Mechanisms Underlying Capsaicin-Stimulated HCO₃⁻ Secretion in the Stomach: Comparison with Mucosal Acidification

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ABSTRACT

The effects of capsaicin and mucosal acidification on gastric HCO₃⁻ secretion were compared in wild-type and prostacyclin (PGI₂) IP receptor or prostaglandin E receptor EP1 or EP3 knockout C57BL/6 mice as well as rats. Under urethane anesthesia, the stomach was mounted on an ex vivo chamber, perfused with saline, and the secretion of HCO₃⁻ was measured at pH 7.0 using the pH-stat method. Capsaicin or 200 mM HCl was applied to the chamber for 10 min. Capsaicin increased the secretion of HCO₃⁻ in rats and wild-type mice, the response at 0.3 mg/ml being equivalent to that induced by acidification. This effect of capsaicin in rats was abolished by ablation of capsaicin-sensitive afferent neurons and attenuated by indomethacin, N^α-nitro-L-arginine methyl ester (L-NAME), and capsaicine [transient receptor potential vanilloid type 1 (TRPV1) antagonist] but not FR172357 [3-bromo-8-[2,6-dichloro-3-[N(E,E)-4-(N,N-dimethylcarbamoyl) cinnamidoacetyl]-N-methylamino]benzyl amino]-2-methylimidazo[1,2-a]pyridine; bradykinin B₂ antagonist] or the EP1 antagonist. The acid-induced HCO₃⁻ secretion was attenuated by indomethacin, L-NAME, the EP1 antagonist, and sensory deafferentation, but not affected by capsaicine or FR172357. Prostaglandin E₂ (PGE₂), NOR-3 [(±)-(E)-ethyl-2-[(E)-hydroxyimino]-5-nitro-3-hexeneaminio]-5-nitro-3-hexeneamine (NO donor), and bradykinin stimulated the secretion of HCO₃⁻, and the effect of bradykinin was blocked by indomethacin and L-NAME as well as FR172357. The stimulatory effect of capsaicin disappeared in IP (-/-) mice, whereas that of acidification disappeared in EP1 (-/-) mice. Intragastric application of capsaicin increased mucosal PGI₂ but not PGE₂ levels in the rat stomach. These results suggested that both capsaicin and acid increase gastric HCO₃⁻ secretion via a common pathway, involving PG and NO as well as capsaicin-sensitive afferent neurons, yet their responses differ concerning TRPV1 or prostanooid receptor dependence.

The gastric mucosa is kept intact by multiple protective mechanisms including humoral and neuronal factors, despite exposure to acid and other chemical hazards (Flemstrom and Garner, 1982). Capsaicin-sensitive afferent neurons play a central role in the neuronal mechanism of the stomach (Holzer, 1998). These afferent neurons regulate various gastric functions such as secretion, mucosal blood flow, and motility and modulate the mucosal integrity of the stomach (Holzer and Samez; 1986 Holzer, 1998; Takeuchi et al., 1991, 1992). Vanilloid receptor type 1, a nonselective cationic channel, has been recently cloned as the binding site of capsaicin (Caterina et al., 1997) and, more recently, has been shown to be one of the transient receptor potential (TRP) family of ion channels (Clapham et al., 2001). Although the TRP family is activated by a diverse range of stimuli, including depletion of intracellular Ca²⁺ stores (Caterina et al., 1997), the vaniloid receptor type 1 receptor remains the only channel activated by vanilloids such as capsaicin and is now known as TRPV1 (Gunthorpe et al., 2002). 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 by so doing exerts a gastroprotective action (Merchant et al., 1994). CGRP acts on endothelial cells to release NO, and this molecule is also known to mediate, in large part, the action of CGRP (Holzer, 1998). Recent studies also showed that the activation of the bradykinin B₂ receptor leads to the opening of TRPV1 and modifies the action of capsaicin (Shin et al., 2002; Ferreira et al., 2004).

