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ACE INHIBITOR AND AT1 ANTAGONIST STIMULATE DUODENAL HCO_3^- SECRETION MEDIATED BY A COMMON PATHWAY - INVOLVEMENT OF PG, NO AND BRADYKININ -

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Recent study demonstrated that duodenal HCO_3^- secretion is affected by modulation of the renin-angiotensin system. We examined the effects of enalapril (angiotensin-converting enzyme (ACE) inhibitor) or losartan (angiotensin AT1 receptor antagonist) on duodenal HCO_3^- secretion in rats and investigated the mechanisms involved in the renin-angiotensin system-related HCO_3^- response. A proximal duodenal loop was perfused with saline, and HCO_3^- secretion was measured at pH 7.0 using a pH-stat method and by adding 2 mM HCl. Enalapril increased the HCO_3^- secretion in a dose-dependent manner, with a decrease in arterial blood pressure (MBP), and these effects were significantly attenuated by pretreatment with indomethacin, L-NAME and FR172357 (a selective bradykinin B2 receptor antagonist). Although losartan alone did not affect the HCO_3^- secretion, despite reducing MBP, the agent dose-dependently increased the HCO_3^- secretion in the presence of angiotensin II, and this response was totally antagonized by prior administration of FR172357, indomethacin and L-NAME. Bradykinin also dose-dependently increased the HCO_3^- secretion with no change in MBP, though transient, and again the effects were blocked by indomethacin, L-NAME and FR172357. Both prostaglandin (PG) E_2 and the nitric oxide (NO) donor NOR-3 also increased the HCO_3^- secretion, the latter effect being inhibited by indomethacin. These results suggest that both an ACE inhibitor and AT1 antagonist (in the presence of angiotensin II) increase duodenal HCO_3^- secretion via a common pathway, involving bradykinin, NO and PGs. It is also assumed that bradykinin releases NO locally, which in turns stimulates HCO_3^- secretion mediated by PGs.

Key words: *duodenal* HCO_3^- *secretion, angiotensin, AT1 antagonist angiotensin-converting enzyme (ACE), ACE inhibitor, rat*

INTRODUCTION

Duodenal mucosal HCO_3^- secretion is a key process that aids in preventing acid-peptic injury. Small amounts of HCO_3^- protect the mucosa against large amounts of acid by neutralizing H^+ ions that diffuse back into the mucus layer (1), and hence duodenal HCO_3^- secretion is thought to play an important role in the pathogenesis of experimental and clinical duodenal ulcers (2-4). The secretion of HCO_3^- is regulated by multiple pathways, including paracrine and endocrine factors as well as neural mechanisms (3, 5).

Recently, the involvement of the renin-angiotensin system in duodenal mucosal defense has been reported by Aneman *et al.* (6), who showed that blockade of the angiotensin-converting enzyme (ACE) by enalaprilate improved mesenteric blood flow and oxygen and HCO_3^- delivery during severe hypovolemic shock and prevented the reduction of duodenal HCO_3^- secretion. Chen *et al.* (7) reported that the ACE inhibitor by itself increased duodenal HCO_3^- secretion via a local bradykinin pathway in rats, involving bradykinin B2 receptors, but not dependent on the extrinsic vagal and splanchnic nerves nor adrenergic transmission. The same group also reported that angiotensin II (ANGII) stimulated duodenal HCO_3^- secretion by activation of the type 2 (AT2) angiotensin receptors located in the mucosa, especially in the presence of AT1 antagonist (8). They also showed that the HCO_3^- secretion stimulated by activation of AT2 receptors is mediated via bradykinin B2 receptors but not endogenous nitric oxide (NO)(7). However, several studies demonstrated that losartan stimulates NO production by AT2 receptor-mediated and bradykinin B2-dependent mechanisms (9-11). Gohlke *et al.* (9) reported that ANGII stimulates the production of cyclic 3', 5' guanosine monophosphate (cGMP) in the vascular wall by an AT2 receptor-dependent mechanism and this effect was abolished by the inhibition of NO synthase as well as the blockade of bradykinin B2 receptors, suggesting the involvement of the bradykinin/NO system in the action mediated by activation of AT2 receptors. Furthermore, since NO stimulates the secretion of HCO_3^- in the rat duodenum through up-regulation of prostaglandin (PG) E_2 production (12, 13), it is possible that endogenous PGE_2 plays a role in the HCO_3^- response induced by activation of AT2. Thus, the involvement of NO and PGs in the modulatory mechanism of HCO_3^- secretion by renin-angiotensin remains controversial.

