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Mechanism of Capsaicin-Stimulated Gastric HCO_3^- Secretion – Comparison with Mucosal Acidification

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<http://dx.doi.org/10.5772/58336>

1. Introduction

The gastric mucosa is maintained in an intact state by multiple protective mechanisms including humoral and neuronal factors, in spite of its exposure to acid and other chemical hazards [1]. Capsaicin-sensitive afferent neurons play a central role in neuronal mechanisms taking place in the stomach [2]. These afferent neurons have been shown to regulate various gastric functions such as secretion, mucosal blood flow (GMBF), and motility, and modulate the mucosal integrity of the stomach [2-5]. Vanilloid type 1 receptor (VR1), a nonselective cationic channel, has recently been cloned as the binding site of capsaicin [6], and more recently, has been identified as a member of the transient receptor potential (TRP) family of ion channels [7]. Although the TRP family is activated by a diverse range of stimuli, including the depletion of intracellular Ca^{2+} stores [6], the VR1 receptor remains the only channel activated by vanilloids such as capsaicin and is now known as TRPV1 [8]. Capsaicin stimulates these afferent neurons via TRPV1, resulting in the release of calcitonin gene-related peptide (CGRP), the predominant neurotransmitter of spinal afferents in the rat stomach, and thus, exerts a gastroprotective action [9]. CGRP has been shown to induce endothelial cells to release nitric oxide (NO), and this molecule is known to strongly mediate the action of CGRP [2]. Other studies have also demonstrated that activation of the bradykinin B2 receptor caused the opening of TRPV1 and modified the action of capsaicin [10, 11].

The secretion of HCO_3^- from surface epithelial cells is a protective mechanism in the stomach, with HCO_3^- working in collaboration with mucus gel, which adheres to the surface of mucosa [1]. We previously reported that capsaicin increased duodenal HCO_3^- secretion mediated by endogenous prostaglandins (PGs) and NO as well as capsaicin-sensitive afferent neurons [12, 13]. We also demonstrated that PGE_2 stimulated HCO_3^- secretion through EP1 receptors in the stomach and EP3/EP4 receptors in the duodenum [14, 15], while the action of capsaicin in the duodenum required the presence of prostacyclin (PGI_2) IP receptors [16]. However, few studies have examined the mechanisms involved in gastric HCO_3^- secretion in response to capsaicin.

We here described the regulatory mechanism underlying capsaicin-induced gastric HCO_3^- secretion, in relation to sensory neurons, TRPV1, PGs, NO, and bradykinin B2 receptors, and compared it to that of the acid-induced response. In addition, because we found that responses to capsaicin and acid in the duodenum differed concerning PGI_2/IP dependency [16], we also examined these responses in the stomach using mice lacking EP1, EP3 or IP receptors.

2. Methods

ANIMALS: Male SD rats (220-260 g, Nippon Charles River, Shizuoka, Japan) and male C57BL/6 mice (25-30 g) were used. Mice lacking the EP1, EP3, or IP receptors were generated as described previously [17, 18]. These rats and knockout mice were deprived of food, but allowed free access to tap water for 18 hr before the experiments. Studies were performed under urethane anesthesia (1.25 g/kg, i.p.) using 4~8 animals per group.

