Direct Evidence for Central Action of PCPGABA to Stimulate Gastric Acid Secretion by Intracisternal Injection

Katsuya YAMASAKI* and Yoshiaki GOTO
Department of Pharmacology, Tokushima Bunri University, Tokushima 770, Japan
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Abstract—PCPGABA, injected into the cisterna magna, significantly stimulated gastric acid secretion in the perfused rat stomach preparation. This secretagogue action was dose-dependent (0.5–2 μg/rat). The peak response occurred within 60 min and lasted up to 100 min. The secretagogue action by PCPGABA was completely reduced by truncal vagotomy. Intracisternal injection of 5-aminovaleric acid, a GABA_B-receptor antagonist, did not alter basal gastric acid output, and it also failed to antagonize the acid secretory response to intracisternal PCPGABA. These results demonstrate that intracisternal PCPGABA caused hypersecretion of acid through vagal dependent mechanisms partially independent of GABA_B-receptors.

2-(p-Chlorophenyl)-4-aminobutyric acid (PCPGABA), a lipophilic analogue of GABA, is a GABA_B receptor agonist (1) and has various pharmacological actions (2–4). It has been also reported that peripheral administration of PCPGABA stimulated gastric acid secretion in many species (5–7) including man (8). Pharmacological approaches demonstrated that PCPGABA induced stimulation of gastric acid output was not attributed to alteration in blood glucose level, unlike the action of insulin (6). The evidence that truncal vagal dissection abolished PCPGABA-induced enhancement of the acid secretion (5–6) seems sufficient to suggest that central mechanisms are involved in this action. This hypothesis has been supported with our recent studies that peripheral PCPGABA enhanced the vagal efferent discharges (9). However, there little evidence for the central action of GABA to stimulate gastric acid. Furthermore, which types of GABA receptors might be involved in the secretagogue action has not been studied.

Our aim in the present study was to obtain direct evidence for the central action of PCPGABA on gastric secretion by intracisternal injection. We also attempted to clarify the possible role of central GABA_B receptors in the acid secretory response.

Materials and Methods
Animals: Male Wistar/ST-strain rats weighing 180–220 g were used. Rats were housed under a 12:12 day-night cycle (lights on at 7:00 A.M.) at an ambient temperature of 23±1 °C and fed with standard rodent chow and water ad libitum for at least 1 week before initiation of the experiment. Rats were fasted for 18 hr prior to each experiment but allowed free access to water.

Measurement of gastric acid secretion: Animals were anesthetized with urethane (1.25 g/kg, i.p.). Tracheostomy was followed by ligation of the upper esophagus to avoid reflux of the perfusate. Laparotomy was followed by the ligation of the pylorus. A dual polyethylene cannula was inserted into the gastric lumen through a small incision of the forestomach for continuous perfusion of the stomach. The gastric lumen was perfused with saline by means of a peristaltic pump at a flow rate of 5 ml/min. Acid production was measured by titrating the perfusate with 0.02 N NaOH to the end point of pH 5.5 using an automatic titrator (TOA Electronics Co., TSB-10A, TSC-10A, Japan) and continuously re-
corded in terms of every 2-min acid response via a zero suppression adaptor (TOA Electronics Co., C96667T, Japan). Ten minute-acid output was calculated by adding five 2-min responses and expressed as H⁺μEq/10 min. The drugs were given intracisternally or intravenously. Intracisternal injection was performed according to the previously described method (10).

Vagotomy was performed by bilateral transection of the vagi at the subdiaphragmatic level, namely, by seromyotomy at the cardiac portion of the esophagus after the introduction of an acute gastric fistula (5).

Drugs: (±)-PCPGABA was a gift from Ciba Geigy. 5-Aminovaleric acid was purchased from Nacalai Tesque, Japan. Test drugs were dissolved in saline. These compounds were administered in a volume of less than 10 μl in the case of intracisternal injection and 0.1 or 0.2 ml/100 g body weight, for peripheral administration.

Statistical analysis: Results are expressed as the mean±S.E.M. Differences between groups were assessed by two way analysis of variance (ANOVA) with Dunnett's multiple range test or one way ANOVA with Scheffe's multiple test. A difference of P<0.05 was regarded as significant.