ABBREVIATIONS: TRP, transient receptor potential; TRPV1, transient receptor potential vanilloid type 1; CGRP, calcitonin gene-related peptide; PG, prostaglandin; PGI₂, prostacyclin; PGE₂, prostaglandin E₂; FR172357, 3-bromo-8-[2,6-dichloro-3-[N(E,E)-4-(N,N-dimethylcarbamoyl) cinnamidoacetyl]-N-methylamino]benzyl amino]-2-methylimidazo[1,2-a]pyridine; L-NAME, N^α-nitro-L-arginine methyl ester; NOR-3, (±)-(E)-ethyl-2-[(E)-hydroxyimino]-5-nitro-3-hexeneaminio]-5-nitro-3-hexeneamine; CMC, carboxymethylcellulose solution; ONO-8711, 6-[[2(S,3S)-3-(4-chloro-2-methylphenylsulfonamylamino)methyl]-bicyclo(2,2,2) octan-2-yl]-5Z-hexenoic acid; NO, nitric oxide.
The secretion of HCO₃⁻ from surface epithelial cells is a protective mechanism in the stomach, the HCO₃⁻ working in collaboration with mucus gel that adheres to the surface of mucosa (Flemstrom and Garner, 1982). We previously reported that capsaicin increased duodenal HCO₃⁻ secretion mediated by endogenous prostaglandins (PGs) and NO as well as capsaicin-sensitive afferent neurons (Sugamoto et al., 2001; Kagawa et al., 2003). We also reported that prostaglandin (PG) E₂ (PGE₂) stimulates HCO₃⁻ secretion through EP₁ receptors in the stomach and EP3 receptors in the duodenum (Takeuchi et al., 1997; Aoi et al., 2004), whereas the action of capsaicin in the duodenum requires the presence of prostacyclin (PGI₂) IP receptors (Nakashima et al., 2004). However, few studies have examined the mechanisms involved in gastric HCO₃⁻ secretion in response to capsaicin.

In the present study, we investigated the regulatory mechanism of capsaicin-induced gastric HCO₃⁻ secretion, in relation to sensory nerves, TRPV₁, PGs, NO, and bradykinin B₂ receptors, and compared it with that of the acid-induced response. In addition, since we have found that the responses to capsaicin and acid in the duodenum differ concerning PGI₁/IP dependence (Nakashima et al., 2004), we also examined these responses in the stomach using mice lacking EP₁, EP₃, or IP receptors.

Materials and Methods

Animals. Male Sprague-Dawley 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 (Oida et al., 1995; Ushikubi et al., 1998). No abnormality was detected in general body appearance or in the morphological feature of the gastroduodenal mucosa. The distribution of the EP₁, EP₃, and IP receptor genes was verified by Northern-blot hybridization, which failed to detect mRNAs encoding the respective receptors in EP₁ (−/−), EP₃ (−/−), and IP (−/−) mice. These rats and knockout mice were deprived of food but allowed free access to tap water for 18 h before the experiments. Studies were performed under urethane anesthesia (1.25 g/kg, i.p.) using approximately four to eight animals per group. All experimental procedures were approved by the Experimental Animal Research Committee of Kyoto Pharmaceutical University.

Determination of Gastric HCO₃⁻ Secretion. The secretion of HCO₃⁻ was measured in the chambered stomach as described previously (Takeuchi et al., 1992). The abdomen was incised and the stomach was exposed, mounted on a chamber (exposed area, rat, 3.1 cm²; mouse, 0.7 cm²), and superfused with saline that was gassed with 100% O₂ and kept in a reservoir. The secretion of HCO₃⁻ 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. To unmask the mucosal surface epithelial cells, the stomach was exposed, mounted on a chamber (exposed area, rat, 3.1 cm²; mouse, 0.7 cm²), and superfused with saline that was gassed with 100% O₂ and kept in a reservoir. The secretion of HCO₃⁻ 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.

Measurement of Mucosal PGE₂ and PGI₂ Levels. The mucosal PGE₂ and PGI₂ (6-keto PGF₁α) levels in the stomach were measured after application of capsaicin (0.3 mg/ml) for 10 min. Thirty minutes later, the stomach was removed, weighed, and put in a tube containing 100% methanol plus 0.1 M indomethacin (Futaki et al., 1994). Then, the samples were minced with scissors, homogenized, and centrifuged at 12,000 rpm for 10 min at 4°C. The supernatant of the samples was used for measuring levels of PGE₂ and 6-keto PGF₁α, by enzyme immunoassay with PGE₂- and 6-keto PGF₁α-kits (Cayman Chemical, Ann Arbor, MI), respectively.