In the present study, we examined the effects of ANGII, the ACE inhibitor enalapril, and the AT1 antagonist losartan on duodenal HCO_3^- secretion in rats and investigated the mechanisms involved in the HCO_3^- response modulated by the renin-angiotensin system, focusing on the interaction of bradykinin, NO and PG.

MATERIALS AND METHODS

Animals

Male Sprague Dawley rats (220~260 g, Nippon Charles River, Shizuoka, Japan) were used. They were kept alone in cages with raised mesh bottoms, and deprived of food but allowed free access to tap water for 18 hr prior to the experiments. Studies were carried out using 4~7 rats per group and performed under uretane anesthesia (1.25 g/kg, i.p.). All experimental procedures described here were approved by the Experimental Animal Research Committee of Kyoto Pharmaceutical University.

Determination of Duodenal HCO_3^- Secretion

HCO_3^- secretion was determined in a duodenal loop, according to a previously published method (3). The abdomen was incised, and a duodenal loop (1.5 cm) was made between the pyloric ring and the area just above the outlet of the common bile duct, in order to exclude the influences of bile and pancreatic juice. Then the loop was perfused at a flow rate of 1 ml/min with saline (154 mM NaCl) that was gassed with 100% O_2, heated at 37°, and kept in a reservoir. The secretion of HCO_3^- was measured at pH 7.0 using the pH-stat system (Hiranuma Comtite-8, Tokyo, Japan) and by adding 2 mM HCl to the reservoir. After basal HCO_3^- secretion had well stabilized, enalapril (0.3-3 mg/kg) or losartan (3 and 10 mg/kg) was given as a single i.v. injection, while ANGII (0.25~7.5 μg/kg/hr) was infused i.v. continuously in the augmented-dose fashion. Indomethacin (5 mg/kg) was given s.c. 1 hr before the treatment with enalapril or losartan, while the bradykinin receptor B2 antagonist FR172357 (1 mg/kg)(14) was given i.v. 15 min before. On the other hand, N^G-nitro-L-arginine methyl ester (L-NAME: 20 mg/kg) was given s.c. 3 hr before the treatment, because this agent acutely increased the HCO_3^- secretion through a neural reflex due to an increase of blood pressure (13, 15, 16). In separate studies, the effects of PGE_2, NOR-3 the NO donor, and bradykinin on duodenal HCO_3^- secretion were examined. PGE_2 (1 mg/kg) or bradykinin (30 μg/kg) was given i.v. as a single injection, while NOR-3 (10^{-3} M) was applied to the mucosa for 10 min. In some cases, indomethacin (5 mg/kg) was given s.c. 1 hr before administration of NOR-3 or bradykinin, L-NAME (20 mg/kg) was given s.c. 3 hr before bradykinin, and FR-172357 (1 mg/kg) was given i.v. 15 min before NOR-3 or bradykinin. In the cases of the treatments with enalapril or losartan plus ANGII, with or without indomethacin, L-NAME or FR-172357, arterial blood pressure was monitored via the femoral artery by a pressure transducer and amplifier system (TP-200TL, AP-100F, RTA-1100A Nihon Koden).