Results

Influence of intracisternal PCPGABA: Microinjection of saline into the cisterna (10 μl) did not alter the basal gastric acid secretion in the rat, and acid output was less than 2.5 H⁺μEq/10 min during the experiment. Figure 1 shows the typical recording of gastric acid secretion in response to intracisternal injection of PCPGABA. The onset of the hyperacidity induced by PCPGABA (2 μg/rat, i.c.) was very rapid, and the latency of the response was less than 10 min. The maximal response was obtained at 45-60 min after PCPGABA, and acid secretion returned to basal level by 100 min (Fig. 1).

Intracisternal injection of PCPGABA, 0.5–2 μg/rat, stimulated gastric acid secretion in a dose-dependent manner (Fig. 2). The integrated net increment in acid output for 90 min was 9.1±3.3, 38.8±11.4 and 90.7±16.6 H⁺μEq/90 min at 0.5, 1 and 2 μg/rat, respectively. No significant action was observed after i.v. injection of 2 μg of PCPGABA, i.e., the total net increment in acid output for 90 min was 2.3±0.9 H⁺μEq/90 min (N=6).

Truncal vagotomy: The effect of vagotomy on gastric acid response to 2 μg intracisternal PCPGABA is shown in Fig. 3. Surgical vagotomy at the subdiaphragmatic level was
performed 30 min before PCPGABA administration. Hypersecretion of gastric acid induced by intracisternal PCPGABA was completely abolished by vagotomy.

Influence of 5-aminovaleric acid: 5-Aminovaleric acid, at the intracisternal dose of 1.1 μg (equimolar dose to 2 μg of PCPGABA), did not antagonize the secretagogue action of PCPGABA (Fig. 4). Therefore, higher doses of 5-aminovaleric acid (10 and 100 μg/rat) were also tested for their ability to antagonize the PCPGABA effect. Again these doses had no significant effect on basal and PCPGABA-stimulated gastric acid secretion (Fig. 5, Table 1).

Fig. 3. Inhibition of intracisternal injection of PCPGABA-induced stimulation of gastric acid secretion by subdiaphragmatic truncal vagotomy. Surgical denervation of the vagus was performed 30 min before PCPGABA injection. Arrow indicates intracisternal dosing of PCPGABA at 2 μg/rat. Each point represents the mean±S.E. * and **: Significant difference from the PCPGABA alone group at P<0.05 and 0.01 by Dunnett's test, respectively. The number of rats is indicated in parenthesis. PCPGABA: 2-(p-chlorophenyl)-4-aminobutyric acid.

Fig. 4. No antagonistic effect was seen between equimolar doses of 5-aminovaleric acid and PCPGABA in response to gastric acid secretion.

Fig. 5. Effect of intracisternal injection of 5-aminovaleric acid on basal acid secretion (left) and on PCPGABA-stimulated acid secretion (right) in rats. Each point represents the mean±S.E. The number of rats is indicated in parenthesis. 5-AVA: 5-Aminovaleric acid, PCPGABA: 2-(p-chlorophenyl)-4-aminobutyric acid.
Table 1. Effect of 5-aminovaleric acid on PCPGABA stimulated gastric acid secretion in urethane-anesthetized rats

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dose (µg)</th>
<th>N</th>
<th>Gastric acid secretion (H⁺Eq/90 min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>7</td>
<td>9.9±3.1</td>
</tr>
<tr>
<td>5-AVA</td>
<td>10</td>
<td>5</td>
<td>11.4±1.6</td>
</tr>
<tr>
<td>PCPGABA</td>
<td>100</td>
<td>5</td>
<td>9.1±3.3</td>
</tr>
<tr>
<td>5-AVA+PCPGABA</td>
<td>100+2</td>
<td>6</td>
<td>103.6±17.3**</td>
</tr>
<tr>
<td>5-AVA+PCPGABA</td>
<td></td>
<td></td>
<td>138.9±16.3**</td>
</tr>
</tbody>
</table>

5-AVA: 5-aminovaleric acid, PCPGABA: 2-(p-chlorophenyl)-4-aminobutyric acid. Each compound was given intracisternally to the rat. Control animals received saline at a volume of 10 µl/rat, intracisternally. Gastric acid secretion was summed cumulatively from 0 to 90 min after the compound injection for each rat. **: Significant difference from the control at P<0.01 by Scheffe's test. No significant difference was observed between the PCPGABA alone group and the 5-AVA+PCPGABA group.