Preparation of Drugs. Drugs used were urethane (Tokyo Kasei, Tokyo, Japan), capsaicin and bradykinin (Nacalai Tesque, Kyoto, Japan), PGE₂ (Funakoshi, Tokyo, Japan), capsazone, l-NAME, and indomethacin (Sigma-Aldrich, St. Louis, MO), ONO-8711 (Ono Pharmaceutical Co., Osaka, Japan), NOR-3 (Dainippon Sumitomo, Japan), omeprazole (Astra Hásse AB, Möln达尔, Sweden) and FR172357, terbutaline (Buricanyl; Fujisawa Pharmaceutical, Osaka, Japan), and aminophylline (Neophylline; Eizai, Tokyo, Japan). Capsaicin was dissolved in a Tween 80-ethanol solution (10% ethanol, 0.03 mg/ml) or bradykinin B₂ antagonist (Asano et al., 1997), capsazone (a TRPV₁ antagonist), or chemical ablation of capsaicin-sensitive afferent neurons were examined on the secretion of HCO₃⁻ induced by the above agents or the 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, whereas l-NAME (20 mg/kg) was given s.c. 3 h before because this agent acutely increased HCO₃⁻ secretion through a neural reflex due to an increase of blood pressure (Takeuchi et al., 1993; Aihara et al., 2005). Capsazepine (2.5 mg/ml) was applied to the chamber for 20 min starting from 10 min before capsaicin or acid treatment (Kagawa et al., 2003) or applied for 20 min followed by i.v. injection of bradykinin 10 min later. 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 (Holzer and Sametz, 1986; Takeuchi et al., 1992). All the injections were performed under ether anesthesia, and the rats were pretreated with terbutaline (0.1 mg/kg i.m.) and aminophylline (10 mg/kg i.m.) to counteract the respiratory impairment associated with capsaicin. To check for the effectiveness of the treatment, a drop of capsaicin solution (0.1 mg/ml) was instilled onto one eye of each rat, and wiping movements were counted as previously reported. In most of the above experiments, blood pressure was concomitantly measured via the femoral via using a pressure transducer and amplifier system (TP-200TL, AP-100F, and RТА-1100A; Nihon Koden, Tokyo, Japan).

Results

Effect of Capsaicin on Gastric HCO₃⁻ Secretion. Under urethane anesthesia, the rat stomach spontaneously secreted HCO₃⁻.
creted HCO$_3^-$ at a steady rate of approximately 0.2 to 0.4 μEq/10 min during a test period. 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 the dose of 0.1 mg/ml or greater; the ΔHCO$_3^-$ output at 0.1 and 0.3 mg/ml was 0.9 ± 0.1 and 1.4 ± 0.3 μEq/h, respectively (Fig. 1). In the subsequent experiments, we used capsaicin at 0.3 mg/ml.

The stimulatory effect of capsaicin on HCO$_3^-$ was significantly attenuated by the chemical ablation of capsaicin-sensitive afferent neurons as well as prior administration of indomethacin (5 mg/kg s.c.) or l-NAME (20 mg/kg s.c.) (Fig. 2). The ΔHCO$_3^-$ output was 0.2 ± 0.1, 0.1 ± 0.2, and 0.1 ± 0.1 μEq/h, respectively, in the animals pretreated with indomethacin, l-NAME, or capsaicin, all of which were significantly less than that (1.4 ± 0.3 μEq/h) of the vehicle-treated animals. Likewise, the stimulatory action of capsaicin was potently inhibited by coapplication of capsazepine, the TRPV1 antagonist, the ΔHCO$_3^-$ output being 0.3 ± 0.2 μEq/h, equivalent to that of the control group without capsaicin treatment (Fig. 3). In contrast, both the EPI1 antagonist ONO-8711 (10 mg/kg s.c.) and the bradykinin B2 antagonist FR172357 (1 mg/kg i.v.) did not significantly affect gastric HCO$_3^-$ secretion in response to capsaicin (Fig. 2).

**Effect of Mucosal Acidification on Gastric HCO$_3^-$ Secretion.** The secretion of HCO$_3^-$ in the stomach was increased in a concentration-dependent manner when the mu-
cosa was acidified by exposure to approximately 50 to 200 mM HCl for 10 min; the ΔHCO₃⁻ output induced by acidification at 200 mM HCl was 1.7 ± 0.2 μEq/h (Fig. 4). The response to 200 mM HCl was significantly prevented by indomethacin, L-NAME, and capsaicin pretreatment, but not FR172357, the degree of inhibition being 61.6, 61.6, and 49.9%, respectively (Fig. 5). The EP1 antagonist ONO-8711 also significantly inhibited the gastric response induced by mucosal acidification. However, the acid-induced HCO₃⁻ secretion in the stomach was not significantly affected by co-application of capsaazepine, and the Δ HCO₃⁻ output was 1.9 ± 0.6 μEq/h, almost equivalent to that (1.8 ± 0.5 μEq/h) of the vehicle-treated group (Fig. 6).

