

Central Site of Inhibitory Action of Bombesin on Gastric Acid Secretion in Rats

Yasunobu OKUMA, Kunihiro YOKOTANI and Yoshitsugu OSUMI

Department of Pharmacology, Kochi Medical School,
Nankoku, Kochi 781-51, Japan

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Abstract—Sites of inhibitory action of bombesin on gastric acid secretion were examined in rats anesthetized with urethane. Intracerebroventricular administration of bombesin (3–1000 pmole) dose-dependently inhibited the increase in gastric acid secretion induced by electrical stimulation of the vagus nerve (1 mA, 0.5 msec, 3 Hz). On the other hand, intrathecal (direct lumbar puncture) administration of bombesin, even in the large dose of 1 nmole, had no effect on the vagally stimulated gastric acid secretion. Three pmoles of bombesin microinjected into the preoptic area, the anterior hypothalamus and the paraventricular nucleus inhibited the vagus stimulated gastric acid secretion. Microinjection of this peptide into the ventromedial nucleus, the dorsomedial nucleus and the lateral hypothalamic area were without effect. A large electrolytic lesion of the anterior hypothalamus, including the preoptic-anterior hypothalamic area and the paraventricular nucleus, abolished the inhibitory effect of intracerebroventricularly applied bombesin, but a lesion restricted to the preoptic-anterior hypothalamus or the paraventricular nucleus was without effect. We propose that the preoptic area, the anterior hypothalamus and the paraventricular nucleus are all involved in the inhibitory effect of bombesin on gastric acid secretion.

Central inhibitory effects of bombesin, a tetradecapeptide, on gastric acid secretion were first reported by Tache et al. in 1980 (1). This inhibition induced by intracisternal administration of bombesin was not vagally mediated and was abolished by spinal cord transection (1, 2). We found that the inhibition of gastric acid secretion induced by intracerebroventricularly (i.c.v.) applied bombesin was almost completely blocked by both bilateral cutting of the greater splanchnic nerves and bilateral adrenalectomy in 6-hydroxydopamine-pretreated animals (3). These findings suggested that this bombesin-induced inhibition of gastric acid secretion was due to a central activation of the sympatho-adrenomedullary system. However, the site of action of this peptide in the brain remains obscure.

In the present study, the central site of the inhibitory action of bombesin on gastric

acid secretion was examined in rats anesthetized with urethane.

Materials and Methods

Male Wistar rats weighing 250–300 g were maintained in a room at 22–24°C under a constant day-night rhythm for 7–10 days and given food (laboratory chow, CE-2, Japan Clea Co.) and tap water, ad libitum. Prior to each experiment, all food but not water was withheld for 16 hr. Under urethane anesthesia (1.1 g/kg, i.p.), the femoral vein was cannulated, and physiological saline was infused at a rate of 1.6 ml/hr. The abdomen was opened by a midline incision, and a round-tip cannula (5 cm long, 0.5 cm outer diameter) connected to a polyethylene tube was inserted into the stomach via an incision into the duodenum (1 cm distal from the pyloric sphincter). To remove solid contents, the stomach was flushed with

saline, taking care to avoid distention. After repeated washings, two ml of solution prewarmed to 38°C was placed in the stomach at the beginning of each 15 min collection period. The composition of this solution was a 1:5 (v/v) mixture of glycine and mannitol adjusted to 300 mOsmolar and pH 3.5 by the addition of 0.1 N HCl, according to Blair et al. (4).

Acid output was determined by titration of gastric samples to pH 7.0 with 0.01 N NaOH, using a pH meter. The animal was placed in a stereotaxic instrument.

To increase gastric acid output, the vagus nerve was electrically stimulated. The vagus nerves were then cut bilaterally at the cervical level; the peripheral end of the left vagus nerve was placed on a ring platinum electrode and stimulated continuously throughout the experiments. Stimulus parameters used were square-wave pulses of 0.5 msec duration, at 3 Hz, 1 mA, by means of an electronic stimulator (Model 3201, Nihon Kohden Ltd., Japan).

