Selective Regulation of Acid, Pepsinogen, and Bicarbonate Secretion in the Stomach by Different C-Fiber Populations of Vagus Nerve

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Acute experiments on anesthetized rats showed that group B nerve fibers of the subphrenic portion of the vagus nerve do not participate in the regulation of gastric secretion. Gastric acid production is mainly controlled by fast C-fibers (2.11±0.09 m/sec), while secretion of pepsinogen and bicarbonates depends on activity of both fast and slow (0.95±0.11 m/sec) C-fibers. Some fast conduction C-fibers stimulating the release of bicarbonates in the stomach are capsaicin-sensitive afferents. The local effect of these afferents depends on cholinergic transmission and most probably it is mediated via its modulation.

Key Words: *stomach; secretion; vagus nerve; capsaicin-sensitive afferents*

The subphrenic branches of the vagus nerve (VN) consist mainly of unmyelinated C-fibers. The myelinated fibers account only for 0.3-0.5% total number of VN fibers. The diameter of C-fibers varies in a wide range from 0.1-0.2 to 1.4-1.6 μ, which may attest to possible functional differences between fibers of different caliber [5]. This hypothesis was not tested experimentally although it is also corroborated by the fact that individual fibers in VN contain different set of cotransmitters, and they terminate on enteric neurons also differing in the transmitter content [10,12]. It should be kept in mind that electrical stimulation of VN (widely used to study the neural control of gastric secretion) activates the afferent fibers, which compose the most part (80-90%) of VN [3]. It was recently shown that some afferent terminals contain peptide transmitters capable to affect the visceral functions including those in the stomach [9]. However, the role of these C-fiber afferents to the secretory response remains unclear.

Our aim was to identify the individual populations of the fibers in abdominal VN by electrophysiological methods, to reveal their specific role in the regulation of acid, bicarbonates and pepsinogen secretion in the stomach, and to elucidate the possibility of selective modulation of individual parameters of gastric secretion via different populations of nervous fibers.

MATERIALS AND METHODS

Experiments were carried out under intraperitoneal urethane and chloralose (Sigma) anesthesia (800 and 100 mg/kg, respectively) on male Sprague—Dawley rats (n=26) weighing 300-340 g. After tracheostomy and catheterization of the femoral vein and artery, the midline laparotomy was made to perfuse the stomach with physiological saline (37°C, pH 6.0) at the perfusion rate of 0.7 ml/min as described in details elsewhere [1,2]. Molar concentrations of H^+ and HCO_3^- were calculated on the basis of pH and Pco_2 measured in gastric perfusate [1,7]. The current values of pH and Pco_2 were fed into a computer and averaged every 30 sec. The concentration of pepsinogen was determined in 15-min perfusate samples with hemoglobin spectrophotometry. The concentrations of gastric juice components measured in gastric effluent were used to calculate the total production of acid, bicarbonates, and

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pepsinogen as the secretion surplus over the baseline level. These components were measured 35 min (acid and bicarbonates) or 45 min (pepsinogen) after the start of stimulation, and the data were normalized to the stomach weight.

In all experiments, the subphrenic right-side vagotomy, bilateral transection of the major celiac nerves, and ligation of left adrenal gland were performed. Left VN was cut on the neck and divided into bundles. Some bundles were placed on Ag—AgCl bipolar recording electrodes. The stimulating electrodes were placed on the nerve below the diaphragm. The nerve was stimulated for 10 min with rectangular electrical pulses (0.05, 0.1, and 1.0 msec duration, 4-6 V amplitude, 8 Hz repetition rate). Simultaneously, evoked potentials (EP) were recorded in the cervical part of the left VN. Conduction velocity in the bundle was calculated from EP latency.

Atropine sulfate (0.1 mg/kg intravenously, Sigma) was used for inhibition of vagal secretory response. Conduction in unmyelinated afferents was blocked by 10-min application of capsaicin (33 mM, Sigma) dissolved in a Twin-ethanol mixture.

In group 1 rats (n=10), the left subphrenic VN was stimulated successively with 0.05-, 0.1-, and 1.0-msec pulses. Then atropine was injected intravenously to some rats and the nerve was stimulated again with 1.0-msec pulses. In group 2 rats (n=16), stimulation with 0.1-msec pulses (n=8) and 1.0-msec pulses (n=8) was performed in the initial (control) state and 45 and 90 min after 10-min perivagal application of capsaicin.

