Inhibition by Central Alpha-2 Adrenergic Mechanism of Thyrotropin-Releasing Hormone-Induced Gastric Acid Secretion in the Rat

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Accepted June 15, 1984

Abstract—Influences of central alpha-2 adrenergic agonists on thyrotropin-releasing hormone (TRH)-stimulated gastric acid secretion were examined in the perfused stomach of anesthetized rats. Clonidine, an alpha-2 adrenergic agonist, given subcutaneously or intracerebroventricularly inhibited the gastric acid secretion stimulated by intracerebroventricular TRH. Intracerebroventricular injection of norepinephrine tended to reduce the acid secretion, while phenylephrine, serotonin and quipazine (serotonin agonist) did not influence the acid secretion. Subcutaneous clonidine enhanced the acid secretion peripherally stimulated by electrical vagus stimulation. The inhibitory effect of clonidine on TRH-induced acid secretion was reversed by yohimbine, an alpha-2 adrenergic antagonist, and phentolamine. In conclusion, the present study suggests that the central alpha-2 adrenergic receptor system participates in the TRH-mediated central nervous system control of gastric acid secretion in anesthetized rats.

In recent years, considerable attention has been focused on the central peptide-mediating systems in gastrointestinal function. Thyrotropin-releasing hormone (TRH) influences the gastrointestinal tract in animals and man in many ways (1-3). Although the exact mechanism of action of TRH, which elicits a vagus-mediated stimulation of gastric acid secretion (4, 5), remains to be identified, the hypothalamus has been shown to participate in brain control of gastric secretion (6) in rats. These facts indicate the possibility that there are interactions between the TRH and the hypothalamic monoaminergic neuron involved in brain modulation of gastric acid secretion. In fact, inhibition by dopamine agonists of TRH-stimulated gastric acid secretion has been observed (7). On the other hand, the central noradrenergic inhibition of acid secretion in electrical stimulation of the lateral hypothalamic area of rats (8) and the inhibitory effect of clonidine (9-13), a central alpha-2 adrenoceptor agonist, on gastric acid secretion suggest the possibility for central noradrenergic inhibition of TRH-stimulated acid secretion.

In the present study, interactions between the TRH action and the central aminergic systems, noradrenergic and serotonergic systems, in gastric acid secretion were examined in the rat.

Materials and Methods
Male Wistar rats (ST. substrain from Sankyo Lab. Co., Ltd.), weighing 220-250 g, were anesthetized with urethane (1.25 g/kg, i.p.) after a 24 hr fast, but were allowed free access to water. A gastric acid secretion assay was performed as described previously (7). Total amount of the secreted acid was expressed in terms of μeq HCl/60 min per animal. Basal secretion was low and almost constant during the experimental periods. Therefore, the data indicate the value of acid output after drug treatments subtracted by the respective basal secretion. Intracerebroventricular drug administration was carried out by the technique of Noble et al. (14). The
vagus nerve was stimulated by a pair of platinum electrodes over a time period of 10 min as in our earlier study (15). Vagus stimulation was repeated at about 60–90 min intervals. All drugs were dissolved in saline and administered subcutaneously or intraperitoneally in a volume of 1 ml/kg. The volume of all intracerebroventricular injections except TRH (10 μl/rat) was 5 μl/rat. Doses of drugs are expressed as the amount of salt.

**Drugs:** Drugs used were clonidine HCl (Tokyo Kasei), dl-norepinephrine HCl (Nakarai), phentolamine (Ciba), phentolamine HCl (Tokyo Kasei), quipazine maleate (Miles Lab.), serotonin creatinine sulfate (Merck), thyrotropin-releasing hormone (Protein Res. Foundation), urethane (Nakarai) and yohimbine HCl (Nakarai).

**Statistical analysis:** All data are presented as mean±S.E. Data were analyzed by Student’s t-test and a paired t-test. All data differences were considered significant at P<0.05 or P<0.01.

### Results

Intracerebroventricular TRH (10 μg/rat, i.c.v.) produced a remarkable increase in gastric acid secretion. The repeated administration of TRH induced reproducible acid secretion. Pretreatment with clonidine 15 min before the second TRH injection inhibited the gastric secretion in a dose-related manner (0.05–0.5 mg/kg, s.c.) as shown in the typical pattern illustrated in Fig. 1 and in Table 1. Clonidine did not influence basal secretion at less than 0.5 mg/kg in the present experiment.

