glass micropipette (30 μm diameter) that was connected to a 1-μl microsyringe (Hamilton, Reno, NV) by a PE-50 catheter filled with silicone oil. We used a silicon oil-filled microinjection system to be able to ensure a constant microinjection pressure and the complete microinfusion of the required drug volume. The micropipette was left in place for 2 min after injection to allow diffusion of the injected solution in the tissue surrounding the tip of the micropipette.
Experimental Procedure

The experiments were performed following the procedure described previously in detail (29, 36). Briefly, all experiments were started between 0830 and 0900. An intravenous indwelling catheter was placed in a femoral vein for infusion of saline and pentagastrin (PG). Starting 30 min after the end of the surgical procedure, animals were infused intravenously with 0.15 M saline at a rate of 0.06 ml/min (basal period). Gastric juice was collected every 10 min by flushing the stomach via the gastric cannula two times with 5 ml of physiological saline followed by 5 ml air. After collecting gastric juice at 50 min of the basal period, cerebral microinjections were performed as described above. Thereafter, the stomach was flushed, but this lavage was expunged. After gastrolavage, the stimulation of gastric acid secretion by PG infused at a dose of 16 μg·kg⁻¹·h⁻¹ was started (stimulation period). Subsequently, gastric acid secretion was determined every 10 min by gastrolavage for an observation period of 120 min. Acid secretion per 10 min was quantified by titration of the gastrolavages with 0.1 N NaOH to pH 7.0.

**Study I: effect of kainate microinjected in the ARC on PG-stimulated gastric acid secretion.** After the end of the basal period, cerebral microinjections were performed. At first, 0.15 M saline (100 nl/side) was microinjected bilaterally in the PVN, and 15 min later either vehicle (100 nl of 0.15 M saline or double-distilled water) or kainate (60 pmol·100 nl⁻¹·side⁻¹) was microinjected bilaterally in the ARC. After cerebral microinjections, the stimulation period was started by intravenous infusion of PG as described above.

**Study II: effect of pretreatment with NPY-Y₁, -Y₂, and CRF1/2 receptor antagonists in the PVN on kainate in ARC-induced inhibition of PG-stimulated gastric acid secretion.** In this study, specific Y receptor antagonists that are selective for the Y₁ (BIIE-3226, 200 pmol·100 nl⁻¹·side⁻¹) and the Y₂ receptor (BIIE-0246, 120 pmol·100 nl⁻¹·side⁻¹), or a specific nonselective CRF receptor antagonist (astressin, 30 pmol·100 nl⁻¹·side⁻¹), was microinjected bilaterally in the PVN. In previous microinjection studies, these dosages have been shown to effectively block cerebral CRF and NPY pathways (8, 25, 32). Subsequently, kainate (60 pmol·100 nl⁻¹·side⁻¹) was microinjected in the ARC (see above), and gastric acid secretion was quantified as described above (see Experimental Procedure).

Brain Histology

The anesthetized animals were transcardially perfused with PBS (0.1 M, pH 7.4) followed by 3% formaldehyde in PBS at the end of each experiment. The brains were removed, postfixed in formaldehyde, and cryoprotected in 25% sucrose for at least 24 h. Coronal brain sections of 30 μm were examined after performing a Nissl staining, and the visualization of the tip of the needle track was considered the microinfusion site and marked on plates reproduced from the atlas of Paxinos and Watson (31). The criteria for including values in data analysis was microinjection within 300 μm from the nucleus based on the radius of spreading under the conditions of microinjections, as described previously (27; Fig. 1). Animals were excluded from data analysis when microinjections where only unilaterally placed within the PVN or the ARC and when it was not possible to follow the needle tract to clearly confirm that the injection was confined to the target nucleus. On the basis of these criteria, 16 animals were excluded for inaccurate injections.

Data Analysis

For the time course of the study, the data are expressed as average gastric acid output during each of the 10-min sampling intervals of the whole (180 min) observation period. For the data expressed as 1-h gastric acid outputs, the average gastric acid output during the time period before PG infusion (basal period: 10–60 min) and during the first (stimulated: 70–120 min) and second (stimulated: 130–180 min) 1-h period after starting the PG stimulation was calculated. Rats that had received only unilaterally correct microinjections in the ARC or the PVN and animals that received injections in the third ventricle were excluded from data analysis.

