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Inhibitory Effects of B-HT 920 on Gastric Acid Secretion Induced by Vagal Stimulation in Rat

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Abstract□Effects of B-HT 920 on the vagally stimulated gastric acid secretion were studied in anesthetized and gastric fistula rats. When the gastric acid secretion was increased by stimulation of the vagus nerve, B-HT 920 significantly inhibited the vagally induced gastric acid secretion. This inhibitory effect of B-HT 920 was partially attenuated by prazosin, α_1-adrenoceptor antagonist and virtually abolished by yohimbine, α_2-adrenoceptor antagonist. On the other hand, when the gastric acid secretion was increased by the infusion of bethanechol, a muscarinic parasympathetic stimulant, B-HT 920 had no effect on the bethanechol-induced gastric acid secretion. These results suggest that B-HT 920 inhibits vagally induced gastric acid secretion by activation of presynaptic α-adrenoceptors located on the vagally stimulated pathways in the gastric wall and this effect of B-HT 920 is more related to α_2-adrenoceptors than α_1-adrenoceptors.

Keywords□B-HT 920, Vagally stimulated gastric acid secretion, Gastric fistula rat, Presynaptic α-adrenoceptor.

B-HT 920 causes a biphasic change, initial hypertension followed by a prolonged hypotension, in mean arterial blood pressure and a sustained bradycardia in the cat[1]. The initial hypertensive effect results from stimulation of sympathetic peripheral α-adrenoceptors[2], and prolonged hypotension is due to the reduction in sympathetic discharge induced by stimulation of α-adrenoceptors at the level of brain stem, and the simultaneous sustained bradycardia results from both the reduction in sympathetic discharge and the enhancement of vagally mediated reflux[1,3]. Peripherally, B-HT 920 inhibits tachycardic response by activating presynaptic α-adrenoceptors in pithed rats[2]. From these pharmacological features, B-HT 920 has been considered to be acting as typical "clonidine-like drug"[1,2].

There are contradictory reports about the effects of clonidine-like drugs on gastric acid secretion. Inhibitory effects of clonidine on the acid secretion have been reported in animal[4-7] and in human[8]. Pascaud and Roger suggested that the antisecretory effect of clonidine was of central origin since intracerebroventricular injection of clonidine inhibited gastric acid secretion induced by i.v. infusion of 2-deoxy-D-glucose[6]. In addition to this, Jennewein demonstrated that clonidine reduced gastric acid secretion induced by vagus nerve stimulation in vagi-sectioned rats, suggesting a peripheral site of action[7]. On the other hand, it has been reported that clonidine inhibited gastric acid secretion by both central and peripheral mechanism, and that clonidine also stimulated acid secretion by a stimulation of histamine H_2-receptors[9-11].

Recently Yokotani *et al.* proposed that splanchnic nerve stimulation inhibits bethanechol-induced gastric acid secretion through α_1-adrenoceptors and inhibits the vagally stimulated gastric acid secretion through α_2-adrenoceptors[12]. With these results, they suggested that α_1-adrenoceptors are located on the structures peripheral to the parasympathetic nerve terminals and α_2-adrenoceptors are located on the vagally stimulated pathway in the gastric wall.

B-HT 920, chemically quite different from clonidine, was found to be a selective α_2-adrenoceptor agonist and has been often used as a probe to aid classification of α-receptor subtypes[2,14]. However, the effect of B-HT 920 on gastric acid secretion has not yet been demonstrated.

The purpose of the present study was to investigate further whether α-adrenoceptors related to the inhibition of neurotransmitter release are on parasympathetic nerve innervating the stomach, and to find out whether one or more types of α-adrenoceptors are involved in parasympathetic pathway, if any. For this study, effect of B-HT 920, a selective α_2-adrenoceptor agonist, was examined on gastric acid secretion induced by stimulation of vagus nerve and infusion of bethanechol in anesthetized and gastric fistula rats.

