β-Phenyl-β-Alanine Prevents the Activation of Vagal Efferent Discharges Evoked by Baclofen and GABA in Rats

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ABSTRACT — The effects of DL-β-phenyl-β-alanine (BPBA) on vagal efferent discharges elicited by γ-aminobutyric acid (GABA) and baclofen were investigated in rats. When given alone, BPBA (40 mg/kg, i.v.) caused no significant change in vagal nerve response and did not elicit any convulsions. Pretreatment with BPBA (40 mg/kg, i.v.) resulted in 70% and 80% reductions in the vagal efferent discharges induced by GABA (400 mg/kg, i.v.) and baclofen (4 mg/kg, s.c.), respectively. The present results suggest that BPBA may be a novel GABA antagonist with respect to vagal activation mechanisms in the CNS.

Keywords: GABA, Baclofen, β-Phenyl-β-alanine (BPBA)

γ-Aminobutyric acid (GABA)-mimetics injected systemically have been shown to produce significant increases in gastric acid secretion in a number of species including rats (1, 2), dogs (3) and humans (4); and these secretagogue effects were totally abolished by truncal vagotomy and atropine (1, 2). Moreover GABA at acid secretagogue doses caused a marked activation of vagal efferent discharges at the cervical level (1). Although GABA actions in the CNS are strongly implicated in these effects, until now, the underlying mechanisms, including receptor subtypes, have been unclear (5).

Intravenous administration of DL-β-phenyl-β-alanine (BPBA) has recently been shown to prevent baclofen-induced gastric acid secretion without altering the response to bethanechol in stomach perfused rat (6, 7). However, the general pharmacodynamic profile of BPBA is poorly described. Studies employing the monitoring of vagal efferent discharges are important for determining the site of the blocking effect of BPBA with respect to gastric acid secretion, whether it be in the periphery or at a central site. Therefore the aim of the present study was to determine whether the systemic administration of BPBA could prevent the baclofen-induced activation of vagal efferent discharges recorded at the cervical level and whether such antagonism by BPBA is specific for baclofen or not.

Adult male Wistar/ST-strain rats weighing 180−220 g were used in this experiment. They were housed in a controlled environment, exposed to a 12-hr light-dark cycle and fed with standard rodent chow and water ad libitum for at least 1 week before initiation of the experiment. Rats were fasted for at least 18 hr prior to each experiment but allowed free access to water.

Rats were anesthetized with urethane (1.25 g/kg, i.p.). The cervical skin was incised, and a tracheotomy was performed, followed by exposure of the vagal trunks. The cavity was then filled with warm paraffin. The left vagus nerve was mounted on a bipolar platinum electrode for recording vagal efferent activity. The peripheral portion of the nerve was crushed to exclude the possibility of contamination by afferent discharges. Using a biophysical amplifier, the neural activity was displayed on an oscilloscope and averaged with a window discriminator, which could distinguish impulses of efferent discharges from background noise. The rate of firing in spikes per second was displayed on a rectilinear recorder.

We tested the following drugs: GABA (Nacalai Tesque, Ltd., Japan), baclofen (Ciba-Geigy, USA) and DL-β-phenyl-β-alanine (BPBA, Aldrich Chem. Co., Inc., USA). GABA was intravenously administered at a dose of 400 mg/kg. Baclofen was injected sub-
cutaneously at a dose of 4 mg/kg. These doses have previously been shown to produce maximal activation of acid secretagogue effects and vagal nerve activity in rats (1, 8). A bolus intravenous administration of BPBA (5–40 mg/kg) was given 10 min prior to baclofen or GABA.

The ANOVA followed by Dunnett’s test was used for statistical analysis of the data.

Figure 1 shows typical extracellular recordings of vagal efferent potentials in the rat. The rate of spontaneous firing before medication could be minimized. Following baclofen (4 mg/kg, s.c.) stimulation, long-lasting potent vagal activation was obtained. The activation of vagal nerve discharges was detected 5 min after baclofen (4 mg/kg, s.c.) and peaked at 20 min with a prolonged duration of more than 90 min. The injection of BPBA (5–40 mg/kg, i.v.) did not significantly alter the spontaneous firing activity of vagal efferent neurons, but dose-dependently prevented the neural activation induced by baclofen (4 mg/kg, s.c.) (Table 1). No convulsions were observed at these doses of BPBA.