Statistical Analysis

Results are expressed as means ± SE. Comparisons between multiple groups were performed using one-way ANOVA followed by a Student-Newman-Keuls multiple-range test. P < 0.05 was considered statistically significant.

RESULTS

**Study I: Effect of Kainate Microinjected in the ARC on PG-Stimulated Gastric Acid Secretion**

There was no significant difference between rats bilaterally microinjected with the different vehicles (0.15 M saline or double-distilled water) inside the PVN plus 0.15 M saline (vehicle) in the ARC. Thus these data were pooled and used as control values (Table 1). In the control group microinjected with vehicle in the PVN and the ARC, infusion of PG at a dose of 16 μg·kg⁻¹·h⁻¹ increased the average gastric acid secretion fourfold during the first and more than fivefold during the second hour of the stimulation period (Table 1).

Bilaterally microinjections of kainate in the ARC at a total dose of 120 pmol/rat in animals pretreated with vehicle microinjected in the PVN significantly reduced the PG-stimulated gastric acid secretion in Urethane-anesthetized rats (Table 1). In comparison with the control group, the average PG-stimulated acid output per hour was diminished significantly by 61% during the first and 64% during the second hour.

We did not observe differences in the kainate-induced inhibition of gastric acid secretion between microinjections localized in rostral or caudal sites within the ARC. Kainate microinjections localized outside of the ARC did not affect gastric acid secretion (data not shown).

**Study II-1: Effect of the CRF1/2 Receptor Antagonist A stressin Microinjected in the PVN on Kainate in the ARC-Induced Inhibition of PG-Stimulated Gastric Acid Secretion**

Pretreatment with the specific CRF receptor antagonist astressin, which is nonselective for the CRF1 or -2 receptor, bilaterally microinjected in the PVN (30 pmol/side) 15 min before kainate administered bilaterally in the ARC totally blocked the kainate-induced inhibition of PG-stimulated gastric acid secretion (Figs. 2 and 3; Table 1). A stressin microinjected in other hypothalamic areas outside of the PVN did not
Fig. 1. Diagram of coronal sections of the brain showing representative microinjection sites. Microinjection sites outside 300 μm from the nucleus based on the radius of spreading under the conditions of microinjections were excluded from data analysis. ▲, Injection site outside 300 μm; ●, injection site inside 300 μm. Plates are adopted from the atlas of Paxinos Watson (31).

Table 1. Effect of kainate microinjected in the ARC on pentagastrin-stimulated gastric acid secretion (1st and 2nd h) and modulation of kainate in ARC: effect of specific Y₁ receptor antagonist (BIBP-3226), specific Y₂ receptor antagonist (BIIE-0246), and CRF1/2 receptor antagonist (astressin) microinjected in the PVN

<table>
<thead>
<tr>
<th>ARC-MI</th>
<th>PVN-MI</th>
<th>n</th>
<th>Basal</th>
<th>1st h</th>
<th>2nd h</th>
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<tbody>
<tr>
<td>Vehicle</td>
<td>Vehicle</td>
<td>18</td>
<td>6.5 ± 1.1</td>
<td>25.3 ± 3.5*</td>
<td>32.6 ± 4.3*</td>
</tr>
<tr>
<td>Kainate</td>
<td>Vehicle</td>
<td>7</td>
<td>5.7 ± 1.7</td>
<td>9.8 ± 0.7†</td>
<td>11.6 ± 1.1†</td>
</tr>
<tr>
<td>Kainate</td>
<td>Aestrin</td>
<td>7</td>
<td>6.3 ± 0.7</td>
<td>23.9 ± 3.92‡</td>
<td>32.2 ± 3.82‡</td>
</tr>
<tr>
<td>Kainate</td>
<td>Aestrin</td>
<td>6</td>
<td>6.9 ± 0.6</td>
<td>11.4 ± 1.3</td>
<td>15.4 ± 1.9</td>
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<tr>
<td>Kainate</td>
<td>BIBP-3226</td>
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<td>6.4 ± 0.8</td>
<td>23.1 ± 2.82‡</td>
<td>29.5 ± 3.12‡</td>
</tr>
<tr>
<td>Kainate</td>
<td>BIBP-3226</td>
<td>5</td>
<td>6.3 ± 1.2</td>
<td>8.3 ± 1.2</td>
<td>11.0 ± 0.9</td>
</tr>
<tr>
<td>Kainate</td>
<td>BIIE-0246</td>
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<td>5.8 ± 0.9</td>
<td>12.0 ± 2.5</td>
<td>10.3 ± 2.2</td>
</tr>
<tr>
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<td>BIIE-0246</td>
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<td>6.0 ± 0.6</td>
<td>13.8 ± 3.3</td>
<td>15.3 ± 4.2</td>
</tr>
</tbody>
</table>