EXPERIMENTAL METHODS

Male Sprague-Dawley rats weighing 270-300 g were maintained at a room temperature of 22-24 °C and given food (laboratory chow, Sam-Yang Co. Seoul, Korea) and tap water *ad libitum*. Before each experiment, all food was withheld for 16 hr, but water was provided. Under urethane anesthesia (1.0 g/kg i.p.), both femoral veins were cannulated to administer drugs and the femoral artery was cannulated to record the systemic blood pressure. Details of the experimental procedure for measuring acid secretion were as described by Yokotani *et al.*[12,13] Briefly, the esophagus was carefully ligated at the cervical portion and the trachea was cannulated in case of bethanechol infusion. The abdomen was opened by a middle incision and a round-tip polyethylene cannula was inserted into the stomach via an incision in duodenum, and then the abdominal incision was sutured. The stomach was flushed with saline to remove solid contents. During this operation, care had been taken to avoid distention. After repeated washings, 2.0 m*l* of the gastric solution prewarmed at 38 °C was instilled in the stomach. The gastric solution was composed of 0.45 g of glycine and 5.47 g of mannitol which were dissolved in 100 m*l* of distilled water (adjusted to 300 m Osmol), and adjusted to pH 3.5 by addition of 0.1 N HCl, according to the method of Blair *et al.*[15]. After all these procedures, 1 hr was allowed to elapse before the start of each experiment and during this time the basal acid secretion had reached a steady level. The gastric solution was replaced with the fresh solution every 15 min. The gastric acid secretion was determined as follows; the total volume of the gastric solution recovered from the stomach every 15 min and 2.0 m*l* of gastric fresh solution (pH 3.5) were titrated to pH 7.0 with 0.01 N NaOH, using a pH meter. By subtracting the latter from the former titration, acid contents secreted for 15 min were calculated and expressed as microequivalents/15 min. Blood pressure was recorded from femoral artery by a pressure transducer (Narco RP-1500) connected to strain gauge coupler (Narco 7179) on physiograph recorder (Narco MK-III-P).

After stabilization of the basal acid secretion, the gastric acid secretion was parasympathetically stimulated. In the first series of experiments, the acid secretion was stimulated by continuous stimulation of vagus nerve after the left and right vagus nerve were carefully separated from the carotid artery and cut centrally at the cervical level. The peripheral end of the vagus nerve placed on bipolar platinum electrodes was stimulated continuously throughout the experiments by square-wave pulses of 0.5 msec duration, 3 Hz, 9 V. In the second series, gastric acid secretion was stimulated by i.v. infusion of supramaximal dose of bethanechol (10.0 μg/kg/min) through the right femoral vein. In all experiments, two successive collections were carried out before stimulation of the vagus nerve or i.v. infusion of bethanechol to ascertain the basal level of acid secretion.

B-HT 920 (30 μg/kg) was infused for 30 min through the left femoral vein to observe effects on gastric acid secretion evoked by vagal stimulation or infusion of bethanechol. Prazosin (1.0 mg/kg) or yohimbine (2.0 mg/kg) was given i.p. 30 min before the start of stimulation of the vagus nerve or infusion of bethanechol.

The following drugs were used: B-HT 920 (Boehringer Ingelheim), prazosin hydrochloride (Taito-Pfizer Co.), yohimbine hydrochloride (Nakarai Chemicals Co.) and atropine sulfate (Wako pure Chemicals).

Because the absolute values of acid secretion varied with the individual animal, the effects of infusion of B-HT 920 on the parasympathetically stimulated gastric acid secretion were expressed as a percentage of the values of control collection period immediately before such treatments. The results given are the mean ± S.E. Statistical significance was compared with the values of corresponding control rats using Student's *t* test for unpaired comparisons.