DETERMINATION OF GASTRIC HCO_3^- SECRETION: The secretion of HCO_3^- was measured in the chambered stomach as described previously [5]. The abdomen was incised and the stomach was exposed, mounted on a chamber (exposed area, rat: 3.1 cm^2 , mouse: 0.7 cm^2), and superfused with saline that was gassed with 100% O_2 and kept in a reservoir. The secretion of HCO_3^- was measured at pH 7.0 using a pH-stat method (Hiranuma Comtite-8, Mito, Japan) and by adding 2 mM HCl to the reservoir. Acid secretion was completely inhibited by omeprazole given i.p. at a dose of 60 mg/kg to unmask HCO_3^- in the stomach. After basal HCO_3^- secretion had stabilized, animals were subjected to the following treatment. Capsaicin (0.03~0.3 mg/ml) or NOR-3 (a NO donor: 3 mg/ml) was topically applied to the chamber for 10 min, while PGE_2 (1 mg/kg) or bradykinin (30 $\mu\text{g/kg}$) was given i.v. as a single injection. The secretion of HCO_3^- was also stimulated by exposing the mucosa to 50~200 mM HCl (rat) or 50 mM HCl (mouse) for 10 min. The effects of indomethacin, N^G -nitro L-arginine methyl ester (L-NAME), ONO-8711 (an EP1 antagonist) (Aoi et al., 2004), FR172357 (a bradykinin B2 antagonist) [19], capsazepine (a TRPV1 antagonist), or the chemical ablation of capsaicin-sensitive afferent neurons were examined on the secretion of HCO_3^- induced by the above agents or mucosal acidification. Indomethacin (5 mg/kg), ONO-8711 (10 mg/kg) or FR172357 (1 mg/kg) was given s.c. 30 min or i.v. 15 min before each treatment, while L-NAME (20 mg/kg) was given s.c. 3 hr before because this agent was previously shown to acutely increase HCO_3^- secretion through a neural reflex due to an increase in blood pressure [20-22]. Capsazepine (2.5 mg/ml) was applied to the chamber for 20 min, starting from 10 min before the capsaicin or acid treatment [13], or applied for 20 min followed by an i.v. injection of bradykinin 10 min later. The chemical ablation of capsaicin-sensitive afferent neurons was achieved with repeated s.c. injections of capsaicin (total dose; 100 mg/kg) once daily for 3 days, 2 weeks before the experiment [3, 5].

MEASUREMENT OF MUCOSAL PGE_2 AND PGI_2 LEVELS: Mucosal PGE_2 and PGI_2 (6-keto $\text{PGF}_{1\alpha}$) levels in the stomach were measured after the application of capsaicin (0.3 mg/ml) for 10 min. The stomach was removed 30 later, weighed, and put in a tube containing 100% methanol plus 0.1 M indomethacin [23]. The samples were then minced with scissors, homogenized, and centrifuged at 12000 g for 10 min at 4°C. The supernatant of each sample was

used to measure PGE_2 and 6-keto $\text{PGF}_1 \alpha$ levels by EIA with PGE_2^- and 6-keto $\text{PGF}_1 \alpha$ -kits (Cayman Chemical Co., Ann Arbor, MI).

PREPARATION OF DRUGS: The drugs used in the present study were urethane (Tokyo kasei, Tokyo, Japan), capsaicin and bradykinin (Nacalai Tesque, Kyoto, Japan), prostaglandin E_2 (PGE_2 : Funakoshi, Tokyo, Japan), capsazepine, N^G -nitro L-arginine methyl ester (L-NAME), and indomethacin (Sigma Chemicals, St. Louis, Mo, USA), ONO-8711 (Ono Pharmaceutical Co., Osaka, Japan), NOR-3 [(±)- (E)-ethyl-2- [(E)-hydroxyimino]-5-nitro-3-hexeneamine] (Dojindo, Kumamoto, Japan), omeprazole (Astra Zeneca, Möndal, Sweden), FR172357, and terbutaline (Buricanyl[®], Fujisawa, Osaka, Japan), and aminophylline (Neophylline[®], Eizai, Tokyo, Japan). Capsaicin was dissolved in a Tween 80-ethanol solution (10% ethanol, 10% Tween, and 80% saline, w/w: Wako, Osaka, Japan) for the s.c. injection, while it was suspended in a 0.5% carboxymethylcellulose solution (CMC: Nacalai Tesque) for the topical application. PGE_2 or NOR-3 was first dissolved in absolute ethanol or dimethyl sulfoxide (DMSO), respectively, and diluted with saline to the desired concentrations. Omeprazole was suspended in a 0.5% CMC solution. Other agents were dissolved in saline. Each agent was prepared immediately before use and given in a volume of 0.5 ml per 100 g body weight in the case of the i.p. or s.c. administration, in a volume of 0.1 ml per 100 g body weight in the case of the i.v. administration, or applied topically to the chamber in a volume of 2 ml per rat or 0.7 ml per mice. Control animals received saline or CMC instead of active agents.

