GABA_A and GABA_B-Receptor Agonists Evoked Vagal Nerve Efferent Transmission in the Rat

Katsuya Yamasaki*, Yoshiaki Goto, Nobuyuki Hará and Youichi Hará

Department of Pharmacology, Tokushima Bunri University, Tokushima 770, Japan
1Research Laboratories, Sumitomo Pharmaceuticals Co., Osaka 554, Japan

Received April 5, 1990 Accepted September 26, 1990

ABSTRACT—The effect of GABA agonists on vagal efferent activity was studied in anesthetized rats. PCPGABA, a GABA_B agonist (4 and 8 mg/kg, s.c.), markedly activated the neural efferent discharge of the vagus. Muscimol, a GABA_A agonist (0.1 and 0.3 mg/kg, i.v.), also facilitated vagal activity. Both agonists caused significant gastric acid hyperacidity. Bicuculline (0.25 mg/kg, i.v.) or picrotoxin (0.5 mg/kg, i.v.) given 10 min prior to each agonist had no effect on the frequency of vagal nerve firing elicited by PCPGABA (4 mg/kg, s.c.) or muscimol (0.3 mg/kg, i.v.). Pretreatment with scopolamine (0.25 mg/kg, i.v.) abolished PCPGABA stimulated vagal activity and gastric acid secretion. Methscopolamine (0.25 mg/kg, i.v.) inhibited only the hyperacidity evoked by PCPGABA, but not vagal activation. These results suggest that PCPGABA and muscimol may cause gastric acid secretion through central cholinergic descending mechanisms that are resistant to GABA_A and GABA_B antagonists.

GABA is present in the central nervous system and is recognized as an inhibitory neurotransmitter (1). The inhibitory action of GABA is due to an increase in intracellular Cl^- transport (2). However, in peripheral sites, it has been reported that GABA has stimulatory actions on the enteric nervous systems of the ileum, large intestine (3-5), and myenteric plexus (6). These effects were antagonized by the GABA_A antagonist bicuculline. On the other hand, the stimulatory effects of centrally or peripherally administered GABA and GABA-mimetics on gastric secretion have been observed in several species, rats (7-9), dogs (10) and humans (11). The secretagogue effects were abolished by truncal vagotomy or atropine (7-10). However, little is known about the mechanisms of the secretagogue action of GABA. The aim of our study was to investigate whether the effect of the systemically administered GABA-mimetics on gastric acid secretion was mediated through central cholinergic mechanisms. Furthermore, the possible involvement of GABA receptor subtypes was also extensively studied by using the GABA antagonists bicuculline and picrotoxin.

MATERIALS AND METHODS

Animals

Male Wistar/ST-strain rats, weighing between 180 and 260 g, were fed with a standard pellet diet and water ad libitum. They were housed in conditions of controlled temperature at 23 ± 1°C and lighting cycle (light periods...
6:00 A.M. – 6:00 P.M.). Rats were fasted for 18–24 hr prior to each experiment but allowed free access to water.

**Recording of truncal vagal efferent activity**

The rats were anesthetized with urethane (1.25 g/kg, i.p.). The left vagus nerve was exposed in the cervical region and manipulated for vagal efferent activity recording as described previously (12). As shown in Fig. 1, the peripheral portion was crushed to exclude the input of the afferent discharges. Extracellular records were obtained from the divided cranial segment using bipolar platinum electrodes in a mineral oil pool. By means of the biophysical amplifier, potentials were displayed on an oscilloscope and monitored in terms of a window discriminator which could distinguish impulses of efferent discharges from the noise level.

**Measurement of gastric acid secretion**

Rats were anesthetized with urethane (1.25 g/kg, i.p.). A tracheostomy was performed, and the esophagus was ligated to avoid the reflux of the perfusate. Laparotomy was followed by ligation of the pylorus. A dual polyethylene gastric cannula was intubated into the gastric lumen through a small incision of the forestomach. The gastric lumen was continuously irrigated with saline by means of a peristaltic pump at a flow rate of 5 ml/min. Acid production was determined 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).

**Drugs**

2-(p-Chlorophenyl)-4-aminobutyric acid (PCPGABA) was a gift from Ciba Geigy Pharmaceutical and injected subcutaneously. Muscimol, bicuculline methiodide, picrotoxin, scopolamine hydrobromide and methscopolamine bromide were purchased from Sigma Chemical Co. (St. Louis, MO) and injected intravenously. Test drugs were dissolved in saline. These compounds were administered in a volume of 0.1 or 0.2 ml/100 g body weight.

**Statistics**

Statistical analysis of the data was performed by means of one-way analysis of variance (ANOVA) followed by Dunnett's test.