Bombesin (Peptide Institute, Inc., Japan) was dissolved in artificial cerebrospinal fluid. The composition of the artificial cerebrospinal fluid, as described by Falcon et al. (5), was 7.3 mg NaCl, 1.9 mg NaHCO₃, 0.3 mg MgSO₄, 0.2 mg CaCl₂, 0.2 mg NaH₂PO₄ and 0.8 mg glucose in 1 ml of deionized water. The solutions containing bombesin were applied into the lateral cerebral ventricle (AP: 7.5, L: 1.1, H: 4.0 mm from the cortical surface) in a volume of 10 μ l through a stainless steel micropipette (0.35 mm outer diameter). Intrathecal injections were given at the L5-L6 level of the spinal cord into the subarachnoid space in a volume of 10 μ l, followed by a 5 μ l flush with artificial cerebrospinal fluid through a Hamilton microliter syringe fitted with a 27 gauge needle. For the microinjection study, 0.5 μ l of solution was applied through a glass micropipette (70 μ m outer diameter). The coordinates of the sites of microinjection were as follows: the preoptic area (AP 6.5, L 0.5, H 2.2), anterior hypothalamus (AP 6.0, L 0.5, H 2.2), paraventricular nucleus (AP 5.6, L 0.3, H 2.9), lateral hypothalamic area (AP 4.8, L 1.6, H 1.8), ventromedial hypothalamus (AP 4.6, L 0.5, H 1.4) and

dorsomedial hypothalamus (AP 4.6, L 0.5, H 2.2), according to the brain atlas by König and Klippel (6).

Bilateral lesions were made by passing a 1.5 mA cathodal DC current between a stainless steel electrode (0.25 mm diameter) and a nasal anode for 20 sec. For a large lesioning of the anterior hypothalamus, including the preoptic-anterior hypothalamic area and the paraventricular nucleus, currents were passed at four different sites: 1) AP 6.5, L 0.5, H 2.2; 2) 0.5 mm ventral site of (1); 3) AP 5.8, L 0.3, H 2.9; and 4) 0.5 mm ventral site of (3).

The brain was removed, fixed in 10% formalin, and the frozen sections cut at 30 μ m were stained with cresyl violet for microscopic study of microinjection and lesion sites. The results were expressed with the corresponding values of the control rats, using Student's *t*-test for unpaired comparisons.

Results

The mean basal gastric acid output obtained from rats under urethane anesthesia was 2.64 ± 0.23 μ Eq/15 min (*n*=73). When the vagus nerve was continuously stimulated (3 Hz, 0.5 msec, 1 mA), gastric acid output increased and reached a steady level within 60 min. Acid output at the 60 min collection period by continuous electrical stimulation of the vagus nerve reached 35.3 ± 1.2 μ Eq/15 min (*n*=73).

Effects of i.c.v. and intrathecally applied bombesin on the increase in gastric acid output induced by electrical stimulation of the vagus nerve: Intracerebroventricular application of bombesin dose-dependently inhibited the increase in gastric acid output induced by electrical stimulation of the vagus nerve (Fig. 1). However, intrathecal application of this peptide even in a large dose of 1 nmole did not alter the vagally stimulated gastric acid output.

Effects of intrahypothalamic administration of bombesin on the vagally stimulated gastric acid secretion: Bombesin in a dose of 3 pmole, a dose which was without effect when given i.c.v., was microinjected into various hypothalamic regions. When bombesin was applied into the preoptic area, the