STATISTICS: Data are presented as the mean±SE from 4~8 rats or mice per group. Statistical analyses were performed using a two-tailed Dunnett's multiple comparison test, and values of $p < 0.05$ were regarded as significant.

3. Results

3.1. Stimulation by capsaicin and mucosal acidification of HCO_3^- secretion in the rat stomach

Capsaicin (0.03~0.3 mg/ml) applied to the chamber for 10 min increased the secretion of HCO_3^- in a dose-dependent manner, and this effect was significant at a dose of 0.1 mg/ml or greater (Figure 1). The stimulatory effect of capsaicin (0.3 mg/ml) on HCO_3^- was significantly attenuated by the chemical ablation of capsaicin-sensitive afferent neurons as well as the prior administration of indomethacin (5 mg/kg, s.c.) or L-NAME (20 mg/kg, s.c.) (Figure 2). The action of capsaicin was also potently inhibited by the co-application of capsazepine, the TRPV1 antagonist. However, neither the EP1 antagonist, ONO-8711 (10 mg/kg, s.c.) nor the bradykinin B2 antagonist, FR172357 (1 mg/kg, i.v.) had any effect on HCO_3^- secretion in response to capsaicin.

The secretion of HCO_3^- was also increased in a concentration-dependent manner when the mucosa was acidified following its exposure to 50~200 mM HCl for 10 min (Figure 3). The response to 200 mM HCl was significantly prevented by indomethacin, L-NAME, ONO-8711, and capsaicin pretreatments (Figure 4). However, acid-induced HCO_3^- secretion in the stomach was not affected by either capsazepine or FR172357.

Figure 1. Effects of capsaicin on gastric HCO₃⁻ secretion in anesthetized rats. Capsaicin (0.03–0.3 mg/ml) was applied to the chamber for 10 min. In Figure A, data are presented as a % of basal values and represent the mean±SE of values determined every 10 minutes from 4–7 rats. Figure B shows the total net HCO₃⁻ output for 1 hr after the capsaicin treatment, and data are presented as the mean±SE for 4–7 rats. *Significantly different from the control, at P<0.05.

Figure 2. Effects of the pretreatment with various agents and capsaicin on gastric HCO₃⁻ secretion induced by capsaicin in anesthetized rats. Capsaicin (0.3 mg/ml) was applied to the chamber for 10 min. Indomethacin (5 mg/kg), ONO-8711 (10 mg/kg), or L-NAME (10 mg/kg) was administered s.c. 1 or 3 hr before capsaicin, respectively. FR172367 (1 mg/kg) was administered i.v. 15 min before the mucosal application of capsaicin. Capsazepine (2.5 mg/ml) was applied to the chamber for 20 min, starting 10 min before the capsaicin treatment. The chemical ablation of sensory neurons (capsaicin pretreatment) was achieved with 3 consecutive s.c. injections of capsaicin (total dose: 100 mg/kg) 2 weeks before the experiment. The figure shows the total net HCO₃⁻ output for 1 hr after the capsaicin treatment, and data are presented as the mean±SE from 4–6 rats. Significantly different at P<0.05; *from the control; # from saline.

Figure 3. Effects of mucosal acidification on gastric HCO_3^- secretion in anesthetized rats. Acidification was achieved by exposing the mucosa to 50~200 mM HCl for 10 min. In Figure A, data are presented as a % of basal values and represent the mean $\pm$ SE of values determined every 10 minutes from 4~6 rats. Figure B shows the total net HCO_3^- output for 1 hr after acidification, and data are presented as the mean $\pm$ SE for 4~6 rats. *Significantly different from the control, at $P<0.05$.

Figure 4. Effects of the pretreatment with various agents and capsaicin on gastric HCO_3^- secretion induced by mucosal acidification in anesthetized rats. Acidification was achieved by exposing the mucosa to 200 mM HCl for 10 min. Indomethacin (5 mg/kg), ONO-8711 (10 mg/kg), or L-NAME (10 mg/kg) was administered s.c. 1 or 3 hr before acidification, respectively. FR172357 (1 mg/kg) was administered i.v. 15 min before mucosal acidification. Capsazepine (2.5 mg/ml) was applied to the chamber for 20 min, starting 10 min before the capsaicin treatment. The chemical ablation of afferent neurons (capsaicin pretreatment) was achieved with 3 consecutive s.c. injections of capsaicin (total dose: 100 mg/kg) 2 weeks before the experiment. The figure shows the total net HCO_3^- output for 1 hr after the capsaicin treatment, and data are presented as the mean $\pm$ SE for 4~6 rats. Significantly different at $P<0.05$; *from the control; # from saline.

