Activation by Systemic GABA of Vagal Efferent Transmission in the Rat: Correlation to Its Acid Secretagogue Action

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Abstract—Stimulatory effects of systemically administered gamma-aminobutyric acid (GABA) on gastric acid secretion and vagal efferent activity were studied in anesthetized rats. Intravenous injection of GABA (400 mg/kg) significantly increased gastric acid secretion. The secretagogue effect of GABA on the gastric acid secretion was partially attenuated by vagotomy by approximately 60%. Atropine (1 mg/kg, s.c.) completely abolished the stimulatory effect of GABA on the acid output. GABA, at secretagogue doses, enhanced the firing rate of vagal efferent activity. These results suggest that the secretagogue action of intravenously injected GABA is primarily mediated by muscarinic mechanisms and that the stimulation of the central vagal efferent pathway might trigger the excitatory action of systemic GABA.

The role of GABA as a major inhibitory neurotransmitter in the central nervous system has become more appreciated (1). However, in the peripheral site, it has been reported that GABA has significant stimulatory actions on enteric neurons of the ileum (2) and rodent myenteric plexus (3, 4). In addition, the stimulatory effect of GABA and GABA-mimetics on gastric secretion have also been observed in several species such as rats (5), dogs (6) and humans (7). Recent findings that GABA evoked release of 3H-ACh from isolated guinea pig gastrointestinal tract (8) and that GABA may stimulate gastrin release and inhibit somatostatin release from rat antral mucosal fragments (9) prompted us to suggest that GABA may cause hyperacidity through stimulation of postganglionic cholinergic neurons. Although some evidence has clearly demonstrated that GABA and GABA mimetics increase acid output, the sites of its action in the central and peripheral nervous system are not yet clearly defined. The present study was undertaken to investigate the effect of GABA on the gastric secretion; in particular, we determined whether the vagal parasympathetic central pathway may participate in the regulation of gastric acid secretion induced by systemic GABA in rats.

Materials and Methods

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.

Recording of truncal vagal efferent activity: Animals were anesthetized with urethane (1.25 g/kg, i.p.). The cervical skin was incised, and a tracheostomy was performed followed by the procedure for vagal truncal exposure. The cavity was then filled with warm paraffin. The left vagus nerve was mounted on bipolar platinum electrodes for recording of vagal efferent activity as described previously (10). The peripheral portion of the nerve was crushed to exclude the contaminant input of afferent discharges. Neural activity was analyzed in terms of firing rates; i.e., the number of nerve impulses every second. By means of a biological amplifier, the neural activity was displayed on an oscil-
loscope and averaged by using 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.

**Measurement of gastric acid secretion:** Animals were anesthetized with urethane (1.25 g/kg, i.p.). Tracheostomy was followed by the ligation of the upper esophagus to avoid the reflux of the perfusate. Laparotomy was followed by the ligation of the pylorus. Vagotomized animals were submitted to bilateral subdiaphragmatic truncal vagotomy; namely, seromyotomy at the cardiac portion of the esophagus. A dual polyethylene cannula was intubated 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 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., Japan) and continuously recorded as an every 2 min acid response via a zero suppression adaptor (TOA Electronics Co., Japan). Acid output was expressed as microequivalent of hydrogen ions per 10 min (μEq/10 min). In order to quantitate the acid output, we also calculated the integrated acid output for a 90-min period after the stimuli. All animals were allowed a 20-min pre-perfusion, at which time spontaneous basal secretion was lowered and constant, and received a bolus intravenous injection of GABA (400 mg/kg) with or without vagotomy. Gastric acid secretion was continuously measured for more than 90 min. In a separate group, animals received atropine (1 mg/kg, s.c.) 20 min before administration of the bolus injection of GABA.

**Drugs:** Gamma-aminobutyric acid (GABA) and atropine were purchased from Nakarai Chem. Co., Japan. Test drugs were dissolved in saline. These compounds were administered in a volume of 0.1 or 0.2 ml/100 g body weight.