RESULTS

Effect of B-HT 920 on gastric acid secretion induced by stimulation of the vagus nerve

Mean basal acid secretion in 6 rats under urethane anesthesia (1.0 mg/kg) was 8.82 ± 0.5 μEq/15 min. In the preliminary studies, the stimulations of

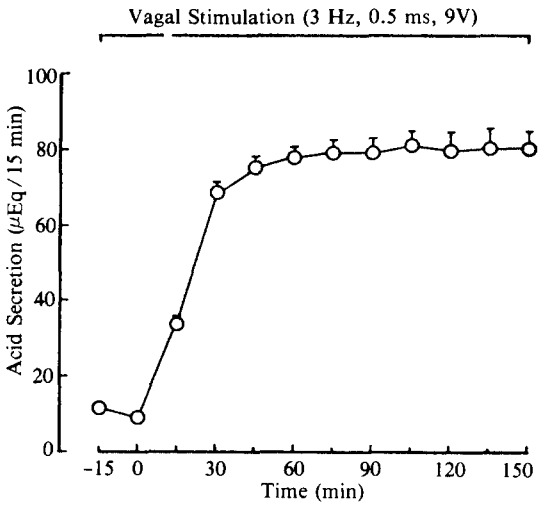

Fig. 1. Gastric acid secretion induced by stimulation of vagus nerve in rats.
Vagus nerve was stimulated at 3 Hz and gastric juice was collected every 15 min. Each value represents the mean ± S.E. of 6 rats.

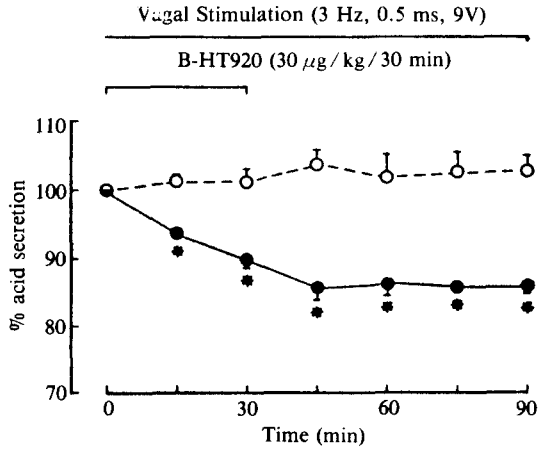

Fig. 2. Effect of B-HT 920 on gastric acid secretion induced by stimulation of vagus nerve.
B-HT 920 was infused i.v. for 30 min from 60 min after the start of stimulation of vagus nerve. Values at different collection periods are expressed as percentage of the value obtained at control collection period (0). Each point represents the mean ± S.E. of 6 rats. ○, control rats; ●, B-HT 920-infused rats (30 μg/kg/30 min). * $p<0.01$ (significantly different from the values obtained at the corresponding period in control rats). The absolute values at control collection period (0) were 78.0 ± 3.13 μEq/15 min for control rats and 72.8 ± 5.9 μEq/15 min for B-HT 920-infused rats, respectively.

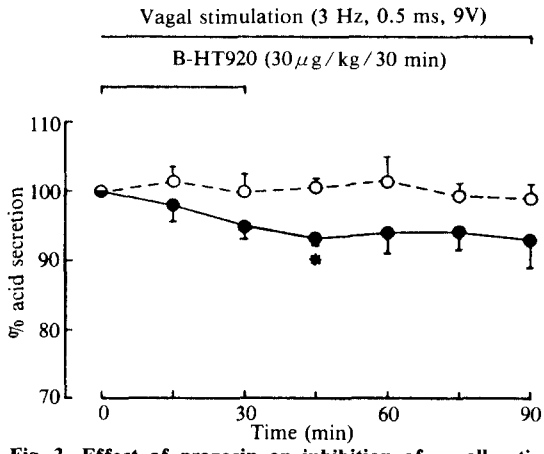

Fig. 3. Effect of prazosin on inhibition of vagally stimulated gastric acid secretion by B-HT 920.
Prazosin was administered i.p. 30 min before the start of stimulation of vagus nerve. Other conditions were as for Fig. 2. ○, Prazosin (1.0 mg/kg)-treated control rats; ●, B-HT 920 (30 μg/kg/30 min)-infused rats with prazosin (1.0 mg/kg). Each point represents the mean ± S.E. of 5 rats. The absolute values at control collection period (0) were 69.7 ± 5.1 μEq/15 min for control rats and 65.0 ± 4.8 μEq/15 min for B-HT 920-infused rats, respectively.