Measurement of Mucosal PGE_2 Contents

The mucosal PGE_2 content in the duodenum was measured after administration of enalapril (1 mg/kg) or losartan (10 mg/kg) in the presence of ANGII (0.25 μg/kg/hr) or bradykinin (30 μg/kg). Each agent was given i.v., and 90 minutes later, the whole tissue of the duodenal loop was removed, weighed, and put in a tube containing 100% methanol plus 0.1 M indomethacin (17). Then, the samples were minced by scissors, homogenized, and centrifuged for 10 min at 12000 r.p.m. at 4°. The supernatant of each sample was used for determination of PGE_2 by EIA using a PGE_2 kit (Cayman Chemical Co., Ann Arbor, MI, USA). Indomethacin (5 mg/kg) or L-NAME (20 mg/kg) was given s.c. 1 hr before administration of the above treatment, while FR172357 (1 mg/kg) was given i.v. 15 min before.

Preparation of Drugs

The drugs used were urethane (Tokyo Kasei, Tokyo, Japan), enalapril, losartan (Banyu, Tokyo, Japan), angiotensin II (Peptide Institute, Osaka, Japan), bradykinin (Nacalai tesque, Kyoto, Japan), indomethacin, N^G-nitro L-arginine methyl ester (Sigma Chemicals, St. Louis, Montana, USA), NOR-3 [(±)-(E)-Ethyl-2-[(E)-hydroxyimino]-5-nitro-3-hexeneamine] (Dojindo, Kumamoto, Japan) and FR172357 (Fujisawa, Osaka, Japan). Indomethacin was suspended in saline with a drop of Tween 80 (Wako, Osaka, Japan). NOR-3 was first dissolved in dimethyl sulfoxide (DMSO) and diluted with saline to a desired concentration. Other agents were dissolved in saline. Each agent was prepared immediately before use and administered i.p. or s.c. in a volume of 0.5 ml per 100 g body weight, or i.v. in a volume of 0.1 ml per 100 g body weight, or infused i.v. in a volume of 1 ml/hr, or applied topically to the loop in a volume of 0.5 ml per rat.

Statistics

Data are presented as the mean±SE for 4~7 rats 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.

RESULTS

Effect of Enalapril on Duodenal HCO_3^- Secretion

Under anesthesia with urethane, the rat duodenum secreted HCO_3^- spontaneously at a rate of 1~1.2 μEq/15 min. Enalapril (0.3-3 mg/kg) given i.v. as a single injection increased HCO_3^- secretion in a dose-dependent manner, and the ΔHCO_3^- output at 1 mg/kg was 1.6±0.2 μEq/hr, which is significantly greater than control values obtained after saline injection (*Fig. 1A* and *1B*). The stimulatory action of enalapril (1 mg/kg) was almost totally attenuated by prior administration of indomethacin (5 mg/kg, s.c.) or L-NAME (20 mg/kg, s.c.) (*Fig. 2A* and *2B*). Likewise, the stimulatory action of enalapril was also significantly mitigated by prior administration of the bradykinin B2 receptor antagonist FR172357 (1 mg/kg, i.v.), the inhibition being 78.4%.

Under urethane anesthesia, systemic blood pressure was maintained at 75~90 mmHg during the test period (*Table 1*). Intravenous administration of enalapril (1 and 3 mg/kg) caused a significant decrease in systemic blood pressure, persisting for the 2 hr-test period, and the reduction observed at 30 min after administration was 26.6% and 25%, respectively. Both indomethacin (5 mg/kg, s.c.) and FR172357 (1 mg/kg, i.v.) had by themselves no effect on blood pressure and did not affect the response to enalapril (1 mg/kg, i.v.) (*Table 2*). By contrast, L-NAME by itself significantly increased blood pressure, but did not abrogate the decrease in blood pressure due to enalapril.

Effects of ANGII and Losartan on Duodenal HCO_3^- Secretion

It has been reported that the AT1 angtagonist losartan increased duodenal HCO_3^- secretion in rats infused i.v. with ANGII (8). To confirm these findings, we examined the effect of ANGII and the AT1 antagonist losartan, either alone or in combination, on duodenal HCO_3^- secretion.

