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Effect of TRPV1 on Activity of Isoforms of Constitutive Nitric Oxide Synthase during Regulation of Bicarbonate Secretion in the Stomach

V. A. Zolotarev, Yu. V. Andreeva, and R. P. Khropycheva

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Application of mild irritants (1 M NaCl; pH 2.0) on the gastric mucosa potentiates the protective secretion of bicarbonates by epithelial cells. This response is mainly mediated by capsaicin-sensitive afferent nerve endings located in the submucosa. It was shown that activation of vanilloid type 1 receptors (TRPV1) induced by exogenous acidification of GM is not sufficient to potentiate the production of HCO_3^-, including production depending on neuronal NO synthase. However, the effect of exogenous acid on TRPV1 leads to activation of endothelial NO synthase that restrict the gastric secretion of HCO_3^-.

Key Words: *stomach; bicarbonate secretion; endothelial and neuronal NO synthases; capsaicin; TRPV1*

Acid back-diffusion from the gastric lumen into the pre-epithelium layer of the mucus is considered as the main chemical signal inducing the protective secretion of bicarbonates by surface epithelial cells [5]. The major molecular sensors of protons, vanilloid type 1 receptor (TRPV1) and acid-sensing ion channels (ASIC), are expressed on the membrane of capsaicin-sensitive afferent nerve fibers (CSN) in the submucosa [14]. Ablation of these primary afferents induced by high doses of alkaloid capsaicin considerably decreased secretion of HCO_3^- in response to intragastric acidification. However, blockade of proton-binding sites (TRPV1 and ASIC) enigmatically had no influence on the production of bicarbonates in the stomach [3,12]. Activation of CSN, particularly in response to application of acid to the gastric mucosa, induces local release of calcitonin gene-related peptide from sensory nerve endings; this peptide, in turn, stimulates endothelial NO synthase (eNOS) and possibly cyclooxygenase in adjacent tissues [7,8]. It is also known that NO in physiological concentrations is released from CSN containing neuronal NO synthase (nNOS) [10]. In the earlier study, we have shown that *in vivo* up-regulation of eNOS or nNOS produced opposite effects on HCO_3^- production [1].

Our aim was to elucidate how TRPV1 mediates the effects of eNOS or nNOS on HCO_3^- secretion in response to intragastric acidification.

MATERIALS AND METHODS

Male Sprague-Dawley rats weighing 300-350 g were used in the experiments (Biocollection, I. N. Pavlov Institute of Physiology; Program for Preservation and Development of Collections of Biological Resources, Russian Federal Agency for Scientific Organizations). The animals were deprived of food for 18 h before the experiment, but had free access to water. The study approved by the Bioethics Committee of the I. N. Pavlov Institute of Physiology. The rats were anesthetized with urethane (1.2 g/kg intraperitoneally) and the stomach was continuously perfused *in situ* at 37°C and perfusion rate of 1 ml/min. The intraluminal pressure was close to zero. Baseline perfusion was performed with buffer-free NaCl solution (154 mM; pH 4.0). Secretion of HCO_3^- was stimulated by application of

I. P. Pavlov Institute of Physiology, Russian Academy of Sciences, St. Petersburg, Russia. ***Address for correspondence:*** vasiliy_zolotarev@hotmail.com. V. A. Zolotarev

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a mild irritants (1 M NaCl; pH 2.0) on the gastric mucosa for 20 min. In the perfusate, pH and carbon dioxide tension (PCO_2) were measured continuously, and production of H^+ and HCO_3^- was calculated every 30 sec [2]. Bilateral subphrenic vagotomy was performed in all experiments.

A selective *in vivo* blocker of nNOS 7-nitroindazole (7-NI, Sigma) or nonselective blocker of eNOS and nNOS Nω-nitro-L-arginine (L-NNA, Sigma) were injected intravenously in a dose of 10 mg/kg 15 min before mucosa irritation with acidic hyperosmotic saline. A TRPV1 receptor antagonist capsazepine (CPZP; Sigma) was administered via retrograde infusion into the splenic artery (0.6 mg×kg^{-1}×h^{-1}) that was started 5 min before irritation and lasted 20 min ACIS blocker amiloride hydrochloride hydrate (AMLR; Sigma) was added to the perfusate in a concentration of 0.2 mM for 15 min before irritation. Control groups received intravenous injections of the solvent, 0.1% DMSO (Vekton) emulsified in saline, in equivalent volumes. Chemical ablation of CSN was performed with repeated subcutaneous injections of CAPS (50 mg/kg; Sigma) once a day for 3 days in a week before the experiment. Satisfactory development of CAPS-induced deafferentation was verified before the experiment by the absence of the corneal chemosensory reflex.

Base rate of secretion was assessed over 5 min before application of mild irritants. Net gastric output of HCO_3^- was calculated using the trapezoidal rule by subtracting basal production from the total production over 55 min after beginning of the irritation. The obtained data normalized to the stomach weight are presented as $M \pm SE$. The sample size (n) is equal to the number of animals in the group, because each animal was challenged with irritant once. The data were processed using Mann—Whitney U test; the differences were significant at $p<0.05$.