GABA (400 mg/kg, i.v.) likewise caused activation of the vagal efferent discharges. Efferent spikes were elicited within 5 min after GABA administration, and the frequency subsequently increased until burst-like

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**Table 1.** BPBA-mediated antagonism of GABAergic ligands-induced excitation of vagal efferent neuronal activity in rats

<table>
<thead>
<tr>
<th>Dose (mg/kg, i.v.)</th>
<th>Maximum response of vagal efferent activity (spikes/sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baclofen (4 mg/kg, s.c.)</td>
</tr>
<tr>
<td>Alone</td>
<td>134 ± 18 (8)</td>
</tr>
<tr>
<td>BPBA</td>
<td>88 ± 15 (6)</td>
</tr>
<tr>
<td>10</td>
<td>39 ± 23 (6)</td>
</tr>
<tr>
<td>15</td>
<td>ND</td>
</tr>
<tr>
<td>20</td>
<td>36 ± 15 (6)*</td>
</tr>
<tr>
<td>40</td>
<td>23 ± 12 (6)*</td>
</tr>
</tbody>
</table>

Values are means ± S.E. Values in parentheses are number of experiments. BPBA (p-β-phenyl-β-alanine) was injected intravenously 10 min prior to GABAergic ligands. *: Statistically significant at P < 0.05 vs. alone group (ANOVA followed by Dunnett’s test). ND: no data.
firing was observed at 20 min after administration (Fig. 1). BPBA also significantly blocked vagal activation by GABA by 69% at a dose of 40 mg/kg, i.v. (Table 1).

The main finding of the present study was the ability of BPBA to suppress the parasympathetic efferent activation induced by baclofen. The inhibitory effect of BPBA was dose-dependent.

The central nervous system is known to play a major role in regulation of gastric acid secretion, where the cephalic phase of gastric acid secretion is mediated by the vagus nerves. Although GABA is a major inhibitory neurotransmitter in the central nervous system (9), nevertheless it has been shown to augment acid secretion in the stomach (1), which raises the possibility that GABA may act as a neurotransmitter that activates vagal efferents, resulting in an increase of gastric acid secretion.

Although the mechanisms through which GABA stimulates acid secretion are not yet completely understood, it seems that a central cholinergic pathway is involved in the secretagogue mechanisms, since these responses were antagonized by pretreatment with atropine and by truncal vagotomy (1). However, there is little information on the correlation between subtypes of GABA receptors and gastric acid response.

We have recently shown that certain GABA mimetic ligands such as isoguvacine, a GABA_A agonist, and 5-aminovaleric acid, a weak GABA_A agonist and a GABA_B antagonist, also stimulate gastric secretion with a potency similar to or greater than that of GABA (2). Baclofen seems to be one of the most potent in stimulating gastric secretion (2). Moreover, intracisternal baclofen (8) at a dose of 2 μg, which is only about one to five hundredths of that used peripherally (2), produces an increase in gastric acid secretion, suggesting that the site of action for baclofen may be located within the CNS. We recently found that phaclofen, a GABA_B-receptor antagonist, has no significant effect on baclofen-induced acid response, and also that a specific GABA_A-receptor antagonist, bicineulline, has no influence on the acid response to baclofen (6). These suggest that the acid stimulatory effect of baclofen might be mediated through non-GABA_A and non-GABA_B-receptors (5). We therefore attempted to find specific or novel types of GABA-receptor antagonists among GABA-related compounds to suppress the acid secretagogue response to baclofen. BPBA was found to reduce the secretagogue action of baclofen dose-dependently (6, 7). BPBA did not inhibit bethanechol-induced gastric acid secretion (6, 7). This suggests that BPBA does not interact with peripheral acetylcholine receptors. However, the possibility that BPBA prevents the baclofen-induced increment in gastric acid secretion by some mechanism within the intragastric nerve plexus cannot be excluded. Thus, the principal aim of the present study was to determine if BPBA blocks baclofen-induced vagal efferent discharges, recorded at the cervical level.

Intravenous injection of BPBA at a dose of 40 mg/kg, i.v. resulted in a significant reduction of vagal efferent discharges evoked by baclofen, which was confirmed to be a sufficient dose to suppress the acid output induced by baclofen (5, 6). BPBA also prevented the vagal response to GABA. These inhibitory effects of BPBA were dose-dependent, with similar potencies against GABA and baclofen. Matheson showed that BPBA had an affinity for the GABA binding site in membranes from cat cerebral cortex with an IC50 value of 35 μM (10). Thus it appears that BPBA acts as an antagonist of the GABA receptor mediating vagal activation which leads to gastric acid hypersecretion. BPBA did not cause convulsions, thus indicating that BPBA might exert a mode of action different from that of bicuculline and picrotoxin. The interaction between BPBA and GABA receptor subtypes in the CNS should be studied further.

In conclusion, we present evidence that BPBA prevents the vagal efferent activations evoked by baclofen and GABA.

REFERENCES

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