Effect of ANGII: Intravenous infusion of ANGII had no effect on the secretion of HCO_3^- at doses less than 0.75 μg/kg/hr, but significantly increased the secretion at doses over 2.5 μg/kg/hr (Figure 3A). At 7.5 μg/kg/hr, the rate of HCO_3^- secretion reached 1.5±0.2 μEq/15 min, about 1.7 times greater than that

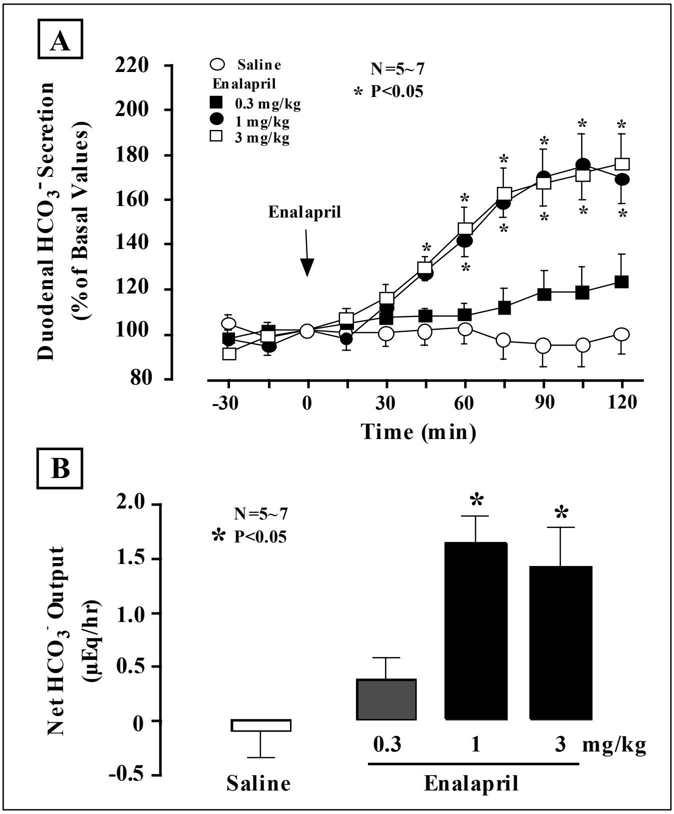

Fig. 1. Effect of enalapril on duodenal HCO_3^- secretion in anesthetized rats. Enalapril (0.3~3 mg/kg) was given i.v. as a single injection. Values are presented as the % of basal values in HCO_3^- secretion and represent the mean±SE of values determined every 15 minutes from 5~7 rats. Fig. B shows the net HCO_3^- output for 1 hr after administration of enalapril and are presented as the mean±SE for 5~7 rats. * Significant difference from saline, at P<0.05.

Table 1. Changes in Arterial Blood Pressure Before and After Administration of Enalapril in Anesthetized Rats

Drugs	No. of Rats	Blood Pressure (mmHg)		
		Before	30 min after	Decrease (%)
Saline	5	74.4±5.0	73.8±5.4	0.8
Enalapril (1mg/ kg)	4	87.5±5.6	64.3±4.0*	26.6
Enalapril (3mg/ kg)	5	94.4±8.4	70.8±6.6*	25.0

Values are presented as the mean±SE for 4~5 rats per group. Blood pressure was continuously measured via the femoral artery with a pressure transducer and amplifier system. Enalapril was given i.v. as a single injection, in doses of 0.3~3 mg/kg. * Significant difference from Before in the corresponding group, at P<0.05

observed in the control group. ANGII treatment caused a significant elevation in blood pressure at 0.75 µg/kg/hr or greater. At 2.5 and 7.5 µg/kg/hr, the blood