RESULTS

Exposure of GM to acidic hyperosmotic solution (1 M NaCl; pH 2.0) partially simulating physicochemical characteristics of the gastric chyme during regular digestion caused a rapid elevation of HCO_3^- secretion that peaked in 15 min after beginning of irritation. Then, secretion returned to the base level within 35-40 min. Infusion of CPZP or AMLR did not significantly change the response, whereas treatment with neurotoxic doses of CAPS almost completely suppressed HCO_3^- output (Fig. 1). These findings support the data that selective activations of the main sensors of acidity, TRPV1 or ASIC, are insufficient for stimulation of bicarbonate secretion in the stomach [3,12]. On the other hand, we confirmed that the increase in gastric production of HCO_3^- in response to acidification of GM almost fully depended on excitation of capsaicin-sensitive primary afferents which main characteristic is TRPV1 receptors [3]. Interestingly, HCO_3^- secretion in the duodenal mucosa induced by acid application is mediated (at least partially) by responses of TRPV1 channels [4].

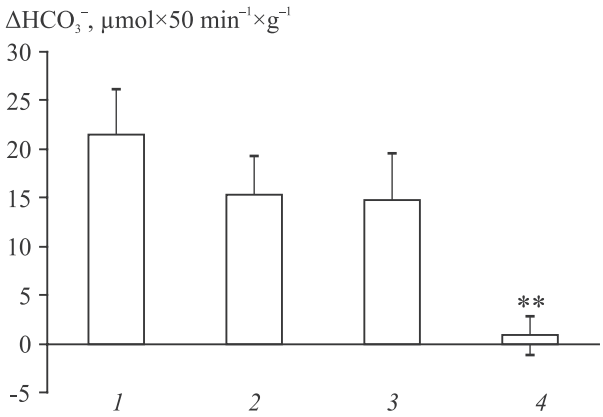

Fig. 1. Specific production of bicarbonates in the stomach induced by irritation of the gastric mucosa with acidic hyperosmotic solution against the background of blockade of potential acid sensors or ablation of capsaicin-sensitive primary afferents. *1*) Control (n=7); *2*) CPZP, (n=8); *3*) AMLP, (n=6); *4*) CAPS (n=7). **$p<0.01$ in comparison with the control.

It is well-known that the gastroprotective effect of CSN, including HCO_3^- secretion and submucosal hyperemia, is largely due to the release of calcitonin gene-related peptide from peripheral capsaicin-sensitive primary afferents; this peptide through special membrane receptors stimulates NO synthesis in endothelial cells. Peripheral endings of CSN are in contact with almost all tissues in the stomach wall. They form the most dense plexus around arteries and arterioles in the submucosa and are also present in ganglia of the myenteric and submucosal nervous plexuses; some of them reach the lamina propria lying near GM [7,8]. All these tissues specifically express eNOS and nNOS [9,11]. It is known [10] that vascular tone can be modulated by NO secreted by CSN containing nNOS. In previous publication, we have reported that nNOS activation caused by application of mild irritants on GM enhanced HCO_3^- production, while selective stimulation of eNOS on the contrary inhibited secretion [1]. Hence, CSN could induce opposite influences on HCO_3^- secretion by stimulating either nNOS, or eNOS. Nonselective inhibition of eNOS and eNOS with L-NNA had no effect on the specific production of HCO_3^- induced *in vivo* by application of acidic hyperosmotic saline on GM (Fig. 2). At the same time, selective nNOS blocker 7-NI markedly attenuated specific production of HCO_3^- ($p<0.05$; Fig. 2). In view of similar affinity of L-NNA to nNOS and eNOS [13] and similar pharmacodynamics and pharmacokinetics of L-NNA and 7-NI [6,15], the effect

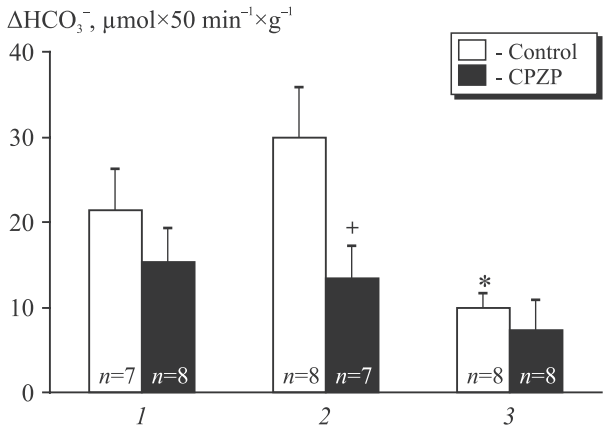

Fig. 2. Effect of NOS blockade on specific production of HCO_3^- in the stomach induced by acidification of the gastric mucosa in the control and against the background of CPZP treatment. *1*) Without NOS blockade; *2*) L-NNA; *3*) 7-NI. **p*<0.05 in comparison with control *1*. +*p*<0.05 in comparison with the corresponding control.

of eNOS activation can be assessed by the difference between the effects of L-NNA and 7-NI. It can be hypothesized that activation of eNOS induced by mild irritants suppresses HCO_3^- production. Earlier, we have shown that local increase of blood flow in the stomach wall related to eNOS activation facilitated washing out of bicarbonate reserve that is accumulated in the submucosa during regular secretion of parietal cells [1]. In the present experiments, inhibition of TRPV1 channels with CPZP substantially decreased specific production of HCO_3^- after administration of L-NNA, but had no influence on HCO_3^- production in the control or in the presence of 7-NI (Fig. 2).

Natural excitation of CSN occurs not only as an result of action of protons on TRHV1 but more likely due to interaction of numerous signals generated during irritation and inflammation of GM. The obtained data suggest that stimulation of TRPV1 induced by application of exogenous acid on GM is insufficient to induce gastric bicarbonate output including that mediated by nNOS. Nevertheless, acidification of GM was a potent stimulus for TRPV1 that activates eNOS and leads to suppression of HCO_3^- secretion.

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