**Statistical analysis:** One-way analysis of variance (ANOVA) with Dunnett’s multiple comparison procedure was used to compare the control and each data for each time group of gastric acid secretion. Comparison of pre- and post-treatment of drug effect was analyzed by the two-tailed paired Student’s t-test. The term “significant” is used to describe difference with a P<0.05 by use of a two-sided significance limit.
GABA on Vagal Efferent Discharges

Fig. 1. Effect of GABA on vagal efferent discharges in rats. A: Photographs from oscilloscope showing representative recordings of action potentials of efferent neurons of a parasympathetic nerve located in the cervical region following intravenous injection of GABA (400 mg/kg). Horizontal calibration is 20 msec and vertical calibration is 20 μV. B: Representative recording of time course of vagal efferent discharges. A recording like this one shown in B was A/D converted by a window discriminator every 10 sec, and these values represent the frequency (impulses/sec).

Fig. 2. Dose-response and time course relationship of intravenous GABA to spontaneous vagal efferent discharges in rats. Each column represents the mean value of activity counts averaged over a 5-min period for 6–7 experiments. Saline was injected in a volume of 0.2 ml/100 g of body weight.
Fig. 3. Reduction of systemically administered-GABA induced gastric hyperacidity by vagotomy or atropine in rat. A bolus intravenous injection of GABA was delivered at the time indicated by the arrow. Gastric acid secretion induced by GABA was decreased substantially by vagotomy, and it was abolished completely by atropine (1 mg/kg, s.c.). Each data indicates the mean±S.E.M. of hydrochloride equivalents secreted/10 min. *P<0.05 vs. GABA alone by Dunnett's test. #P<0.05 and ##P<0.01 vs. pre-value by the paired t-test. N=6-7.

Fig. 4. Effect of vagotomy and atropine on GABA (400 mg/kg, i.v.) induced gastric hyperacidity in stomach perfused rats. Gastric acid output was accumulated up to 90 min after GABA. Each column indicates the mean±S.E.M. of the increment of acid output. *P<0.05 vs. the control group by Dunnett’s test. N=6-7.

gastric output in the vagotomized rat occurred at 50 min. Pretreatment with atropine at a dose of 1 mg/kg, s.c., completely abolished the gastric acid production in response to GABA (400 mg/kg).

The effect of vagotomy and atropine on accumulative gastric acid output up to 90 min during GABA-induced hypersecretion is shown in Fig. 4. Vagotomy significantly suppressed the gastric acid response to GABA by 59%. Atropine inhibited the gastric acid secretion by 93%. The cumulative incline in gastric acid output for 60 min after GABA alone (control), GABA with vagotomy, and GABA plus atropine were 26.1±3.8, 10.6±2.6 (P<0.05 vs. control, Dunnett’s test), and 2.2±1.7 (P<0.05 vs. control, Dunnett’s test), respectively; and the degree of inhibition did not differ from that during the 90 min period.

Discussion

The results of the present study demonstrate that peripherally administered GABA leads to parasympathetic efferent activation which appears to be associated with hypersecretion of acid; this effect could be antagonized by a cholinergic antagonist such as atropine. Vagal activation by GABA occurred prior to hyperacidity, suggesting that GABA may play an essential role to trigger the secre-
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The central nervous system is known to play a major role in regulation of acid secretion and ulcer formation (11). The cephalic phase of gastric acid secretion is mediated by the vagus nerves. It has been shown that electrical stimulation of the lateral hypothalamus (LH) increases gastric acid secretion, and vagotomy abolishes the increased gastric acid secretion due to LH stimulation (12). Furthermore, lesions of the LH lead to a decrease in gastric acid secretion (13). ACh given into the LH induced a remarkable increment in gastric acid output by a mechanism involving the cholinergic muscarinic system (14). The dorsal motor nucleus of the vagus (NDV) contains cell bodies of the efferent cholinergic component, and the neurotransmission of the LH-NDV descending pathway in excitatory regulation of gastric functions has been shown (15). GABA is a primary neurotransmitter shown to play a major role in regulating acid secretion in the stomach. Evidence for the potential physiological significance of GABA in the regulation of gastric acid secretion comes from the observations that GABA neurons are immunohistochemically identified in the hypothalamus (16, 17). These data raise the possibility that GABA may act as a neurotransmitter which activates the vagal efferent, resulting in an increase of gastric acid secretion. The major point addressed in the present study was to determine if GABA causes facilitating actions within the parasympathetic efferent discharges.