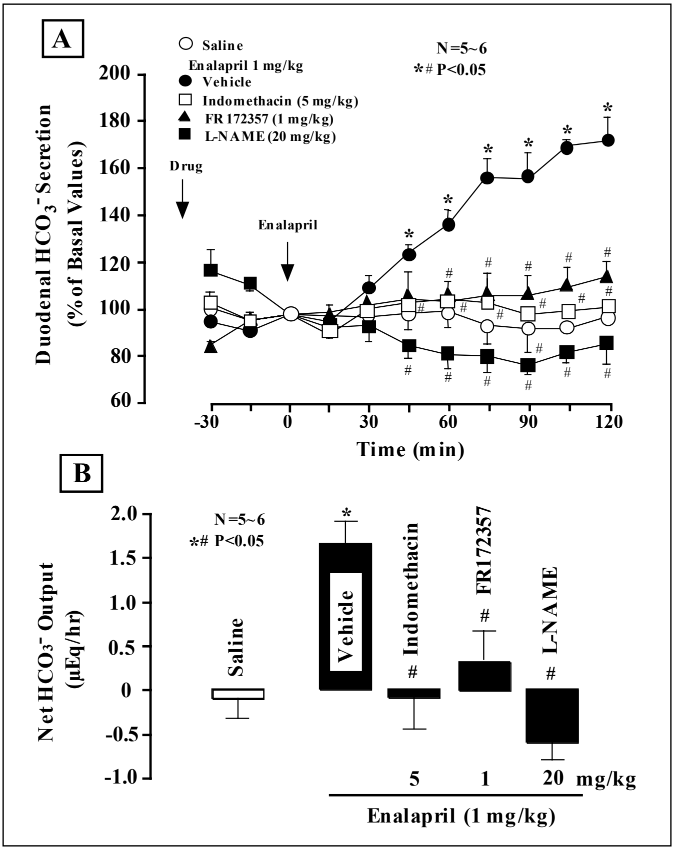

Fig. 2. Effect of various agents on duodenal HCO_3^- stimulatory action of enalapril in anesthetized rats. The secretion of HCO_3^- was stimulated by i.v. administration of enalapril at 1 mg/kg. Indomethacin (5 mg/kg) or L-NAME (20 mg/kg) was given s.c. 1 or 3 hr, respectively, before enalapril, while FR172357 (1 mg/kg) was given i.v. 15 min before. Values are presented as the % of basal values in HCO_3^- secretion and represent the mean±SE of values determined every 15 min from 5~6 rats. Fig. B shows the net HCO_3^- output for 1 hr after administration of enalapril and are presented as the mean±SE for 5~6 rats. Significant difference at P<0.05; * from saline; # from vehicle.

Table 2. Effect of Various Agents on Changes in Arterial Blood Pressure Induced by Enalapril in Anesthetized Rats

Drugs	No. of Rats	Blood Pressure (mmHg)		
		Before	30 min after	Decrease (%)
Saline	5	74.4±5.0	73.8±5.4	0.8
Enalapril (1mg/ kg)				
Vehicle	4	87.5±5.6	64.3±4.0*	26.6
Indomethacin	5	89.4±4.5	62.4±5.7*	23.8
FR172357	5	86.2±4.5	64.0±3.9 *	25.8
L-NAME	5	124.2±10.4#	97.4±8.4*#	21.6

Values are presented as the mean±SE for 5 rats per group. Blood pressure was continuously measured via the femoral artery by a pressure transducer and amplifier system. Enalapril was given i.v. as a single injection, in a dose of 1 mg/kg. Indomethacin (5 mg/kg) or L-NAME (20 mg/kg) was given s.c. 1 or 3 hr, respectively, before enalapril, while FR172357 (1 mg/kg) was given i.v. 15 min before enalapril. Significant difference at P<0.05; * from Before in the corresponding group; # from vehicle.