Values are means ± SE. ARC, accuate nucleus; PVN, paraventricular nucleus; MI, microinjection; CRF, corticotropin-releasing factor. P < 0.05 vs. basal (*), vs. microinjection of vehicle in ARC and vehicle-MI in PVN (†), and vs. microinjection of kainate in ARC and vehicle-MI in PVN (‡).
Study II-2: Effect of the NPY-Y1 Receptor Antagonist BIBP-3226 and the NPY-Y2 Receptor Antagonist BIIE-0246 Microinjected in the PVN on Kainate in the ARC-induced Inhibition of PG-stimulated Gastric Acid Secretion

The specific Y1 receptor antagonist BIBP-3226 bilaterally microinjected in the PVN at a total dose of 400 pmol/rat before microinjection of kainate in the ARC long lastingly reduced the kainate-induced inhibition of PG-stimulated gastric acid secretion (Figs. 4 and 5; Table 1). In the first hour of the stimulation period, BIBP-3226 microinjected in the PVN completely blocked the kainate-induced inhibition of PG-stimulated gastric acid secretion. In the second hour, this pretreatment reduced the kainate-induced effect by 88% (Fig. 5; Table 1). At microinjection of kainate in the ARC and vehicle in the PVN, the average gastric acid output significantly increased from 9.8 ± 0.7 to 23.1 ± 2.8 µmol/10 min during the first hour and from 11.6 ± 1.1 to 28.5 ± 3.1 µmol/10 min in the second hour after starting the intravenous infusion of PG (Fig. 5; Table 1).

In contrast, the Y2 receptor antagonist BIIE-0246 (120 pmol/side) microinjected in the PVN before microinjection of kainate in the ARC had no significant effect on kainate-induced inhibition of PG-stimulated gastric acid secretion (Fig. 6; Table 1). BIBP-3226 and BIIE-0246 microinjected in other hypothalamic areas outside the PVN did not modulate the inhibitory effect of kainate injected in the ARC on gastric acid secretion (Table 1).

DISCUSSION

The arcuato-paraventricular-hypothalamic neuronal system, i.e., the ARC-PVN axis, is involved in the regulatory control of homoeostatic systems in the organism by the brain (20, 22). A physiological role of this
strong neuroanatomical line of neuronal communication within the hypothalamus in the regulation of food intake is well established (15, 20). Recent studies suggest that the ARC-PVN axis is also involved in the CNS control of gastrointestinal function (36). The aim of the present study was to investigate in more detail the role of neuropeptidergic pathways in the mediation of the regulatory influence of the ARC-PVN axis on gastric function, in particular gastric acid secretion.

There is considerable evidence from neuroanatomical and physiological studies in experimental animals that allows speculating that NPY and CRF pathways are involved in the CNS modulation of gastrointestinal function by the ARC-PVN axis (4, 35). Thus we analyzed the role of these neurotransmitters within the arcuato-paraventricular-hypothalamic neuronal system in the CNS control of gastric acid secretion by combining microinjection of the EAA kainate in the ARC and of antagonists to NPY-Y1, - Y2, or CRF1/2 receptors in the PVN. Previous studies suggest that these NPY and CRF receptor subtypes in the ARC-PVN axis are involved in CNS regulation of digestive function, in addition to the far-reaching role of these neuropeptides and their receptors in the hypothalamic regulation of satiety and food intake (1, 11, 13, 21).

The present data confirm our previous observation that bilateral microinjection of kainate in the ARC inhibits the gastric acid secretion stimulated by peripheral PG (36). We were able to show in earlier studies that this effect of kainate on gastric acid secretion is site specific to the ARC, since the EAA had no effect when microinjected in hypothalamic sites outside of this nucleus.

