PHYSIOLOGY

Quantitative Analysis of the Role of Cholinergic, Gastrin, and Histamine Regulatory Mechanisms of Pepsinogen Production in the Stomach of Narcotized Rats

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Pepsinogen secretion in the intraluminally perfused stomach of narcotized rats was induced by electrical stimulation of the vagus nerve or intravenous injection of pentagastrin. Blockade of histamine H_2 receptors inhibited pepsinogen production induced by vagal stimulation by 35%, but caused only a 13% decrease in pentagastrin-stimulated pepsinogen secretion.

Key Words: *pepsinogen; vagus; pentagastrin; histamine*

Practically in all mammals, vagal cholinergic and gastrin effects on parietal cells of the stomach are mediated by histamine-producing cells [4]. Chief cells of the gastric mucosa producing pepsinogen are evolutionary similar to parietal cells [3,10] and functionally depend on their state. However, of mechanisms secretion, nervous and endocrine regulation in these cells differ considerably [5,6,11]. The data on the involvement of main secretagogues in the regulation of pepsinogen production are contradictory due to species specificities and peculiarities of experimental models [9,12]. The role of histamine in mediating vagal and gastrin effects on chief cells of the stomach remains unclear. Here we evaluated the role of cholinergic, gastrin, and histamine mechanisms in the regulation of pepsinogen secretion in the stomach of narcotized rats.

MATERIALS AND METHODS

Experiments were performed on 42 male Sprague-Dawley rats weighing 309±6 g and kept at 20±1°C under a 12-h light/dark schedule and *ad libitum* food supply (Rappolovo R50258-92). One day before operation, the animals were deprived of food, but had free access to water.

The rats were narcotized with intraperitoneal injection of choral hydrate and urethane (800+100 mg/kg, Sigma). Isotonic NaCl containing 3 mM $NaHCO_3$ and 2% glucose was administered intravenously (1 ml/h) to prevent acidosis and dehydration. The animals were heated on a temperature-controlled table with incandescent lamp. Heart rate (HR), blood pressure (BP), and rectal temperature were measured.

After tracheotomy, catheterization of the right femoral artery and vein, and median laparotomy were carried out subdiaphragmatic vagotomy, gastric sympathectomy, and ligation of the left adrenal gland were conducted if required. The stomach was perfused with isotonic NaCl at a flow rate of 0.7 ml/min, 37°C, pH 6.0, and 0 mm Hg. The solution was administered through a catheter and removed via a perforated polyethylene tube introduced through a duodenal section into the pylorus. The peripheral end of the left subdiaphragmatic vagus nerve was demyelinated and stimulated with rectangular pulses (6 V, 1 msec, 8 Hz) delivered through silver electrodes for 10 min.

Perfusate samples were taken at 15-min intervals using a fraction collector (LKB). Pepsinogen concen-

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TABLE 1. Basal Pepsinogen Secretion in Intact and Denervated Rat Stomach (*M±m*)

Splanchnic nerves	Pepsinogen, μg/min/g		*p*, ANOVA
	before vagotomy	after vagotomy	
Intact (*n*=9)	0.79±0.09	0.53±0.08	0.046
Denervated (*n*=12)	1.31±0.09	0.86±0.11	0.001

tration in acidified samples (pH 2.0) was measured spectrophotometrically at 280 nm using hemoglobin as the standard [1]. The calibration curve was constructed using purified porcine prolactin (122 U/g, Fluka). Pepsinogen secretion was expressed in μg/min/g wet weight. The total production of pepsinogen was the difference between stimulated and basal secretion over 45 min after stimulation. The measurements were performed 50 min after initiation of perfusion, when basal secretion, heart rate (HR), and blood pressure (BP) were stabilized. Perfusate samples were taken before the experiment to evaluate basal pepsinogen secretion.

The results were analyzed by ANOVA and expressed as mean±standard error.

RESULTS

Basal pepsinogen secretion in our experiments is consistent with the data obtained on alert animals and isolated mouse stomach (Table 1) [2,12].