Effects of Capsaicin on Mucosal PGE₂ and 6-Keto PGF₁α Levels in Rat Stomach. Mucosal PGE₂ and 6-keto PGF₁α levels in the normal rat stomach were 4.9 ± 0.7 and 5.8 ± 0.9 ng/g tissue, respectively. 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 PGF₁α to about 2.8-fold the control level, the value being 16.9 ± 2.3 ng/g tissue (Fig. 7).

Effect of PGE₂, NOR-3, and Bradykinin on Gastric HCO₃⁻ Secretion. Since the response of HCO₃⁻ to capsaicin or acid in the stomach was attenuated by either indomethacin or L-NAME but not FR172357, it is assumed that both PGs and NO but not bradykinin are involved in the stimulatory mechanism of these agents. To further investigate the interactive role of PGs and NO in the mechanism of HCO₃⁻ secretion in the stomach, we examined the effects of PGE₂, NOR-3 (NO donor), and bradykinin on the secretion, in the absence or presence of indomethacin, L-NAME, or FR172357. Intravenous administration of PGE₂ (1 mg/kg) increased the secretion of HCO₃⁻ in the stomach, and the ΔHCO₃⁻ output was 1.5 ± 0.5 μEq/h, the value being equivalent to
that induced by capsaicin at 0.3 mg/ml (Fig. 8). Likewise, the NO donor NOR-3 (3 mg/ml), applied topically to the mucosa for 10 min, also increased HCO$_3^-$ secretion, the Δ HCO$_3^-$ output being $1.0 \pm 0.2$ μEq/hr. Indomethacin, L-NAME, or FR172357 did not significantly affect the increase of HCO$_3^-$ secretion in response to PGE$_2$ (data not shown). Likewise, gastric HCO$_3^-$ secretion was also stimulated by i.v. administration of bradykinin (30 μg/kg), reaching a maximal value of 160% of the basal level, although this effect was less potent than that of capsaicin or acidification and completely disappeared at 1 h (Fig. 9). The stimulatory effect of bradykinin on HCO$_3^-$ was significantly antagonized by FR172357 and also attenuated by prior administration of indomethacin or L-NAME later. In addition, the stimulatory effect of bradykinin was almost totally blocked by chemical ablation of capsaicin-sensitive afferent neurons but not significantly affected by pretreatment with capsazepine.

**Effect of Capsaicin on Gastric HCO$_3^-$ Secretion in Wild-Type and IP Receptor Knockout Mice.** We previously reported the importance of PGI$_2$/IP receptors in the HCO$_3^-$ stimulatory action of capsaicin in the duodenum (Nakashima et al., 2004). Since capsaicin also stimulated HCO$_3^-$ secretion in the stomach, in an indomethacin-inhibitable manner, we investigated which type of prostanoid receptor is involved in the capsaicin-induced response in the stomach, using EP1, EP3, and IP receptor knockout mice, in comparison with that induced by mucosal acidification.

The mouse stomach spontaneously secreted HCO$_3^-$ at a rate of approximately 0.1 to 0.3 μEq/10 min. No difference was found in the rate of basal HCO$_3^-$ secretion between
approximately four to six rats.

The gastroduodenal mucosa responds to acidification with a significant rise in the secretion of HCO₃⁻, which, in collaboration with mucus, contributes to the mucosal tolerance of luminal acid (Flemstrom and Garner, 1982). We have previously reported that intraluminal application of capsaicin also stimulates the secretion of HCO₃⁻ in these tissues through the activation of capsaicin-sensitive afferent neurons (Takeuchi et al., 1991, 1992; Aoi et al., 2004; Nakashima et al., 2004). The present study confirms that both acid and capsaicin produced an increase of HCO₃⁻ secretion in the stomach mediated by these afferent neurons and clearly showed the difference in their modes of action in terms of sensitivity to TRPV1 and prostanooid receptors. Furthermore, we observed the involvement of endogenous PGs and NO in the stimulatory action of capsaicin in the stomach, essentially similar to the findings in the duodenum.