the vagus nerve (1 to 20 Hz for 10 min) caused frequency-dependent increases of gastric acid secretion. When the vagus nerves were continuously stimulated at 3 Hz, the gastric acid secretion gradually increased and reached a steady level within 60 min, and this steady level was maintained for at least 120 min (Fig. 1). The mean arterial blood pressures before and 60 min after stimulation of the vagus nerve at 3 Hz were 83.7 ± 3.1 mmHg and 83.0 ± 2.6 mmHg (n = 8), respectively. This vagally stimulated gastric acid secretion was abolished by atropine (0.1 mg/kg i.v.) or hexamethonium (2.0 mg/kg i.v.).

When B-HT 920 (30 μg/kg i.v.) was infused for 30 min from 60 min after the start of the vagus nerve stimulation, the gastric acid secretion induced by stimulation of the vagus nerve was significantly reduced (Fig. 2). This inhibitory effect of B-HT 920 reached the maximum at 45 min (reduced to 85.79 ± 2.01% of preinfusion level of B-HT 920) and persisted for more than 45 min.

Effects of prazosin and yohimbine on inhibition of the vagally stimulated gastric acid secretion by B-HT 920

In order to determine whether the inhibitory effect of B-HT 920 on vagal stimulation-induced gas-

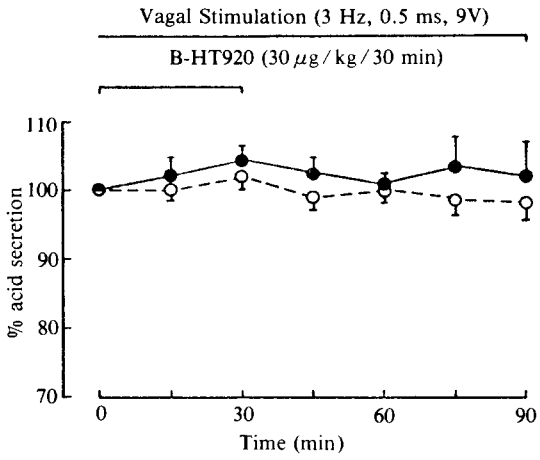

Fig. 4. Effect of yohimbine on inhibition of vagally stimulated gastric acid secretion by B-HT 920.
Yohimbine was administered i.p. 30 min before the start of stimulation of vagus nerve. Other conditions were as for Fig. 2. ○, Yohimbine (2.0 mg/kg)-treated control rats; ●, B-HT 920 (30 μg/kg/30 min)-infused rats with yohimbine (2.0 mg/kg). Each point represents the mean ± S.E. of 4 rats. The absolute values at control collection period (0) were 74.0 ± 6.2 μEq/15 min for control rats and 83.8 ± 5.4 μEq/15 min for B-HT 920-infused rats, respectively.

tric acid secretion was involved in α-adrenoceptor activation, the effect of B-HT 920 was investigated in the presence of α-adrenoceptor antagonists. When yohimbine (2.0 mg/kg i.p.) or prazosin (1.0 mg/kg i.p.) was administered 30 min before stimulation of the vagus nerve, inhibitory effect of B-HT 920 on vagal stimulation-induced gastric acid secretion was partially antagonized by prazosin (Fig. 3) whereas it was virtually abolished by yohimbine (Fig. 4). The inhibition of gastric acid secretion induced by B-HT 920 was reduced by 52% at 45 min after infusion of B-HT 920 in prazosin-treated rats.