Subdiaphragmatic vagotomy suppressed basal pepsinogen secretion in intact and denervated (splanchnicectomy) stomachs by 35%. At the same time, sympathectomy enhanced basal secretion of pepsinogen by 60% before and after vagotomy (Table 1). The data suggest that basal pepsinogen secretion in narcotized rats is simultaneously and independently regulated by stimulatory vagal and inhibitory sympathetic effects.

Electrical stimulation of the peripheral end of the left subdiaphragmatic vagus nerve practically did not affect HR and BP, but sharply increased pepsinogen secretion over the first 15 min (Fig. 1, *a*). Stimulatory vagal effects were mediated by cholinergic reactions, because muscarinic receptor blocker atropine (0.1 mg/kg intravenously, *n*=9) and nicotinic receptor blocker hexamethonium (10 mg/kg intravenously, *n*=6) did not

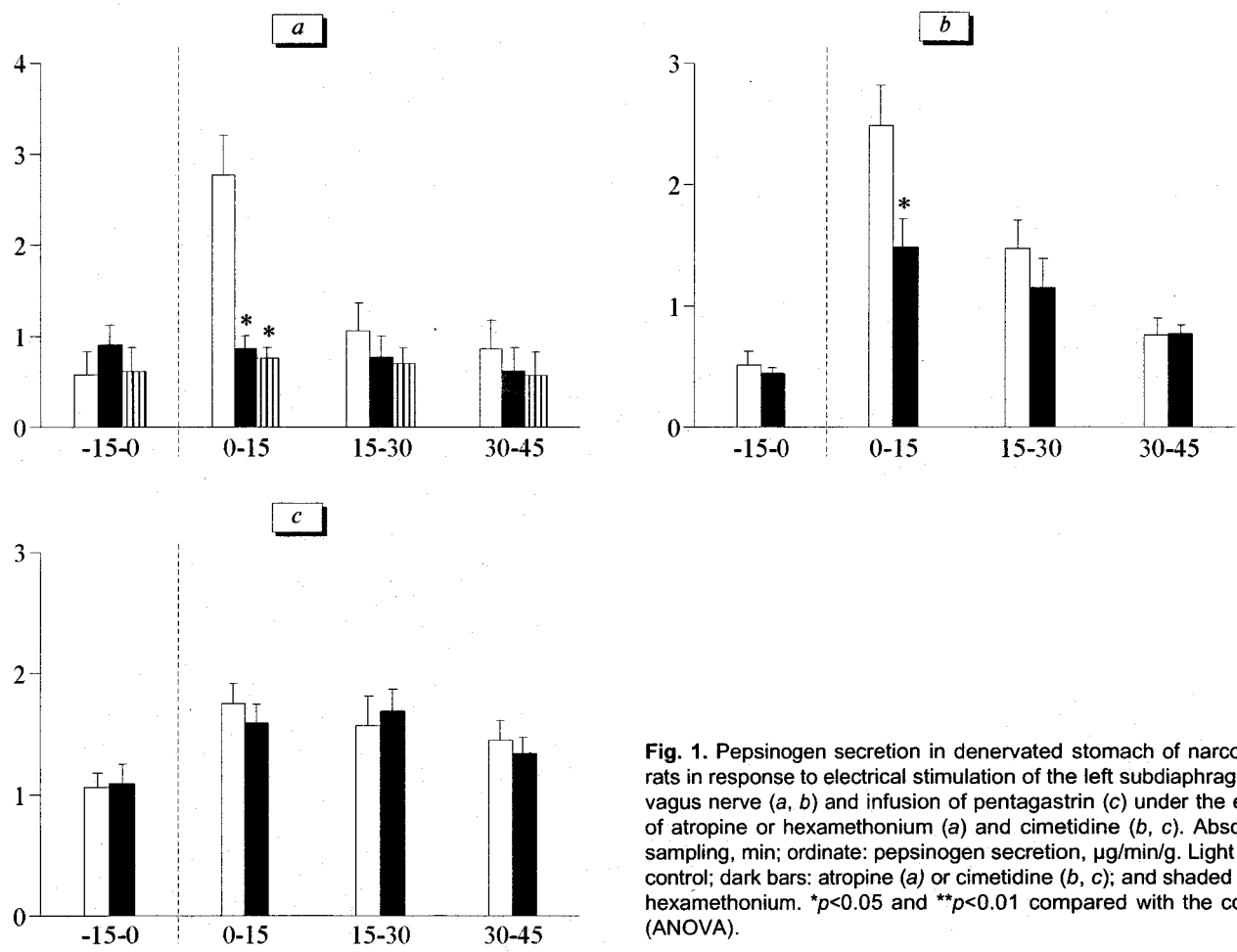

Fig. 1. Pepsinogen secretion in denervated stomach of narcotized rats in response to electrical stimulation of the left subdiaphragmatic vagus nerve (*a*, *b*) and infusion of pentagastrin (*c*) under the effect of atropine or hexamethonium (*a*) and cimetidine (*b*, *c*). Abscissa: sampling, min; ordinate: pepsinogen secretion, μg/min/g. Light bars: control; dark bars: atropine (*a*) or cimetidine (*b*, *c*); and shaded bars: hexamethonium. *$p<0.05$ and **$p<0.01$ compared with the control (ANOVA).

affect basal pepsinogen production, but completely inhibited the secretion induced by electrical stimulation of the vagus nerve (-7.58±7.75 and 0.78±0.06 μg/45 min/g, respectively, *vs.* 44.32±9.19 μg/45 min/g in the control).

Cholinergic influences do not directly act on cholinoceptors on hydrochloric acid-producing parietal cells of gastric glands, but affect histamine-producing cells [4]. However, the data on the role of histamine mechanisms in mediating cholinergic effects on pepsinogen-producing chief cells are ambiguous. Exogenous histamine did not affect pepsinogen secretion in the stomach of awake rats [12] and even inhibited this process in dogs [5]. On the other hand, histamine stimulated pepsinogen secretion in isolated mouse [2] or rat [6] stomach and cultured human gastric mucosal cells [7].