Repeated electrical vagus stimulation for 10 min led to reproducible gastric acid secretion. Although saline given 15 min

![Graph showing effect of clonidine on thyrotropin-releasing hormone (TRH)-stimulated gastric acid secretion.](image)

**Fig. 1.** Effect of clonidine on thyrotropin-releasing hormone (TRH)-stimulated gastric acid secretion. Ordinate: acid output per 2 min. Abscissa: time, chart speed 1 cm/10 min. TRH (10 μg/rat) was intracerebroventricularly injected twice into one rat. Clonidine (CND, mg/kg, s.c.) was given 15 min before the 2nd TRH injection.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose mg/kg, s.c.</th>
<th>No. of rats</th>
<th>Increase in acid output μeq HCl/60 min</th>
<th>1st</th>
<th>2nd</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td></td>
<td>12</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clonidine</td>
<td>0.05</td>
<td>6</td>
<td>112.2±14.0</td>
<td>120.2±11.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.25</td>
<td>6</td>
<td>106.8±15.5</td>
<td>86.2±19.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>6</td>
<td>97.7±14.0</td>
<td>20.8±8.8</td>
<td>5.4±2.5**</td>
</tr>
</tbody>
</table>

The values are mean±S.E.M. of increase in acid output (the acid output after TRH administration minus basal values). Basal secretion is 13.1±2.6 μeq HCl/60 min (n=30). Clonidine dissolved in saline was administered subcutaneously (s.c.) 15 min before the 2nd TRH intracerebroventricular injection (10 μg/rat). ** P<0.01 vs. 2nd TRH control (Student’s t-test). ° P<0.01 vs. corresponding 1st value (paired t-test).
before the vagus stimulation did not significantly influence the gastric secretion, clonidine at the dose (0.5 mg/kg) which remarkably inhibited TRH-stimulated acid secretion significantly enhanced the response (Table 2). Clonidine (0.5 mg/kg) did not increase basal secretion in the preparations in which the vagus nerve was cut.

Table 3 shows the influence of intracerebroventricular injection of clonidine, norepinephrine and phenylephrine on TRH-induced acid secretion. Clonidine (10-20 µg/rat) significantly inhibited the gastric secretion, norepinephrine (5-20 µg/rat) tended to reduce the secretion (not significant), while phenylephrine (20 µg/rat) did not influence it. On the other hand, intracerebroventricular injection of serotonin (20 µg/rat) or quipazine (20 µg/rat) did not produce any changes in the TRH-stimulated gastric acid secretion, as shown in Table 3.

Table 2. Effect of clonidine on acid secretion stimulated by electrical vagus-stimulation

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Dose (µg/kg)</th>
<th>No. of rats</th>
<th>Increase in acid output (µeq HCl/60 min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Before drug</td>
</tr>
<tr>
<td>Saline</td>
<td>1 ml/kg</td>
<td>5</td>
<td>35.6±5.7</td>
</tr>
<tr>
<td>Clonidine</td>
<td>0.5 mg/kg</td>
<td>5</td>
<td>36.8±3.6</td>
</tr>
</tbody>
</table>

Clonidine was administered subcutaneously 15 min before the vagal stimulation. ** P<0.01 vs. before drug (paired t-test). a P<0.05 vs. saline (Student’s t-test).

Table 3. Effects of intracerebroventricular adrenergic and serotonergic agents on TRH-stimulated gastric acid secretion

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Dose (µg/rat, i.c.v.)</th>
<th>No. of rats</th>
<th>Increase in acid output (µeq HCl/60 min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>1st</td>
</tr>
<tr>
<td>Saline</td>
<td></td>
<td>12</td>
<td>106.2±17.6</td>
</tr>
<tr>
<td>Clonidine</td>
<td>5</td>
<td>6</td>
<td>105.7±24.8</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>6</td>
<td>97.1±16.2</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>6</td>
<td>111.7±19.8</td>
</tr>
<tr>
<td>Norepinephrine</td>
<td>5</td>
<td>6</td>
<td>101.6±12.6</td>
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<tr>
<td></td>
<td>20</td>
<td>6</td>
<td>100.9±14.5</td>
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<tr>
<td>Phenylephrine</td>
<td>20</td>
<td>6</td>
<td>106.7±20.7</td>
</tr>
<tr>
<td>Serotonin</td>
<td>20</td>
<td>5</td>
<td>96.3±17.6</td>
</tr>
<tr>
<td>Quipazine</td>
<td>20</td>
<td>5</td>
<td>99.3±11.5</td>
</tr>
</tbody>
</table>

Drugs dissolved in saline were given intracerebroventricually 10 min before 2nd TRH (10 µg/rat, i.c.v.) injection. a P<0.05; b P<0.01 vs. corresponding control (paired t-test). * P<0.05; ** P<0.01 vs. 2nd saline control (Student’s t-test).