The data were analyzed statistically using ANOVA test.

RESULTS

Stimulation of the left subphrenic VN with pulses of 0.05 msec duration and 4.5-5.0 V amplitude induced EP of B-fibers (conduction velocity of 4.26±0.06 m/sec, n=20) recorded in the cervical part of ipsilateral VN. Increasing pulse duration to 0.1 msec and the amplitude to 6 V allowed recording of additional potentials characterized by 2.11±0.09 m/sec conduction velocity (n=20) corresponding to EP of fast C-fibers. Stimulation with 1.0 msec pulses induced generation of EP in C-fibers characterized by conduction velocity of 0.95±0.11 m/sec (n=20), i.e. slow C-fibers. Further increasing the duration of stimulating pulses (>1 msec) in some experiments was not accompanied by prolongation of EP latency.

Selective stimulation of B-fibers did not affect secretion of acid, bicarbonates, and pepsinogen (Fig. 1). Combined stimulation of B- and fast C-fibers with 0.1-msec pulses induced almost maximum acid release (26.5±6.7 μmol/g for 35 min, n=10, Fig. 1, *a*). Simultaneous stimulation of B-fibers and fast and slow C-fibers was not accompanied by significant increase in acid secretion (30.2±4.9 μmol/g for 35 min, n=10, Fig. 1, *a*). These findings indicate that gastric acid secretion is controlled mainly by fast C-fibers.

Assessment of bicarbonate secretion induced by VN stimulation showed that the effects of fast and slow conduction C-fibers are summed. Secretion of HCO_3^- in response to stimulation of B- and fast conduction C-fibers surpassed the baseline by 24% (p<0.05, n=10). Additional stimulation of slow C-fibers 2-fold increased the secretory response compared to stimulation of fast C-fibers only (p<0.05, n=10, Fig. 1, *b*). Summation of the effects produced by stimulation of fast and slow C-fibers was also observed for pepsinogen secretion. The total production of pepsinogen during stimulation of all C-fibers significantly surpassed pepsinogen production induced by stimulation of B-fibers and fast C-fibers (n=10, Fig. 1, *c*). Atropine had no effect on the baseline gastric secretion, but completely blocked the secretory reactions induced by stimulation of subphrenic VN. It can be concluded that secretion of bicarbonates and pepsinogen is controlled by both fast and slow C-fibers, and their regulatory effects are mediated by muscarinic transmission.

Therefore, our study showed that acid, bicarbonate, and pepsinogen secretion in the stomach is controlled by different populations of C-fibers of subphrenic VN characterized by different conduction velocity of nerve impulses. This agrees with the data on abundant preganglionic innervation of the stomach (up to 36% subphrenic efferent fibers travel to gastric branches of VN [11]) and pronounced divergence and convergence of vagal preganglionic fibers in the enteric plexus [8,15].

Neurotoxin capsaicin (a widely known member of the vanilloid family) used in this work for blockade of unmyelinated C-afferent fibers seems to be a unique and highly selective functional marker of unmyelinated sensory fibers: 10-30% afferent C-fibers in gastric branches of VN are sensitive to capsaicin [4,6]. The local effector function of these afferents with respect to acid secretion was only partially studied [13].

This paper is the first detailed study of the effect of antidromic stimulation of capsaicin-sensitive vagal afferents on acid, bicarbonate, and pepsinogen secretion. Perivagal application of capsaicin *per se* produced no significant effect on the baseline secretion of these substances. However, a significant inhibition of VN-stimulated acid secretion (by 50-60% on the average) occurred during the first 45 min after application of capsaicin, and this effect was observed during individual stimulation of fast conduction C-fibers (Fig. 2, *a*) and during combined stimulation of fast and