TRPV1 is a nonselective cation channel responsive to proton as well as capsaicin (Caterina et al., 1997). The binding sites of capsaicin are located at the intracellular site of the receptor protein (Jung et al., 1999), whereas the target of protons is thought to be located on the extracellular surface of the receptor protein (Jordt et al., 2000). In the present study, when the TRPV1 antagonist capsazepine was applied to the mucosa together with capsaicin or acid, it was found that this agent completely blocked the increase in gastric HCO₃⁻ secretion induced by capsaicin but not acid, despite the fact that both responses are mediated by capsaicin-sensitive afferent neurons. These results are consistent with our previous findings in the duodenum, showing that capsazepine significantly mitigated the response induced by capsaicin but not mucosal acidification (Kagawa et al., 2003). Several investigators examined the effect of capsazepine on various events both in vivo and in vitro induced by acidification, but the results were controversial (Seno et al., 1998; Akiba et al., 1999; McIntyre et al., 2001). Akiba et al. (1999) reported that acid in the lumen induced a mucosal hyperemic response in the rat duodenum in a capsazepine-sensitive way and suggested luminal acid as the endogenous ligand for duodenal TRPV1. McIntyre et al. (2001) reported pharmacological differences between the human and rat TRPV1 and demonstrated that capsazepine blocked the human but not rat TRPV1’s response to low pH. At present, the reason for the different results between these studies remains unclear. The present results do not exclude the involvement of TRPV1 in the acid-induced secretion of HCO₃⁻, yet it is likely that the target site of acid is different from that of capsaicin, i.e., the binding site inhibitable by capsazepine. Alternatively, acid might activate these afferent neurons through acid-sensing ionic channels.

Endogenous PGs are particularly important in the local regulation of HCO₃⁻ secretion in the gastroduodenal mucosa. We previously found, using subtype-specific EP agonists and antagonists, that PGE₂ stimulates the secretion of HCO₃⁻ in the duodenum through EP3/EP4 receptors and in the stomach through EP1 receptors (Takeuchi et al., 1999; Aoi et al., 2004; Nakashima et al., 2004). Many investigators reported that mucosal acidification increases HCO₃⁻ secretion in these tissues, with a concomitant rise in mucosal PGE₂ levels (Sugamoto et al., 2001; Kagawa et al., 2003; Nakashima et al., 2004). Capsaicin also stimulated the secretion of HCO₃⁻
in the stomach in an indomethacin-inhibitable manner, suggesting the involvement of endogenous PGs in this action. However, capsaicin was reported to increase PGE2 production in the duodenum but not in the stomach (Kagawa et al., 2003; Takeuchi et al., 2003). Notwithstanding, this agent has a variety of actions in the stomach, such as mucosal protection and hyperemia, mediated by capsaicin-sensitive afferent neurons and also depending on endogenous PGs (Takeuchi et al., 1991, 1992; Boku et al., 2001). The present study confirmed that intragastric application of capsaicin did not in-

Fig. 9. Effect of bradykinin on gastric HCO₃⁻/H₁¹⁰₀₂⁻ secretion in anesthetized rats. Bradykinin (30 μg/kg) was given i.v. after basal HCO₃⁻ secretion had been stabilized. Indomethacin (5 mg/kg) was given s.c. 1 h before bradykinin, whereas FR172357 (1 mg/kg) was given i.v. 15 min before. L-NAME (20 mg/kg) was given s.c. 3 h before bradykinin. Capsazepine (2.5 mg/ml) was applied for 20 min to the chamber 10 min before the administration of bradykinin. Chemical ablation of sensory neurons (capsaicin pretreatment) was achieved with three consecutive s.c. injections of capsaicin (total dose, 100 mg/kg) 2 weeks before the experiment. A, data are presented as percentage of basal values and represent the mean ± S.E. of values determined every 10 min from approximately four to five rats. B, total net HCO₃⁻ output for 1 h after the capsaicin treatment, and the data are presented as the mean ± S.E. for approximately four to five rats. Significant difference at p < 0.05; *, from control; #, from bradykinin + saline.

Fig. 10. Effect 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 h before the capsaicin treatment. A, data are presented as percentage of basal values and represent the mean ± S.E. of values determined every 10 min from approximately four to seven rats. B, total net HCO₃⁻ output for 1 h after the capsaicin treatment, and the data are presented as the mean ± S.E. for approximately four to seven rats. Significant difference at p < 0.05; *, from control wild-type mice; #, from wild-type mice treated with capsaicin + saline.
crease levels of PGE$_2$ but significantly enhanced levels of 6-keto PGF$_{1\alpha}$, the PGI$_2$ metabolite, consistent with the findings in the mouse stomach (Boku et al., 2001). We previously reported that the gastroprotective action of capsaicin against HCl/ethanol was significantly attenuated by indomethacin in wild-type mice but totally disappeared in animals lacking IP receptors (Takeuchi et al., 2003). In the present study, capsaicin increased gastric HCO$_3^-$ secretion in EP1- and EP3-receptor knockout mice, similar to wild-type mice, but not in the animals lacking IP receptors. Consistent with previous findings (Boku et al., 2001; Takeuchi et al., 2003), these results strongly suggest that endogenous PGI$_2$ plays a supportive role in the action of capsaicin in the stomach, probably by sensitizing the sensory neurons through IP receptors.