3.2. Effect of capsaicin and mucosal acidification on PGE₂ and 6-keto PGF₁ α levels in the rat stomach

The intragastric application of capsaicin (0.3 mg/ml) for 10 min did not affect the amount of PGE₂, but significantly increased that of 6-keto PGF₁ α to approximately 2.8-fold that of the control level (data not shown). In contrast, the acidification of the gastric mucosa significantly stimulated the production of PGs and increased PGE₂ or 6-keto PGF₁ α levels to approximately 2 or 3-fold that of basal levels, respectively.

3.3. Stimulation by PGE₂, NOR-3, and bradykinin of HCO₃⁻ secretion in the rat stomach

The intravenous administration of PGE₂ (1 mg/kg) increased the secretion of HCO₃⁻ in the stomach, and this response was equivalent to that induced by capsaicin at 0.3 mg/ml (Figure 5). The NO donor, NOR-3 (3 mg/ml), which was applied topically to the mucosa for 10 min, also increased HCO₃⁻ secretion. Neither indomethacin, L-NAME, nor FR172357 significantly affected the increase in HCO₃⁻ secretion in response to PGE₂ (data not shown). Gastric HCO₃⁻ secretion was also stimulated by the i.v. administration of bradykinin (30 μ g/kg), reached a maximal value of 160% that of the basal level; however, this effect was less potent than that of capsaicin or acidification and completely disappeared after 1 hr (Figure 6). The stimulatory effect of bradykinin on HCO₃⁻ was significantly antagonized by FR172357 and attenuated by the prior administration of indomethacin or L-NAME. In addition, the stimulatory effect of bradykinin was almost completely blocked by the chemical ablation of capsaicin-sensitive afferent neurons, but was not significantly affected by pretreatment with capsazepine.

Figure 5. Effects of PGE₂ and NOR-3 on gastric HCO₃⁻ secretion in anesthetized rats. PGE₂ (1 mg/kg) was administered i.v., while NOR-3 (3 mg/ml) was applied to the chamber for 10 min. Data represent the total net HCO₃⁻ output for 1 hr after the administration of PGE₂ or NOR-3 and are presented as the mean±SE for 4-5 rats. *Significantly different from the control, at P<0.05.

Figure 6. Effects of bradykinin on gastric HCO_3^- secretion in anesthetized rats. Bradykinin (30 $\mu\text{g/kg}$) was administered i.v. after basal HCO_3^- secretion had been stabilized. Indomethacin (5 mg/kg) was administered s.c. 1 hr before bradykinin, while FR172357 (1 mg/kg) was given i.v. 15 min before. L-NAME (20 mg/kg) was administered s.c. 3 hr before bradykinin. Capsazepine (2.5 mg/ml) was applied for 20 min to the chamber 10 min before the administration of bradykinin. The chemical ablation of sensory neurons (capsaicin pretreatment) was achieved with 3 consecutive s.c. injections of capsaicin (total dose: 100 mg/kg) 2 weeks before the experiment. Data show the total net HCO_3^- output for 1 hr after the capsaicin treatment and are presented as the mean $\pm$ SE for 4–5 rats. Significantly different at $P<0.05$; *from the control; # from bradykinin+saline.

3.4. Effect of capsaicin on HCO_3^- secretion in wild-type and IP-receptor knockout mice

We previously demonstrated the importance of PGI_2/IP receptors in the HCO_3^- stimulatory action of capsaicin in the duodenum [16]. Since capsaicin also stimulated HCO_3^- secretion in the stomach, in an indomethacin-inhibitable manner, we attempted to identify the type of prostanoid receptor involved in capsaicin-induced responses in the stomach using EP1-, EP3-, and IP-receptor knockout mice, in comparison with those induced by mucosal acidification.