**RESULTS**

**Effect of GABA agonists on vagal efferent activity**

Figure 2 shows a typical extracellular recording of vagal efferent discharges in the

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**Fig. 1.** Schematic diagram of experimental set-up for extracellular recording of spontaneous efferent signals in rats. Details are described in the text.
anesthetized rats. The noise level of the recorded firing was between 10–20 μV. A few spikes were observed during the pre-medication period. PCPGABA (4 mg/kg, s.c.) caused significant activation of the vagal efferent discharges. Muscimol (0.3 mg/kg, i.v.) also activated the neural responses. The spikes observed are bipolar, and their amplitudes were between 30 μV and 100 μV. Therefore, the slice levels of the window discriminator were set at 30 μV (lower) and at 100 μV (upper).

The excitation of truncal vagal efferent discharges induced by PCPGABA or muscimol was the all or none type (Fig. 3). The stimulatory effects of vagal tone were initiated at 5 min and 1 min after PCPGABA and muscimol administration; and maximal responses were observed within 30 min and 10 min, respectively, and declined to basal levels about 60 min after the treatment (Fig. 2).

The evoked discharges of vagi by these GABA agonists were not abolished by bicu-

![Fig. 2. Typical responses of spontaneous vagal efferent discharges to PCPGABA and muscimol in anesthetized rats. A: Resting conditions (upper traces) and 30 min after treatment with PCPGABA at 4 mg/kg, s.c. or muscimol at 0.3 mg/kg, i.v. (lower traces). The calibrations are the Y axis: 20 μV and the X axis: 20 msec. B: Typical recording of vagal efferent activity before and 60 min after PCPGABA and muscimol administration.](image-url)
Fig. 3. Effect of PCPGABA (left) and muscimol (right) on the vagal efferent discharges in rats. Control (○–○). PCPGABA was given subcutaneously at the doses of 2 mg/kg (●–●), 4 mg/kg (▲–▲) and 8 mg/kg (■–■). Muscimol was given intravenously at the doses of 0.1 mg/kg (○–○) and 0.3 mg/kg (▲–▲). The values shown are the mean of 6 and 7 experiments.

Fig. 4. Effect of bicuculline and picrotoxin on activation of vagal efferent induced by PCPGABA (left) and muscimol (right). Each antagonist was given 10 min before PCPGABA and muscimol. Each data point represents the mean of 6–10 experiments. PCPGABA (4 mg/kg, s.c.) or muscimol (0.3 mg/kg, i.v.) alone (○–○) in the pretreatment with bicuculline 0.25 mg/kg, i.v. (▲–▲) or picrotoxin 0.5 mg/kg, i.v. (■–■).
Table 1. Effects of PCPGABA and muscimol on vagal efferent discharge and gastric acid secretion and modification of these actions by various antagonists

| Compound        | Dose (mg/kg, i.v.) | PCPGABA (4 mg/kg, s.c.) | Muscimol (0.3 mg/kg, i.v.) |
|-----------------|--------------------|-------------------------|---------------------------|
|                 | VED<sup>a</sup>    | GAS<sup>b</sup>         | VED<sup>a</sup>          |
| Control         | -                  | 108 ± 21 (30)           | 134 ± 26 (15)             |
| Bicuculline     | 0.25               | 95 ± 34 (20)            | 135 ± 26 (25)             |
| Picrotoxin      | 0.5                | 110 ± 25 (35)           | 107 ± 39 (25)             |
| Scopolamine     | 0.25               | 19 ± 10 (20)            | 12 ± 3 (10)               |
| Methscopolamine | 0.25               | 109 ± 15 (30)           | 159 ± 25 (20)             |

<sup>a</sup>: vagal efferent discharge (Hz); figures in parentheses represent the time (min) after compound administration at which the maximum response (calculated as the mean of measurements over a 5-min period) was observed. <sup>b</sup>: gastric acid secretion (μEq/90 min), increment of acid-output for 90 min. *: P < 0.05 by Dunnett's test, Mean ± S.E., N = 5–10.

culline (0.25 mg/kg, i.v.) or picrotoxin (0.5 mg/kg, i.v.) (Fig. 4 and Table 1). Scopolamine (0.25 mg/kg, i.v.) inhibited the vagal activities (P < 0.05, Dunnett's test), but methscopolamine (0.25 mg/kg, i.v.) did not (Fig. 5 and Table 1).

Effect of GABA agonists on gastric acid secretion

PCPGABA (4 mg/kg, s.c.) or muscimol (0.3 mg/kg, i.v.) increased gastric acid secretion. Neither bicuculline (0.25 mg/kg, i.v.) nor picrotoxin (0.5 mg/kg, i.v.) reduced gastric acid secretion in response to PCPGABA and muscimol. However, both scopolamine (0.25 mg/kg, i.v.) and methscopolamine (0.25 mg/kg, i.v.) completely abolished gastric acid production induced by these agonists (P < 0.05, Dunnett's test) (Table 1).