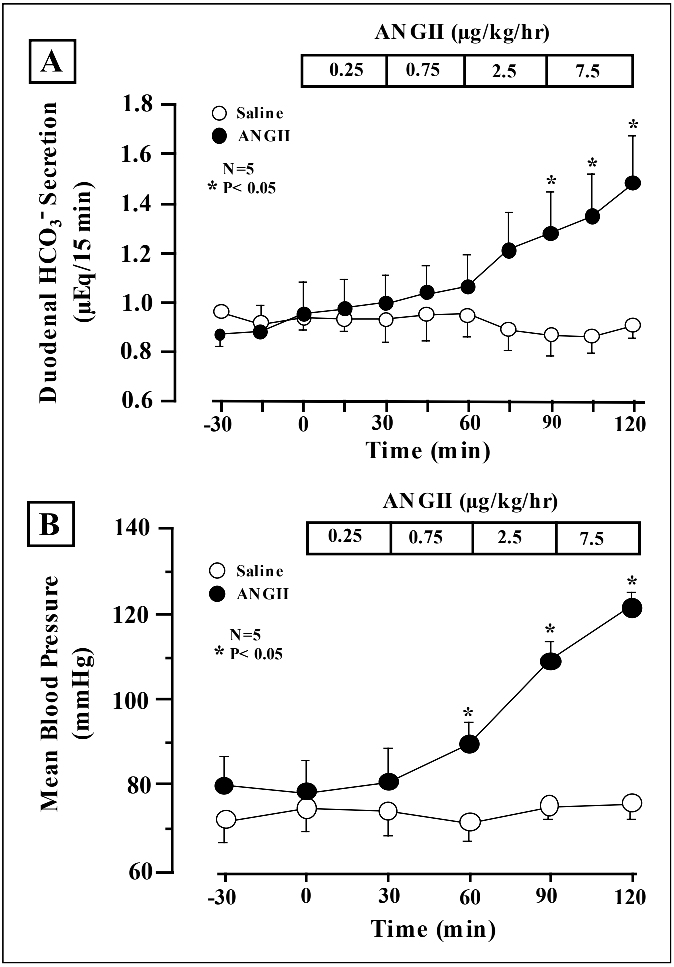

Fig. 3. Effect of angiotensin II (ANG II) on duodenal HCO_3^- secretion (A) and arterial blood pressure (B) in anesthtized rats. ANG II was infused i.v. in augmented doses of 0.25~7.5 µg/kg/hr. Data are presented as the mean±SE of values determined every 15 min from 5 rats. * Significant difference from saline, at P<0.05.

pressure reached 109±2.3 mmHg and 123±2.1 mmHg, respectively, both of which being significantly greater than that in saline-infused rats (*Fig. 3B*).

Effect of losartan with or without ANG II infusion: The AT1 antagonist losartan alone had no effect on duodenal HCO_3^- secretion even at 10 mg/kg given i.v. (not shown), although this agent significantly decreased arterial blood pressure from 83.0±2.8 mmHg to 58.5±2.4 mmHg 30 min after the administration (*Table 3*). However, when losartan (3 and 10 mg/kg, i.v.) was administered in the presence of ANGII (0.25 µg/kg/hr), the secretion of HCO_3^- was increased in a dose-dependent manner, although ANGII alone had no effect (*Fig. 4A*). The ΔHCO_3^- output caused by ANGII was 1.0±0.3 µEq/hr and 2.2±0.6 µEq/hr, respectively, in the presence of 3 and 10 mg/kg of losartan (*Fig. 4B*). The increase in HCO_3^- secretion caused by ANGII (0.25 µg/kg/hr) plus losartan (10 mg/kg) was significantly attenuated by

Table 3. Changes in Arterial Blood Pressure before and after Administration of Losartan in Anesthetized Rats

Drugs	No. of Rats	Blood Pressure (mmHg)		
		Before	30 min after	Decrease (%)
Saline	5	74.4±5.0	73.8±5.4	0.8
Losartan (3 mg/ kg)	5	80.0±3.3	71.6±4.0*	10.5
Losartan (10 mg/ kg)	5	83.0±2.8	58.5±2.4*	29.5

All values are presented as the mean±SE for 5 rats per group. Blood pressure was continuously measured via the femoral artery with a pressure transducer and amplifier system. Losartan was given i.v. as a single injection, in doses of 3 and 10 mg/kg. * Significant difference from Before in the corresponding group, at P<0.05.