The mouse stomach spontaneously secreted HCO_3^- at a rate of 0.1–0.3 $\mu\text{Eq}/10$ min. Capsaicin (0.3 mg/ml), which was applied to the chamber for 10 min, increased the gastric secretion of HCO_3^- in wild-type mice, and the same effect was also observed in EP1- or EP3-receptor knockout mice, but was absent in mice lacking IP receptors (Figure 7). HCO_3^- secretion was also increased in wild-type mice following the exposure of the mucosa to 50 mM HCl for 10 min, and the same response was also observed in EP3 and IP, but not EP1 receptor knockout animals (Figure 8). The response induced by either capsaicin or acidification in wild-type animals was significantly attenuated by the pretreatment with indomethacin.

Figure 7. Effects of capsaicin on gastric HCO₃⁻ secretion in wild-type, and EP1-, EP3-, and IP-receptor knockout mice under urethane anesthesia. Capsaicin (0.3 mg/ml) was applied to the chamber for 10 min. In some wild-type mice, indomethacin (5 mg/kg) was given s.c. 1 hr before the capsaicin treatment. In Figure A, data are presented as a % of basal values and represent the mean±SE of values determined every 10 minutes from 4–7 rats. Figure B shows the total net HCO₃⁻ output for 1 hr after the capsaicin treatment, and data are presented as the mean±SE for 4–7 rats. Significantly different at P<0.05; *from control wild-type mice; # from wild-type mice treated with capsaicin+saline.

4. Commentary

The gastroduodenal mucosa responds to acidification by significantly increasing the secretion of HCO₃⁻, which, in collaboration with mucus, contributes to the mucosal tolerance of luminal acid [1]. We previously reported that the intraluminal application of capsaicin stimulated the secretion of HCO₃⁻ in these tissues by activating capsaicin-sensitive afferent neurons [4, 5, 15, 16]. The present study confirmed that both acid and capsaicin increased HCO₃⁻ secretion in the stomach, which was mediated by these afferent neurons, and clearly showed the difference in their modes of action in terms of sensitivity to TRPV1 and prostanoid receptors. Furthermore, we observed the involvement of endogenous PGs and NO in the stimulatory action of capsaicin in the stomach, which was consistent with previous findings in the duodenum [21].

TRPV1 is a nonselective cation channel that responds to protons as well as capsaicin [6]. The binding sites of capsaicin are located on the intracellular site of the receptor protein [24], whereas the target of protons is thought to be located on the extracellular surface of the receptor protein [25]. When the TRPV1 antagonist, capsazepine was applied to the mucosa together with capsaicin or acid in the present study, it completely blocked the increase in gastric HCO₃⁻ secretion induced by capsaicin, but not acid, in spite of both responses being mediated

Figure 8. Effects of mucosal acidification on gastric HCO_3^- secretion in wild-type, and EP1-, EP3-, and IP-receptor knockout mice under urethane anesthesia. Acidification was achieved by exposing the mucosa to 50 mM HCl for 10 min. In some wild-type mice, indomethacin (5 mg/kg) was administered s.c. 1 hr before the capsaicin treatment. In Figure A, data are presented as a % of basal values and represent the mean $\pm$ SE of values determined every 10 minutes from 4–8 rats. Figure B shows the total net HCO_3^- output for 1 hr after the capsaicin treatment, and data are presented as the mean $\pm$ SE for 4–8 rats. Significantly different at $P<0.05$; * from control wild-type mice; # from wild-type mice treated with 50 mM HCl+saline.