The inhibition of gastric acid secretion induced by activation of the neurons in the ARC was totally blocked by CRF1/2 and NPY-Y1 receptor antagonists microinjected in the PVN. In contrast, the NPY-Y2 receptor antagonist BIIE-0246 microinjected in the PVN at the same dose as BIBP-3226 had no effect on the inhibition of gastric acid secretion by kainate microinjected in the ARC.

Fig. 4. Effect of the neuropeptide Y (NPY)-Y1 receptor antagonist BIBP-3226 microinjected in the PVN on the inhibitory effect of kainate microinjection in the ARC on PG-stimulated gastric acid secretion/10 min. The selective Y1 receptor antagonist BIBP-3226 bilaterally microinjected in the PVN at a dose of 200 pmol/side significantly reduced the inhibitory effect of kainate microinjection in the ARC on PG-stimulated gastric acid secretion. *P < 0.05 vs. microinjection of BIBP-3226. Data are means ± SE.

Fig. 5. Effect of the Y1 receptor antagonist BIBP-3226 microinjected in the PVN on the inhibitory effect of kainate microinjection in the ARC on PG-stimulated gastric acid secretion. The selective Y1 receptor antagonist BIBP-3226 bilaterally microinjected in the PVN at a dose of 200 pmol/side significantly reduced the kainate in the ARC-induced inhibition of PG-stimulated gastric acid secretion. *P < 0.05 vs. microinjection of vehicle in the ARC and vehicle in the PVN. #P < 0.05 vs. microinjection of kainate in the ARC and vehicle in the PVN. Data are means ± SE of the number of rats (n) group.
Several lines of evidence indicate that the action of the selective antagonist to the Y1 receptor and the nonselective antagonist to the CRF1/2 receptor in the PVN to block the inhibition of gastric acid secretion induced by activation of the neurons in the ARC is site specific. First, it is unlikely that the antagonists act by diffusing to another responsive site, since blockade of the kainate effect, observed at microinjection of CRF1/2 and NPY-Y1 receptor antagonists in the PVN, could not be achieved at microinjection of astressin and BIBP-3226 in other hypothalamic sites outside the boundaries of the PVN, namely into the zona incerta, the anterior area of the hypothalamus, and the lateral area of the hypothalamus. Second, it is also doubtful that the effect of the antagonists microinjected in the PVN results from leakage in the third ventricle. Astressin and BIBP-3226 microinjections in hypothalamic sites located at the same mediolateral level as the PVN but dorsal to the nucleus had no effect on the inhibition of gastric acid secretion by kainate microinjection in the ARC. Third, in previous studies, we found a similar spatial distribution of CRF- and NPY-responsive hypothalamic microinjection sites, namely the PVN, to alter colonic transit and fecal pellet output in conscious rats (27, 28). Taken together, these results indicate that the inhibition of PG-stimulated gastric acid secretion induced by excitatory stimulation of neurons in the ARC is centrally mediated by neuropeptides released in the PVN that unfold their effects via hypothalamic NPY and CRF pathways.

The present results are in good agreement with previous neuroanatomical, pharmacological, and physiological observations (3, 4, 23, 24, 34).

NPY is a potent feeding-stimulatory peptide expressed in the brain predominantly in neurons of the ARC of the hypothalamus that mainly project to the PVN (3, 20, 22). There is considerable evidence that NPY projections from the ARC to the PVN are involved in the CNS regulation of food intake and other physiological functions of the organism by neuroendocrine and autonomic pathways (15). Also, it has been shown that exogenous NPY microinjected in the PVN inhibits gastric acid secretion in experimental animals (16).

Six recognized subtypes of NPY receptors have been described (NPY-Y1 to NPY-Y6). Two of these, NPY-Y1 and -Y2 receptors, are found in high density in the hypothalamus (5). Recent studies have shown that, in particular, NPY-Y1 and NPY-Y2 receptors are involved in the CNS regulation of gastrointestinal function (2, 5, 43). For this reason, we focused on NPY-Y1 and -Y2 receptors in the present study and did not investigate the role of NPY receptor subtypes Y4 and Y5, which are also expressed in the PVN (11). The question of whether NPY-Y4 or NPY-Y5 receptors in the PVN are involved in the CNS control of gastrointestinal function should be examined in future studies.