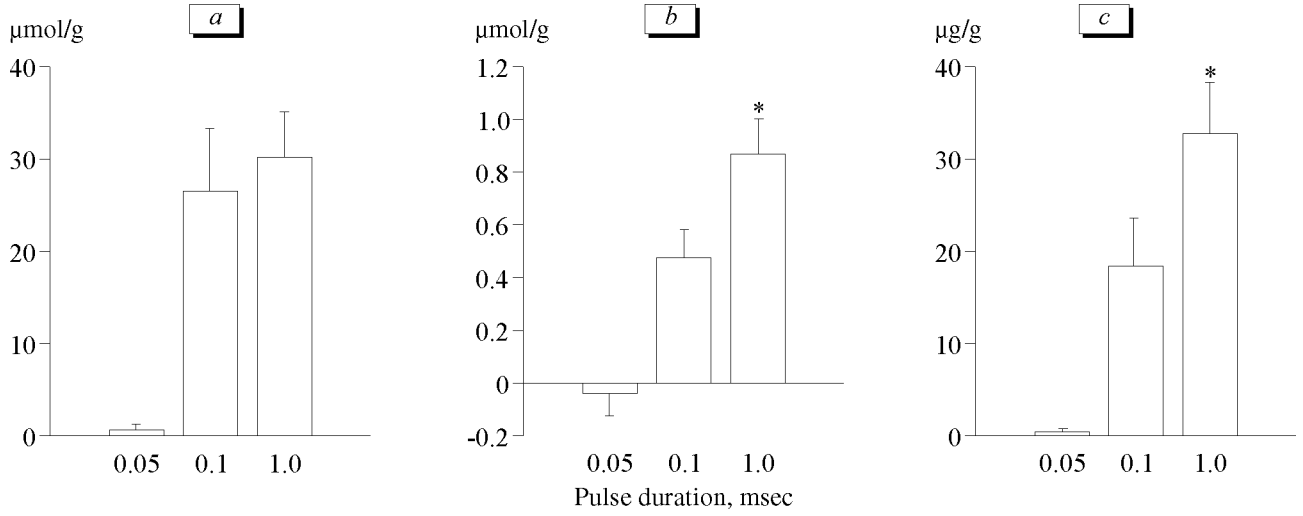

Fig. 1. Gastric secretion of acid (H^+, *a*), bicarbonates (*b*), and pepsinogen (*c*) as a function of duration of stimulating pulses applied to subphrenic vagus nerve. *$p<0.05$ relative to stimulation with 0.05 msec pulses. Here and in Figs. 2 and 3: secretion of acid and bicarbonates was measured for 35 min and that of pepsinogen for 45 min. Secretion was calculated as excess over the baseline secretion.

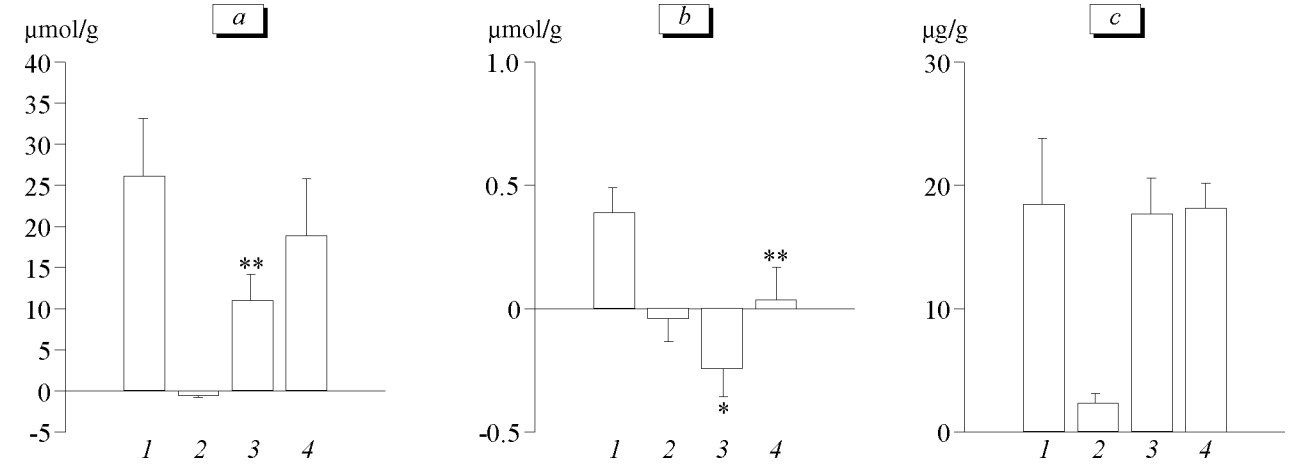

Fig. 2. Effect of perivagal application of capsaicin on gastric secretion of acid (H^+, *a*), bicarbonates (*b*), and pepsinogen (*c*) induced by stimulation of subphrenic vagus nerve with 0.1-msec pulses. Here and in Fig. 3: *1*) control; *2*) capsaicin *per se*; *3*) and *4*) 45 and 90 min after application of capsaicin, respectively. *$p<0.01$ and **$p<0.05$ compared to the control.