by capsaicin-sensitive afferent neurons. These results are consistent with our previous findings in the duodenum, in which capsazepine significantly mitigated the response induced by capsaicin, but not mucosal acidification [13]. Akiba et al [26] reported that acid in the lumen induced a mucosal hyperemic response in the rat duodenum in a capsazepine-sensitive manner, and suggested that luminal acid may be an endogenous ligand for duodenal TRPV1. McIntyre et al [27] described pharmacological differences between the human and rat TRPV1 and demonstrated that capsazepine blocked the response of human, but not rat TRPV1 to low pH. The reason for these different results between studies currently remains unclear. The results of the present study do not exclude the involvement of TRPV1 in the acid-induced secretion of HCO_3^- ; however, the target site of acid may differ from that of capsaicin, i.e., the binding site inhibitable by capsazepine. Alternatively, acid may activate these afferent neurons through acid-sensing ionic channels (ASICs). This proposal has been supported by the recent findings in which the acid-induced duodenal HCO_3^- response was greater in female than male rats, with the different responses being parallel with the intensity of the expression of ASIC3, and ovariectomy suppressed the expression of ASIC3 in the duodenum and abolished such a gender difference in the HCO_3^- response [28].

Endogenous PGs are known to be particularly important in the local regulation of HCO_3^- secretion in the gastroduodenal mucosa. We previously demonstrated, using subtype-specific EP agonists and antagonists, that PGE_2 stimulated the secretion of HCO_3^- in the duodenum

through EP3/EP4 receptors and in the stomach through EP1 receptors [15, 16, 29]. Many previous studies reported that mucosal acidification increased HCO_3^- secretion in these tissues, with a concomitant rise in mucosal PGE_2 levels [12, 13, 16]. Capsaicin also stimulated the secretion of HCO_3^- in the stomach in an indomethacin-inhibitable manner, which suggested the involvement of endogenous PGs. However, capsaicin was shown to increase PGE_2 production in the duodenum, but not in the stomach [13, 30]. Notwithstanding, this agent exhibited various effects in the stomach, such as mucosal protection and hyperemia, mediated by capsaicin-sensitive afferent neurons that also depended on endogenous PGs [4, 5, 31]. We confirmed that the intragastric application of capsaicin significantly enhanced the levels of 6-keto $\text{PGF}_{1\alpha}$, the PGI_2 metabolite, which was consistent with the findings reported in the mouse stomach [21, 31]. We previously demonstrated that the gastroprotective effects of capsaicin against HCl/ethanol were significantly attenuated by indomethacin in wild-type mice, but were completely absent in animals lacking IP receptors [30]. In the present study, capsaicin increased gastric HCO_3^- secretion in EP1- and EP3-receptor knockout mice, similar to wild-type mice, but did not in animals lacking IP receptors. These results strongly suggest that endogenous PGI_2 plays a supportive role in the action of capsaicin in the stomach, possibly by sensitizing sensory neurons through IP receptors.

However, HCO_3^- secretion induced by acidification remained unchanged in IP receptor knockout mice and was absent in animals lacking EP1 receptors. These results further support the response of HCO_3^- induced in the stomach by acidification and capsaicin, but depending on sensory neurons, being mediated by different mechanisms related to PG dependency; the former is mainly mediated by PGE_2 through EP1 receptors, while the latter depends on PGI_2 /IP receptors. Similar results were obtained for the gastric hyperemic response induced by acid or capsaicin [31]. Although gastric hyperemic responses to these treatments were mitigated by the capsaicin pretreatment [2, 32], the response induced by acid required the presence of EP1 receptors [33], while that evoked by capsaicin required the presence of IP receptors [29]. Thus, it is not unreasonable to assume that the presence of different prostanoid receptors may be required for gastric HCO_3^- secretion in response to acid or capsaicin.

We found that capsaicin had no effect on the production of PGE_2 , but significantly increased that of PGI_2 in the stomach. Capsaicin-sensitive afferent neurons are known to be abundantly at peri-vascular sites, and the stimulation by capsaicin releases CGRP/NO, resulting in an increase in mucosal blood flow [2]. Harada et al. [34] reported that the activation of these afferent neurons ameliorated ischemia/reperfusion-induced liver injury by limiting the inflammatory response through an enhancement in endothelial PGI_2 production, and suggested that the CGRP-induced activation of both endothelial NO synthase and cyclooxygenase-1 may be involved in this mechanism. Thus, it is possible that capsaicin increases endothelial PGI_2 production locally in the stomach when applied topically to the mucosa. The reason why capsaicin had different effects on the production of PGE_2 and PGI_2 in the stomach currently remains unknown.