Discussion

The present study provides direct evidence that intracisternal PCPGABA produces an increase of gastric acid output dose-dependently. The stimulatory effect was observed within 10 min after the intracerebral injection and lasted for 100 min. This time course of gastric secretion in response to PCPGABA was quite similar to that obtained by intracisternal thyrotropin-releasing hormone (TRH) (10). The potent acid stimulatory effect of PCPGABA had already been observed when this compound was given subcutaneously (5). However, the peripheral doses to stimulate acid output were high. Previous studies in the rat have shown that more than 2 mg/kg of PCPGABA was required to produce a significant increment in gastric acid secretion (5). The threshold dose of intracisternal PCPGABA to stimulate acid output in this study (0.5 µg/rat) was less than one eight hundredths compared with the subcutaneous dose (5).

A central mechanism of PCPGABA is supported by the fact that very small doses are still active in the case of intracisternal injection and by the fact that surgical denervation of the vagus abolished the acid response to PCPGABA. The latter finding is consistent with the previous observation that subcutaneous PCPGABA-induced stimulation was abolished by subdiaphragmatic vagotomy (5). This also suggests that the parasympathetic nervous system is a crucial neurogenic pathway mediating the central action of PCP-GABA. Systemic administration of PCPGABA increased efferent discharges of the cervical and gastric branches of the vagus (9). Taken together, these findings led us speculate that PCPGABA acts in the CNS to activate vagal efferent outflow and acid output.

Even when bioactive substances are directly injected into the brain, the possibility that some portion of the substances will leak out into the peripheral circulation should be carefully considered. Intravenous injection of PCPGABA (2 µg/rat, approximately equivalent to 10 µg/kg) did not have any significant effect (total net increment in acid output for 90 min was 2.3±0.9 H⁺Eq/90 min), suggesting that intracisternal PCPGABA (2 µg/rat) elicited acid secretagogue action by acting in the CNS. The small possibility that PCPGABA acts through the secondary effect after its leakage into the peripheral circulation could be excluded.

Accumulated pharmacological findings suggest that GABA in the CNS may play substantial roles in the regulation of gastric acid secretion (11, 12). This hypothesis is partially supported by the following findings: 1) muscimol (a GABAₐ agonist) acts within the brain to stimulate gastric function (13) and 2) central administration of the GABAₐ agonist caused hypersecretion of gastric acid. These findings indicate a close interaction between central GABA mechanisms and gastric functions. Immunohistochemistry has shown the presence of GABA neuron terminals and GAD activity in the hypothalamus (14-16) known to regulate gastrointestinal
function (17). Stimulation of the amygdala which contains a high concentration of GABA (14, 18, 19) resulted in hypersecretion of gastric acid (20), and this activation was likely to occur via the projection to the dorsal motor nuclei in the medulla (20). Therefore, it could be suspected that the centrally mediated alteration in autonomic responses induced by the GABA-mimetics are probably through the brainstem pathways, i.e., the dorsal motor nucleus complex (21), if intracisternal PCPGABA could reach to this area. 

Most of the pharmacological actions of PCPGABA can be attributed to interactions with the B type of GABA receptors (1, 22). The evidence that tritiated PCPGABA selectively binds to rat brain synaptic membranes (23), suggests the presence of GABA_B receptors in the CNS. The physiological and pharmacological significances of GABA_B receptors in the CNS are still unknown (22). In our previous paper, we showed that phaclofen, a specific GABA_B-receptor antagonist, failed to counteract the acid secretory effect of PCPGABA, suggesting that mechanisms other than B-receptors might be involved in the secretagogue action of GABAergic agents. Therefore, we employed another type of GABA_B-receptor antagonist in order to confirm this hypothesis. 5-Aminovaleric acid, a putative GABA_B antagonist, both in the peripheral nervous system (24, 25) and the CNS (26–28) failed to antagonize the gastric acid stimulatory response to PCPGABA in this experiment. Intracisternal 5-aminovaleric acid, examined up to the highest dose (100 μg/rat), did not antagonize the acid secretion response to PCPGABA (2 μg/rat). 5-Aminovaleric acid has been reported to be partial agonist for GABA_B receptor; very weak antagonist (28) and slightly potent agonist (29). However, no antagonistic action of 5-aminovaleric acid was observed in the acid response to PCPGABA; and in addition, this compound did not show any secretagogue actions on basal secretion at the doses employed in this experiment.

In conclusion, the present study suggested that intracisternal injection of PCPGABA has a significant stimulatory effect on gastric acid secretion.

References