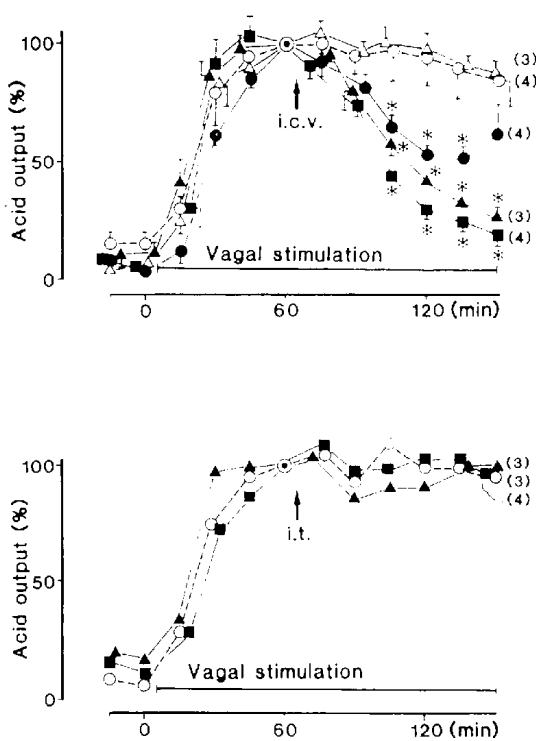


Fig. 1. Effects of intracerebroventricular (i.c.v.; upper panel) and intrathecal (i.t.; lower panel) administration of bombesin on the increase in gastric acid output induced by electrical stimulation of the vagus nerve. The left vagus nerve was stimulated at 3 Hz, 0.5 msec, 1 mA. ○: artificial cerebrospinal fluid (CSF), △: 3 pmole bombesin, ●: 10 pmole bombesin, ▲: 100 pmole bombesin, ■: 1000 pmole bombesin. (): Number of animals in each experiment. * $P < 0.05$ (statistically significant difference from the respective controls with CSF).

anterior hypothalamus and the paraventricular nucleus, the vagally stimulated gastric acid secretion was significantly inhibited (Fig. 2A). Microinjection of this peptide into the ventromedial nucleus, the dorsomedial nucleus and the lateral hypothalamic area were without effect (Fig. 2B).

Effects of hypothalamic lesioning on the bombesin (i.c.v.)-induced inhibition of gastric acid secretion: To determine the possible site of the inhibitory action of bombesin, bilateral electrolytic lesioning of the anterior hypothalamus, including the preoptic-anterior hypothalamic area and the paraventricular nucleus were performed. With electrical

stimulation of the vagus nerve of these lesioned animals, acid output before and 60 min after the stimulation were 3.30 ± 1.17 $\mu\text{Eq}/15$ min and 40.7 ± 2.3 $\mu\text{Eq}/15$ min ($n=8$), respectively. These values were not significantly different from the respective control values. In these lesioned animals, the bombesin (10 pmole, i.c.v.)-induced inhibition of gastric acid output was abolished (Fig. 3). A lesion restricted to the preoptic-anterior hypothalamic area or the paraventricular nucleus did not alter the inhibitory effect of bombesin (data not shown). Subsequent histological examination of the brain from these animals indicated that other hypothalamic nuclei adjacent to the lesion site such as the ventromedial nucleus, the dorsomedial nucleus and the lateral hypothalamic area were intact.

Discussion

Bombesin microinjected into the preoptic area and the anterior and the paraventricular nuclei of the hypothalamus markedly inhibited vagally stimulated gastric acid secretion. The prior bilateral electrolytic destructions of these 3 regions abolished the inhibitory effect of this peptide given i.c.v.

On the other hand, microinjections of bombesin into the lateral hypothalamic area, the ventromedial and the dorsomedial nuclei of the hypothalamus were without effect. Intrathecal administration of this peptide was also without effect, even in a large dose. The ineffectiveness of bombesin within the lateral hypothalamic area and the ventromedial hypothalamus corresponded well with reported findings (7, 8). It was also noted that this bombesin-induced inhibition was unaffected by amygdaloid damage (9). There is now much evidence that the dense bombesin-immunoreactivity and the high affinity binding site of this peptide are localized in the preoptic area and the anterior and the paraventricular nuclei of the hypothalamus (10–13). Therefore, the site of the inhibitory action of bombesin on gastric acid secretion probably resides divergently in the anterior areas of the hypothalamus, including the preoptic area and the anterior and the paraventricular nuclei of the hypothalamus.