The present study also showed that capsaicin-induced HCO_3^- secretion in the stomach was significantly attenuated by L-NAME, which suggested the involvement of endogenous NO in this process, in addition to PGs. Several studies showed that CGRP, the dominant neurotrans-

mitter of spinal afferents, had various pharmacological actions, such as vasodilation, that were mediated by endogenous NO [2, 3, 35]. We demonstrated that the NO donor, NOR-3 stimulated gastric HCO_3^- secretion in the present study, and this was consistent with our previous findings in the duodenum [12]. Nishihara et al [36] reported that capsaicin increased the release of CGRP and NO in the rat stomach. Although we did not measure NO release in the stomach following the capsaicin treatment, it is assumed that capsaicin activated primary afferent neurons, with the assistance of PGI_2 , to liberate CGRP, which in turn stimulated NO release, resulting in an increase in gastric HCO_3^- secretion.

Bradykinin is also known to activate nociceptive-like afferent neurons through metabotropic G protein-coupled bradykinin B2 receptors [37, 38]. A previous study showed that binding to B2 receptors activated an intracellular signaling cascade, which led to the opening of TRPV1 channels [39]. We found that bradykinin itself stimulated the secretion of HCO_3^- in the stomach in the present study. Furthermore, this response was attenuated not only by FR172357, the B2 antagonist, but also by indomethacin and L-NAME, which suggested the involvement of both PGs and NO in the response of HCO_3^- to bradykinin. The stimulatory effect of bradykinin was also significantly mitigated by the chemical ablation of capsaicin-sensitive afferent neurons, but was not affected by capsazepine, a TRPV1 antagonist. The stimulatory effect of bradykinin on HCO_3^- secretion is assumed to be partly mediated by sensory neurons via B2 receptors, but not through the interaction with TRPV1, in addition to endogenous PGs. Since bradykinin also potentiates the activation of TRPV1 by capsaicin through the hydrolysis of endogenous phosphatidylinositol-4, 5-bisphosphate in a phospholipase C-dependent manner [39, 40], it is possible that capsaicin-induced gastric HCO_3^- secretion may be affected by the B2 receptor antagonist. However, the present study showed that the responses induced by capsaicin and acidification were not significantly affected by FR172357, which suggests that endogenous bradykinin has no role in these responses. The reason for these results has yet to be elucidated and is currently under investigation in our laboratory.

5. Summary

Capsaicin is assumed to stimulate the secretion of HCO_3^- in the stomach mediated by endogenous PGs and NO, as well as capsaicin-sensitive afferent neurons, but not bradykinin B2 receptors. Mucosal acidification also increased gastric HCO_3^- secretion through sensory neurons mediated by both PGs and NO, similar to capsaicin; however, their modes of action differed in terms of capsazepine-sensitivity and prostanoid receptor-dependency (Figure 9). Although luminal H^+ played a modulator-type role in the physiological response mediated by capsaicin-sensitive afferent neurons in the stomach, it is likely that this action was not due to the interaction of H^+ with the capsazepine-sensitive site of TRPV1, but resulted from the activation of ASIC3.

Figure 9. Mechanisms underlying stimulation of gastric HCO_3^- secretion induced by capsaicin and acid. Capsaicin stimulated HCO_3^- secretion in the stomach, essentially through capsaicin-sensitive afferent neurons via the activation of TRPV1, and this action was mediated with endogenous PGE₂/EP1 receptors and NO. Mucosal acidification also increased HCO_3^- secretion through the activation of sensory neurons as well as endogenous PGs and NO; however, this action did not result from an interaction between H^+ and the capsazepine-sensitive site of TRPV1, and depended on the PGI₂/IP receptor.

Acknowledgements

The authors are greatly indebted to Professor Shu Narumiya, Kyoto University Faculty of Medicine, for kindly supplying EP1, EP3, and IP receptor-knockout mice and Ono Pharmaceutical for supplying various EP agonists and antagonists. We also thank the undergraduate students at the Department of Pharmacology and Experimental Therapeutics, Kyoto Pharmaceutical University, Kyoto, Japan, for their technical collaboration.

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The authors declare no conflict of interest.

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