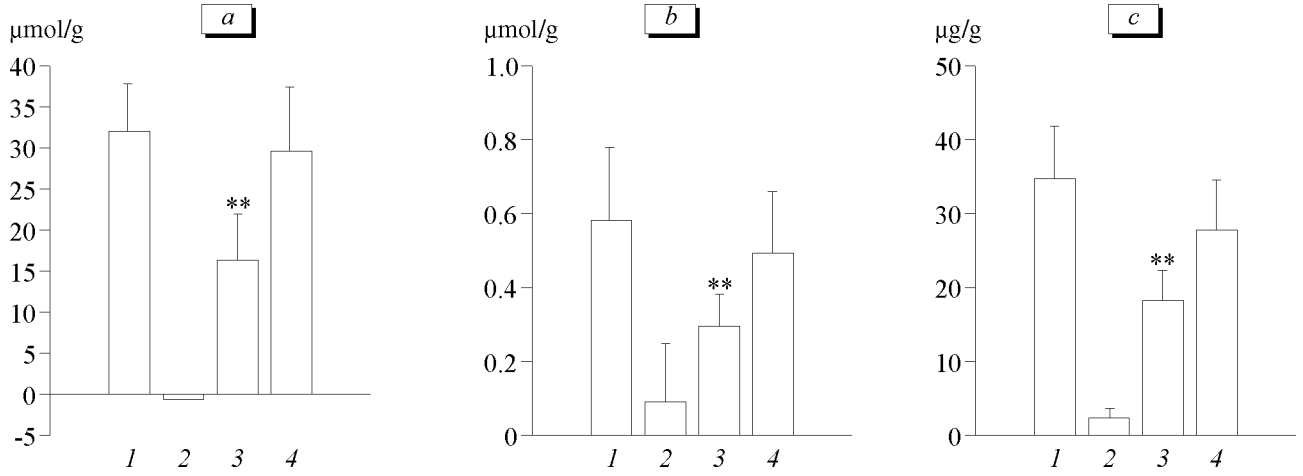

Fig. 3. Effect of perivagal application of capsaicin on gastric secretion of acid (H^+, *a*), bicarbonates (*b*), and pepsinogen (*c*) induced by stimulation of subphrenic vagus nerve with 1.0 msec pulses.

slow conduction C-fibers (Fig. 3, *a*). Capsaicin completely suppressed secretion of bicarbonates stimulated by excitation of fast conduction C-fibers (Fig. 2, *b*) and pronouncedly inhibited production of bicarbonates evoked by simultaneous stimulation of all subphrenic C-fibers (to 51±15% control value, Fig. 3, *b*). The neurotoxin produced no significant changes in pepsin secretion stimulated by fast conduction C-fibers (Fig. 2, *c*), but moderated production of pepsinogen induced by simultaneous stimulation of all subphrenic C-fibers by 48±9% ($p<0.05$, $n=8$, Fig. 3, *c*).

The dynamics of capsaicin effect showed that the release of acid and pepsinogen after initial and seemingly non-specific inhibition [14] is significantly restored after 90 min (Figs. 2, 3). Inhibition of stimulated bicarbonate secretion was maintained for a longer period. It is noteworthy that inhibition of that part of bicarbonate secretion, which was evoked by stimulation of fast conduction C-fibers, was not virtually restored (Fig. 2, *b*). Therefore, the up-regulation of bicarbonate gastric secretion attests to local effector function of capsaicin-sensitive C-fiber vagal afferents triggered by their antidromic excitation. The dependence of the local effect of capsaicin-sensitive vagal afferents in the stomach on cholinergic transmission is a specific feature of these fibers. All secretory reactions were blocked by atropine, which indicates that the effect of vagal gastric afferents is mediated via modulation of activity of intramural cholinergic neurons.

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