The present study suggests that NPY released in the PVN from neurons localized in the ARC unfolds an inhibitory effect on gastric secretion by acting on...
NPY-Y₁ but not on NPY-Y₂ receptors. This could be evidenced by the observation that the NPY-Y₁ receptor subtype acts rather postsynaptically and the NPY-Y₂ receptor rather presynaptically (7, 42). Therefore, the involvement of NPY-Y₂ receptors in the hypothalamic regulation of digestive function cannot be excluded at present.

Synaptic contacts between NPY-positive terminals and CRF-positive neurons in the ARC and PVN have been shown by light and electron microscopy. This suggests that the reciprocal interaction between CRF and NPY mechanisms is of particular importance in the arcuate-paraventricular-hypothalamic neuronal system (22–24). For example, it is assumed that CRF-positive neuronal projections from the parvocellular part of the PVN to the ARC represent one of the downstream systems modulating the regulation of satiety by NPY-immunoreactive neurons in the ARC (33). Conversely, NPY-immunoreactive neurons projecting from the ARC to the PVN appear to gain influence on CRF-immunoreactive neurons in the PVN via direct neuronal contact. This hypothesis is supported by the fact that axonal endings of Y₁ receptor-positive neurons from the ARC have been found in the PVN in close proximity to CRF-immunoreactive-positive cells (3, 23). Li et al. (29) conclude from their anatomic studies that NPYergic neurons of the ARC directly regulate the function of CRF neurons in the PVN. In particular, Y₁ receptor-positive neurons in the PVN, mostly in the parvocellular part of the PVN, appear to play a role. Our data give a first indication of a physiological role of this interaction between NPY and CRF mechanisms at the PVN in the CNS regulation of gastrointestinal function. This idea is supported by data from pharmacological experiments showing that NPY exhibits a stimulatory effect on hypothalamic CRF-immunoreactive neurons. Acute intracerebroventricular administration of exogenous NPY induces an increased CRF gene expression in the PVN, and microinjection of NPY directly in the PVN raises the ACTH and corticosteroid secretion (41).

If one goes along with the hypothesis that CRF-dependent mechanisms are the downstream effector systems for NPY effects in the PVN on gastric acid secretion, it is not surprising that antagonism of CRF receptors in this hypothalamic nucleus is more effective than antagonism of NPY receptors, as demonstrated in the present study. One possible explanation for the higher effectiveness of the CRF1/2 receptor antagonist in blocking the inhibition of PG-stimulated gastric acid secretion by kainate microinjection in the ARC involves a positive CRF feedback on CRF neurosecretory cells in the PVN. The observation that CRF itself could modulate the positive secretory activity of CRF neurosecretory cells in the PVN during stress was first described by Ono et al. (30). Moreover, it has been demonstrated that exogenous CRF activates transcription of the gene encoding the CRF1 receptor selectively within the PVN (18). This effect on CRF1 mRNA, and also the transcription of the CRF gene within the PVN, was completely prevented by pretreatment with a CRF antagonist (30, 34). These observations suggest the existence of CRF-CRF synaptic connections at the level of the PVN (4, 34). Therefore, the activation of CRF neurons in the PVN by exogenous CRF may represent a positive feedback loop mediated through a specific CRF receptor subtype that is expressed selectively among neuroendocrine cells of the PVN (4). We have shown in previous studies that the effect of various stressors on colonic motor function can be blocked by pretreatment with a CRF1/2 receptor antagonist microinjected in the PVN (26, 27). In addition, we have observed before that the stimulatory effect of exogenous NPY in the PVN on colonic transit is mediated by hypothalamic CRF pathways (28). Thus the present data are in accordance with the idea that neuronal projections from the ARC to the PVN, in particular NPYergic projections, are involved in activation and modulation of a positive CRF-CRF feedback regulation in the PVN that plays a role in the CNS regulation of gastrointestinal function.

In summary, stimulation of neurons in the ARC leads to a long-lasting inhibition of peripherally stimulated gastric acid secretion. Neuronal projections from the ARC to the PVN, and NPY-Y₁ as well as CRF1/2 receptors in the PVN, appear to be involved in the central mediation of this effect (Fig. 7). One might hypothesize that NPY released by neurons projecting from the ARC to the PVN activates CRF neurons in this brain area that are part of a positive CRF feedback loop. This hypothesis must, however, be examined in further receptor binding studies. The present study gives the first indication that endogenous NPY from ARC neurons released in the PVN influences gastric acid secretion via central CRF mechanisms and Y₁ receptors.

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DISCLOSURES

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