However, it should be noted that the HCO$_3^-$ secretion induced by acidification was unaltered in IP receptor knockout mice and disappeared in the animals lacking EP1 receptors. These results further support the idea that the response of HCO$_3^-$ induced in the stomach by acidification and capsaicin, although in both cases depending on the sensory neurons, is mediated by different mechanisms concerning PG dependence; the former is mainly mediated by PGE$_2$ through EP1 receptors, whereas the latter depends on PGL$_2$/IP receptors. Similar results were obtained for the gastric hyperemic response induced by acid or capsaicin (Takeuchi et al., 2003). Although gastric hyperemic responses to these treatments were mitigated by capsaicin pretreatment (Holzer, 1998; Mimaki et al., 2002), the response induced by acid required the presence of EP1 receptors (Takeuchi et al., 2002), whereas that evoked by capsaicin required the presence of IP receptors (Takeuchi et al., 2003). Thus, it is not unreasonable that the presence of different prostanoid receptors is required for gastric HCO$_3^-$ secretion in response to acid or capsaicin.

We observed that capsaicin had no effect on PGE$_2$ production but significantly increased PGL$_2$ generation in the stomach. Capsaicin-sensitive afferent neurons are known to distribute abundantly at perivascular sites, and the stimulation by capsaicin releases CGRP/NO, resulting in increase of mucosal blood flow (Holzer, 1998). Harada et al. (2002) reported that the activation of these afferent neurons ameliorated ischemia/reperfusion-induced liver injury by increasing hepatic blood flow and by limiting inflammatory response through enhancement of endothelial PGI$_2$ production and suggested that the CGRP-induced activation of both endothelial NO synthase and cyclooxygenase-1 is involved in this mechanism. Thus, it is possible that capsaicin increases endothelial PGI$_2$ production locally in the stomach when applied topically in the mucosa. At present, the reason why capsaicin showed different effects on the production of PGE$_2$ and PGI$_2$ in the stomach remains unknown, yet there may be species or tissue differences in this action.

The present study also showed that the capsaicin-induced HCO$_3^-$ secretion in the stomach was significantly attenuated by L-NAME, suggesting the involvement of endogenous NO in this process, in addition to PGs. Several studies showed that CGRP, the dominant neurotransmitter of spinal afferents, had various pharmacological actions, such as vasodilation, mediated by endogenous NO (Holzer and Sametz, 1986; Lambrecht et al., 1993; Holzer, 1998). We demonstrated in this study that the NO donor NOR-3 stimulated gastric HCO$_3^-$ secretion, in agreement with our previous findings in the duodenum (Sugamoto et al., 2001). Nishihara et al. (2002) 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 capsaicin treatment, it is assumed that capsaicin activates primary afferent neurons, with the assistance of PGL$_2$, to liberate CGRP, which in turn stimulates NO release, resulting in an increase in gastric HCO$_3^-$ secretion.

Bradykinin, a product of the kinin-kallikrein system often associated with inflammation, is also known to activate no-
acid-like afferent neurons through metabolotropic G protein-coupled bradykinin B2 receptors (McGuirk and Dolphin, 1992; Maubach and Grundy, 1999). A recent study showed that the binding to B2 receptors activates an intracellular signaling cascade leading to the opening of TRPV1 channels (Chuang et al., 2001). We observed in the present study that bradykinin itself stimulated the secretion of $\text{HCO}_3^-$ in the stomach. Furthermore, this response was attenuated not only by FR172357, the B2 antagonist, but also by indomethacin and L-NAME as well, suggesting the involvement of both PGs and NO in the response of $\text{HCO}_3^-$ to bradykinin. The stimulatory effect of bradykinin was also significantly affected by FR172357, suggesting no role for en- dogenous bradykinin in these responses. The reason for these results remains unexplained at present, and this point is currently under investigation in our laboratory.

Given the findings in the present study, we concluded that capsaicin stimulates the secretion of $\text{HCO}_3^-$ in the stomach mediated by endogenous PGs and NO as well as capsaicin-sensitive afferent neurons but not bradykinin itself. The reason for the reason for these results remains unexplained at present, and this point is currently under investigation in our laboratory.

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