DISCUSSION

A number of clinical and experimental studies on the significance of the CNS with respect to the pathogenesis of gastrointestinal lesions or ulcers have been studied (13–17). Modified sham feeding seems to adequately reflect normal physiological vagal activation of gastric secretion (18), which was confirmed by the observation that complete vagotomy abolished the acid response to sham feeding (19, 20). The pathological mechanisms of stress ulceration have often been attributed to gastric hypersecretion (21–23). Pharmacological studies of brain neurotransmitters suggest that central GABAergic mechanisms are involved in the stimulation of gastric acid secretion in several species (7–11) and also participate in the aggravation of stress-induced ulceration.

It has been proposed that GABA receptors in the central and peripheral nervous systems are not homogeneous and can be divided into two subtypes: GABA<sub>A</sub> and GABA<sub>B</sub> (24). The results in the present study show that systemic administration of either an A or B type GABA agonist increased gastric acid secretion via activation of central cholinergic efferent mechanisms. The vagal efferent activation of PCPGABA, a GABA agonist acting on bicuculline-insensitive GABA<sub>B</sub> receptors (24), was the all or none type, but the acid secretory response to PCPGABA was dose-dependent (7). The vagal efferent response to PCPGABA at the dose of 4 mg/kg, s.c. preceded gastric acid secretion. The acid secretagogue response to PCPGABA was more potent than that in response to histamine or bethanechol (9). Muscimol, an agonist acting selectively at bicuculline-sensitive GABA<sub>A</sub> receptor sites (25), also caused an activation in vagal efferent transmission more rapidly than PCPGABA, followed by gastric hypersecretion which is in accordance with previous findings using pylorus ligated rats (26). Although
the degrees of their effects on vagal activities were almost the same, PCPGABA was a more potent secretagogue than muscimol. The difference in the latency and potency of the responses between PCPGABA and muscimol may be explained by the difference in the administration route.

The precise mechanism and site of action by which PCPGABA increases vagal nerve activity is still a subject of speculation. Our results, in which the systemically administered anti-cholinergic drug, scopolamine, inhibited both the vagal activation and the gastric hyperacidity induced by PCPGABA, suggest that the PCPGABA-mediated response is cholinergic. Methscopolamine, a quaternary derivative of scopolamine that does not cross the blood brain barrier (27), suppressed the acid secretion, but did not change the vagal efferent discharge. This may indicate that vagal activations by GABA agonists involve central vagal-cholinergic mechanisms and the inhibitions of gastric acid secretion were due to the blockade of muscarinic receptors at the peripheral site. This finding, however, does not always indicate that cholinomimetic factors are definitely involved in PCPGABA-induced vagal activation at the central site. Recent reports (28, 29) have suggested that the paraventricular nucleus of the hypothalamus may be involved in

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![Fig. 5. Effect of scopolamine (Scop) and methscopolamine (MeScop) on excitatory responses of vagal nerve to PCPGABA at 4 mg/kg, s.c. (A) or muscimol (Mus) at 0.3 mg/kg, i.v. (B). Each antagonist was given intravenously at a dose of 0.25 mg/kg.](image-url)
the central regulation mechanisms of gastric secretion. Furthermore, it was reported that central muscarinic mechanisms existed within the dorsal complex for excitation of gastric functions (30) and that there are indirect polysynaptic fibers connecting the hypothalamus to the dorsal nucleus of the vagus (31).

In order to clarify the involvement of the subtypes of GABA receptors, we investigated if vagal activation and secretagogue action were challenged by a GABA antagonist, bicuculline or picrotoxin. The doses used here are adequate for the pharmacological actions of these drugs, since repetitive high voltage spikes and wave complexes in EEG have been reported (32) at the same doses. Indeed, bicuculline (0.25 mg/kg, i.v.) and picrotoxin (0.5 mg/kg, i.v.) caused slight physical convulsions in this study and it did not antagonize the GABA agonists-induced vagal activation and gastric acid hypersecretion. When given subcutaneously at larger doses, both bicuculline and picrotoxin inhibited PCPGABA-induced acid hypersecretion (33) but not vagal activation (Y. Goto et al., unpublished data) coincident with marked convulsion. High doses of bicuculline and picrotoxin also abolished bethanechol-induced gastric acid secretion. These inhibitions of acid secretion by large doses of the GABA antagonists might be attributable to a non-specific effect, because of serious convulsion followed by respiratory insufficiency. Moreover, it has been recently shown that the GABA\textsubscript{A} selective antagonists 5-aminoovaleric acid (34) and phaclofen (33) had no effect on PCPGABA induced gastric secretion. The reason why picrotoxin did not antagonize PCPGABA induced vagal activation in this study is not understood at present.

In conclusion, the present study suggests that the cholinergeric mediation of GABA mimetic-induced gastric stimulation is of central origin to regulate the parasympathetic tones, whereas the GABA mimetic-induced stimulation of vagal nerve activity is refractory to GABA\textsubscript{A} and GABA\textsubscript{B} receptor antagonists. Further studies must be performed to elucidate the sites and mechanisms of actions of the vagal activation induced by GABA agonists.

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