As a critical step in assessing the activation of parasympathetic transmission, we examined the influence of intravenous GABA on vagal responses. Intravenous injection of GABA caused a marked facilitation in vagal efferent discharge, and the stimulatory effect was rapid in onset and dose-dependent, suggesting that GABA may have a role as a neurotransmitter to modulate vagal parasympathetic outflow.

Onset of vagal efferent firing induced by GABA was observed within 5 min and had a prolonged duration of more than 90 min, which coincided with the prolonged type of gastric acid response. The time course of gastric acid secretion by GABA was correlated with that of vagal excitation. Furthermore, pretreatment with atropine abolished the gastric acid stimulatory effect of GABA, suggesting that the mechanisms of acid hypersecretion by GABA may involve muscarinic receptors in the final process.

Vagal activation by GABA was, however, not altered by pretreatment with atropine, suggesting that the mechanism of activation of cholinergic transmission may involve a central site, but not a peripheral site.

It has been generally accepted that systemically administered GABA seldomly penetrates the blood-brain barrier (18). The possibility that a small amount of GABA could penetrate into the central nervous system can not, however, be excluded. Recently, i.c.v. administration of the GABA<sub>A</sub> agonist muscimol at the dose of 1 μg, which is only about one hundred thousandth that used in present study, was shown to produce an increase in gastric acid secretion (19). In our preliminary study, a lipophilic derivative of a GABA<sub>B</sub> agonist baclofen, also increased gastric acid output at an intracisternal dose of 2 μg, which is about four hundredth of the peripheral effective dose (4 mg/kg, s.c.) (20). This stimulatory effect was abolished by atropine, cimetidine and truncal vagotomy (6). These findings support the concept that GABA agonists act within the central nervous system to modify the regulation mechanisms of gastric acid secretion. However, there is little information on the correlation between subtypes of GABA receptors and gastric acid response, and it is unclear whether these two classes of agonists have similar effects on gastric function.

The present study did not define the site of action of GABA in the brain and also precise mechanisms of its action. The rapid onset of vagal activation by GABA and the suppression of its secretagogue action by highly selective surgical vagotomy may suggest that neural activation to the stomach might be crucial for GABA action. Recently, GABA and GABAergic synapses, however, have also been identified in peripheral organs (21, 22), and the involvements of peripheral GABAergic mechanisms have become appreciated (22, 23). The possibility that GABA might affect gastric acid secretion via a peripheral action was suggested by in vitro studies in which GABA
affected the release of gastrin, somatostatin, and acetylcholine from rat gastric antrum (9, 23). Initial studies demonstrated GABA receptors on peripheral autonomic nerve terminals in the small intestine (24). More recently, many investigators have begun to describe peripheral GABAergic functions (27-31). The presence of GABA and a high-affinity transport system for GABA in guinea pig gallbladder and the demonstration of neural release of GABA suggest that GABA is a neurotransmitter in the gallbladder (32). Harty and Franklin (33) have reported that GABA acted on postganglionic neurons to alter the release of antral hormones through stimulation of the cholinergic neurotransmitter acetylcholine. Recently, it has been reported that GABA induced acid secretion in isolated everted whole guinea pig stomach via the A type of GABA receptor (34). The present studies, including vagal efferent discharges and vagotomy, suggest that GABA is capable of stimulating central vagal-cholinergic mechanisms to affect gastric acid secretion. This result, however, do not exclude the possibility that peripheral cholinergic mechanisms may also be implicated in the action of GABA, because bilateral truncal vagotomy did not totally suppress the secretagogue action. Surgical vagotomy could not abolish the gastric acid response completely, but reduced it by 59%. This suggests that GABA may affect physiological functions via both central and peripheral mechanisms and that central vagal outflow to the stomach may contribute to approximately 60% of GABA-induced hypersecretion.

In conclusion, GABA may play a role in regulating gastric acid secretion by at least two mechanisms: one is mediated by central parasympathetic outflow and the other by vagal activation at the level of the intragastric nerve plexus.

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