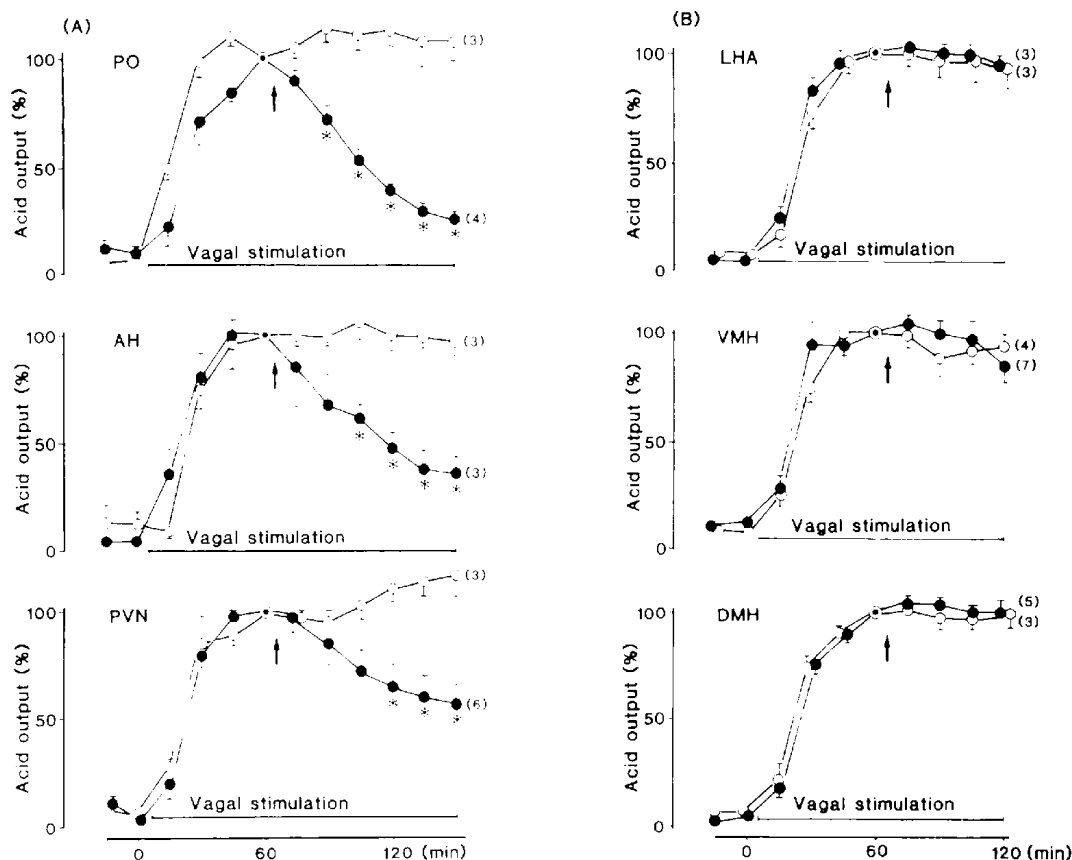


Fig. 2. Effects of bombesin microinjected into hypothalamic regions on the vagally stimulated gastric acid output. \circ : CSF, \bullet : 3 pmole bombesin. (A) PO: the preoptic area, AH: the anterior hypothalamus, PVN: the paraventricular nucleus, (B) LHA: the lateral hypothalamic area, VMH: the ventromedial hypothalamus, DMH: the dorsomedial hypothalamus. The other conditions are the same as those in the Fig. 1.

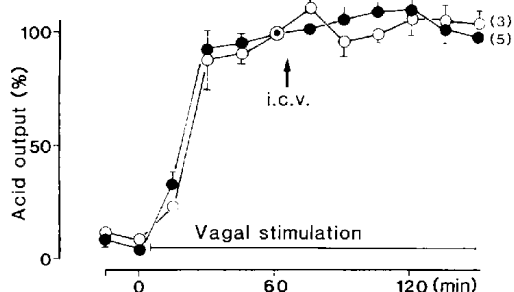


Fig. 3. The effect of bilateral lesioning of the preoptic area and the anterior hypothalamus and the paraventricular nucleus on the bombesin-induced inhibition of gastric acid output. Test solution was applied i.c.v. \circ : CSF, \bullet : 10 pmole bombesin. The other conditions are the same as those in the Fig. 1.

As for the role of bombesin-reactive neuronal regions in the regulation of gastric acid secretion, it is known that electrical low intensity stimulation of both the anterior hypothalamus and the preoptic area consistently increase gastric acid secretion (14–18). Pertaining to the paraventricular nucleus, there are direct hypothalamic-vagal fiber connections originating from the paraventricular nucleus down to the dorsal vagal complex in the medulla (19). With electrical stimulation of this hypothalamic nucleus, changes in a variety of gastrointestinal functions occur; i.e., an increase in gastric acid secretion (20), excitation of gastric-vagal solitary neurons (21), and inhibition of gastric motility (22). The modes of action of

bombesin on neuronal activities of these brain regions and its physiological roles in inhibiting gastric acid secretion will be an interesting subject for future investigations.

In conclusion, the site of action of bombesin in inhibiting gastric acid secretion is probably within the anterior areas of the hypothalamus, including the preoptic area and the anterior and paraventricular nuclei.

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