Blockade of histamine H_2 receptors with cimetidine (30 mg/kg intravenously, n=9) showed that vagal effects on chief cells of the gastric glands in narcotized rats are only partially mediated by histamine (Fig. 1, *b*). The total production of pepsinogen in response to vagal stimulation after H_2 receptor blockade decreased by 35% suggesting that only one-third of the vagal stimulatory effect on pepsinogen secretion is mediated by histamine.

Gastrin is another paracrine factor of gastric secretion. Despite the presence of gastrin receptors on all gastric secretory cells, its effects on hydrochloric acid secretion in the stomach of rabbits, rats, and pigs are mediated by histamine [8,13]. However, gastrin effects on pepsinogen production in biopsy samples of human gastric mucosa are not completely mediated by histamine [7].

In our experiments, pentagastrin (10-100 μg/kg intravenously, n=8) led to a dose-dependent inhibition of pepsinogen secretion, but did not change HR and BP. Repeated injection of pentagastrin in a dose of 70 μg/kg at a 60-min interval caused similar secretory response (Fig. 1, *c*). Pepsinogen applied in this dose significantly enhanced pepsinogen production (p<0.05), but this effect did not exceed 50% of that observed after electrical stimulation of the vagus nerve. Therefore, pentagastrin is a less potent stimulator of chief cells. Blockade of histamine H_2 receptors with cimetidine (30 mg/kg intravenously) did not considerably inhibit pentagastrin-stimulated pepsinogen secretion (Fig. 1, *c*) and decreased the total pepsinogen production by only 13%. The data suggest that direct gastrin mechanisms regulating pepsinogen secretion dominate over histamine-mediated effects.

Thus, two-thirds of the stimulatory effect of the vagus nerve on the production of pepsinogen are due to direct cholinergic pathways, whereas one-third is mediated by histamine release (as distinct from hydrochloric acid secretion regulated by histamine-producing cells). Gastrin is relatively weak stimulator of pepsinogen secretion, and its effects on chief cells are not mediated by histamine.

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