Inhibition by subcutaneous clonidine on TRH-stimulated acid secretion was partly prevented by the pretreatment with yohimbine (2.5 mg/kg, i.p.) or phentolamine (1 mg/kg, i.p.), as shown in Fig. 2. Yohimbine and phentolamine alone did not influence the TRH-induced response at 2.5 mg/kg and 1 mg/kg, respectively. Higher dose of phentolamine (2.5 mg/kg) significantly reduced the response.

Figure 3 summarizes the influences of alpha-adrenergic blocking agents on the antisecretory effects of intracerebroventricular clonidine in TRH-treated rats. Pretreatment with yohimbine (2.5 mg/kg, i.p.) or phentolamine (1 mg/kg, i.p.) partly reversed the action of clonidine.

Discussion

Although the interactions between the TRH action and monoamines in the central...
nervous system have been obscure, the central effects of TRH such as release of growth hormone (16) and gastric acid stimulation (7) were modified by dopamine receptors, and the antinociceptive properties of TRH were resistant to modifications of the noradrenergic system (17). To our knowledge, the present work represents the first observation of the interaction of central alpha-2 adrenergic receptor and TRH.

Subcutaneous or intracerebroventricular injection of clonidine, an alpha-2 adrenergic agonist (18), inhibited TRH-stimulated gastric acid secretion, and intracerebroventricular norepinephrine, but not phenylephrine (alpha-1 agonist) (19), tended to reduce the secretion. The antisecretory effect of clonidine was prevented by yohimbine, an alpha-2 antagonist (18, 20, 21), or phentolamine, an alpha-1 and -2 antagonist (18). Clonidine also has no ganglionic blocking effect (22). These results indicate that the action of TRH on gastric acid secretion can be modified by central alpha 2 adrenergic receptor stimulation, and these results have close associations with previous findings of the central noradrenergic inhibition of acid secretion in electrical stimulation of the lateral hypothalamic area (8) and of the inhibitory effect of clonidine on 2-deoxy-D-glucose- or insulin-stimulated acid secretion (9–11).

On the other hand, the potentiating effect of clonidine on vagal stimulation-induced acid secretion can be due to peripheral actions. This drug enhanced bethanechol-induced (10, 11, 23) and basal (9–11) acid secretion. Since the stimulatory effect of clonidine might be mediated by histamine (10, 11, 24, 25), the potentiating effect of clonidine may be explained by the hypothesis that endogenous agonists such as acetylcholine, histamine and gastrin stimulate their specific receptors to produce synergistic effects on acid secretion (26).

Some authors postulated that in the rat, 5HT is an inhibitory neurotransmitter in the amygdala for gastric acid secretion (27). 5HT given intracerebroventricularly reduced acid secretion more strongly than by the intraperitoneal route (28). These facts have led us to examine the central serotonergic as well as noradrenergic inhibitory mechanisms in the TRH-induced gastric acid secretion. However, the acid secretion was not influenced by intracerebroventricular 5HT or quipazine, a central serotonergic agonist (29, 30), in the present study.

Clonidine has dual actions (increasing and decreasing) on blood pressure which have
both peripheral and central origins (31, 32). It has been reported that gastric acid secretion did not decrease in reserpinized rats with low blood pressure (33), and there were no correlations between the influences of some adrenergic drugs on gastric secretion and on hemodynamics (34). These results suggest that blood pressure changes must not interfere with gastric secretion in the present study.

In conclusion, the present study suggests that the alpha-2 adrenergic receptor system participates in the TRH-mediated central regulatory mechanisms of gastric acid secretion in the anesthetized rat.

References


Parsons, M.E.: Studies on the effect of clonidine on histamine H$_2$ receptors in the uterus, heart and gastric mucosa. Agents Actions 8, 402–403 (1978)