Effect of B-HT 920 on gastric acid secretion induced by infusion of bethanechol

To determine whether inhibitory effect of B-HT 920 on the gastric acid secretion induced by stimulation of vagus nerve was caused by actions on postsynaptic α-adrenoceptors in the gastric wall, the influence of B-HT 920 on gastric acid secretion induced by bethanechol infusion was examined. After the start of infusion of bethanechol (10.0 μg/kg/min i.v.), the gastric acid secretion gradually increased and reached a steady level within 90 min

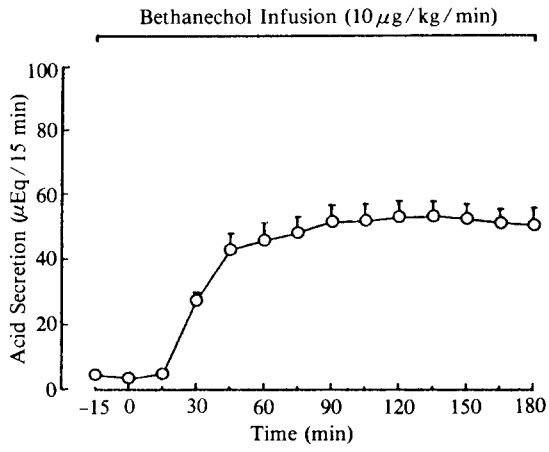

Fig. 5. Gastric acid secretion induced by bethanechol infusion in rats.
Bethanechol was infused at 10 μg/kg/min i.v. and gastric juice was collected every 15 min. Each value represents the mean ± S.E. of 4 rats.

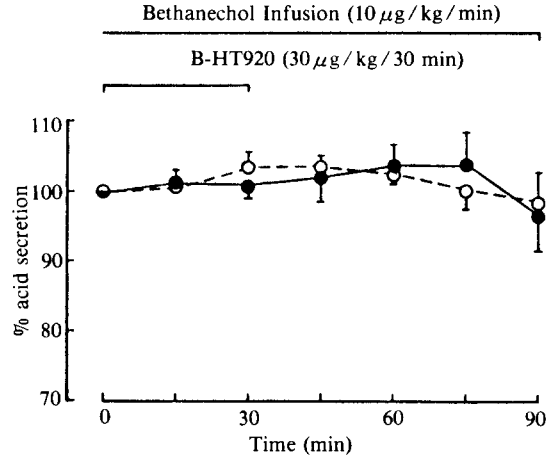

Fig. 6. Effect of B-HT 920 on gastric acid secretion induced by infusion of bethanechol.
B-HT 920 was infused i.v. for 30 min from 90 min after the start of bethanechol infusion. Other conditions were as for Fig. 2. ○, control rats; ●, B-HT 920-infused rats (30 μg/kg/30 min). Each point represents the mean ± S.E. of 5 rats. The absolute values at control collection period (0) were 51.6 ± 5.1 μEq/15 min for control rats and 52.9 ± 5.5 μEq/15 min for B-HT 920-infused rats, respectively.

(Fig. 5). Infusion of B-HT 920 (30 μg/kg i.v.) was carried out for 30 min from 90 min after the beginning of bethanechol infusion. The bethanechol-induced gastric acid secretion was abolished by atropine (0.1 mg/kg i.v.), but was not influenced

by hexamethonium (2.0 mg/kg i.v.). In this experiment, infusion of bethanechol (10.0 μg/kg/min) produced a transient fall in the mean arterial blood pressure from 82.5 ± 3.5 to 75.3 ± 2.0 mmHg ($n = 7$), subsequently returned to the preinfusion level.

When B-HT 920 (30 μg/kg i.v.) was infused for 30 min, there was no effect on the gastric acid secretion stimulated by infusion of bethanechol (Fig. 6).

DISCUSSION

In this study, two different procedures have been used to induce parasympathetic stimulation of gastric acid secretion. One is stimulation of the cervical vagus nerve, which involves the entire pathway of parasympathetic nerve from the preganglionic parasympathetic nerve to the parietal cells. The other is the infusion of bethanechol, which stimulates the postganglionic parasympathetic effector systems in the gastric wall. To confirm the existence of α-adrenoceptor-mediated inhibitory mechanisms, B-HT 920, a selective α_2-adrenoceptor agonist, was administered under conditions in which gastric acid secretion was elevated by vagus nerve stimulation or bethanechol infusion. In anesthetized and gastric fistula rats, the gastric acid secretion induced by low frequency of vagal stimulation was significantly inhibited by B-HT 920 (Fig. 2). This inhibitory effect of B-HT 920 on the gastric acid secretion induced by stimulation of vagus nerve was virtually abolished by yohimbine, a relatively selective α_2-adrenoceptor antagonist[16], whereas it was partially antagonized by prazosin, a highly selective α_1-adrenoceptor antagonist[17] (Fig. 3,4). These results suggest that the inhibitory effects of B-HT 920 would be more related to α_2-adrenoceptors than α_1-adrenoceptors since B-HT 920 has a higher selectivity for α_2-adrenoceptors than α_1-adrenoceptors[2,3]. On the other hand the gastric acid secretion induced by infusion of bethanechol, a muscarinic parasympathetic stimulant, was not decreased by B-HT 920. From these results, B-HT 920 seems to inhibit parasympathetic transmission in the rat stomach not by actions on postsynaptic α-adrenoceptors in the gastric wall, but by a decrease of the liberation of acetylcholine from pre- and/or postganglionic cholinergic nerve endings. No effort was made to distinguish between these possibilities.

In recent years evidences have been accumulated for existence of α_2-adrenoceptors on the cholinergic fiber endings that innervate the stomach[9,12,18]. It has been found that vagally stimulated gastric acid secretion was inhibited by clonidine[9,12], a relatively selective α_2-adrenoceptor agonist[19,20], and that this inhibitory effect was abolished by phentolamine[9] or yohimbine[12] but not by labetalol[9] or prazosin[12]. On the other hand, Hong and Sohn demonstrated that guanabenz, a clonidine-type substance, also reduced gastric acid secretion induced by vagus nerve stimulation, and that this inhibitory effect was blocked by yohimbine[18]. Thus, these observations may suggest that α_2-adrenoceptors are located on the parasympathetic nerve terminal to the stomach.

It is generally accepted that B-HT 920 acts on α_1-adrenoceptors though it is a selective α_2-adrenoceptor agonist at post- as well as presynaptic α-adrenoceptor sites[2,21], and that yohimbine is a relatively selective α_2-adrenoceptor blocker[16,22] but antagonizes both α_1- and α_2-adrenoceptors, with about 25-fold greater selectivity for the latter[23]. In the present experiment the inhibitory effect of B-HT 920 on vagally stimulated gastric acid secretion was abolished by yohimbine and partially antagonized by prazosin which is a selective α_1-adrenoceptor antagonist, indicating that the possible existence of α_1-adrenoceptors other than α_2-adrenoceptors on the vagally stimulated pathway in the gastric wall may be considered. Further studies are necessary to find out whether α_1-adrenoceptors other than α_2-adrenoceptors are involved in parasympathetic nerve pathway in the gastric wall.

In summary, the gastric acid secretion induced by low frequency of vagal stimulation was significantly inhibited by B-HT 920, whereas the gastric acid secretion induced by infusion of bethanechol was not decreased by B-HT 920. These results suggest that B-HT 920 inhibits vagally induced gastric acid secretion by activation of presynaptic α-adrenoceptors located on the vagally stimulated pathways in the gastric wall, and this action of B-HT 920 is related more to α_2-adrenoceptors than to α_1-adrenoceptors.

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