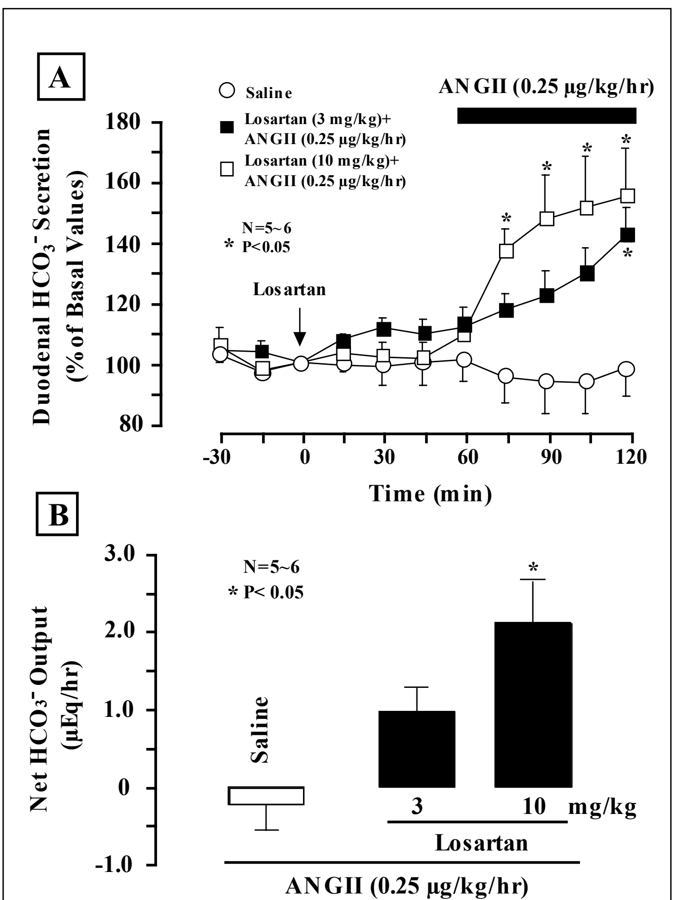

Fig. 4. Effect of losartan on duodenal HCO_3^- secretion in anesthetized rats, in the presence of ANG II. Losartan (3 and 10 mg/kg) was given i.v. as a single injection, while ANG II (0.25 µg/kg/hr) was infused i.v., starting from 1 hr after the injection of losartan. Values are presented as the % of basal values in HCO_3^- secretion and represent the mean±SE of values determined every 15 min from 5~6 rats. Fig. B shows the net HCO_3^- output for 1 hr after the onset of ANGII infusion and are presented as the mean±SE for 5~6 rats. * Significant difference from saline, at P<0.05.

either indomethacin (5 mg/kg), L-NAME (20 mg/kg) or FR172357 (1 mg/kg), the degree of inhibition being over 70% in all cases (*Fig. 5A*). The ΔHCO_3^- output caused by ANGII plus losartan was 2.3±0.3 μEq/hr in the control group, and this value decreased to 0.3±0.5, 0.6±0.6 and 0.5±0.5 μEq/hr, respectively, with prior administration of indomethacin, L-NAME and FR172357 (*Fig. 5B*). The hypotensive effect of losartan (10 mg/kg, i.v.) was not affected by i.v. infusion of ANGII at a dose of 0.25 μg/kg/hr, and the blood pressure was decreased about 30%. Indomethacin and FR172357 had no effect on basal blood pressure and did not significantly modify the decrease in blood pressure due to losartan plus ANGII (*Table 4*). Although L-NAME significantly raised basal blood pressure, it did not affect the response to losartan plus ANGII, the decrease being 30.6%.

Effects of PGE_2, NOR-3 and Bradykinin on Duodenal HCO_3^- Secretion

The present study showed that the stimulatory action of enalapril or losartan plus ANG II on duodenal HCO_3^- secretion is mediated by endogenous PGs and

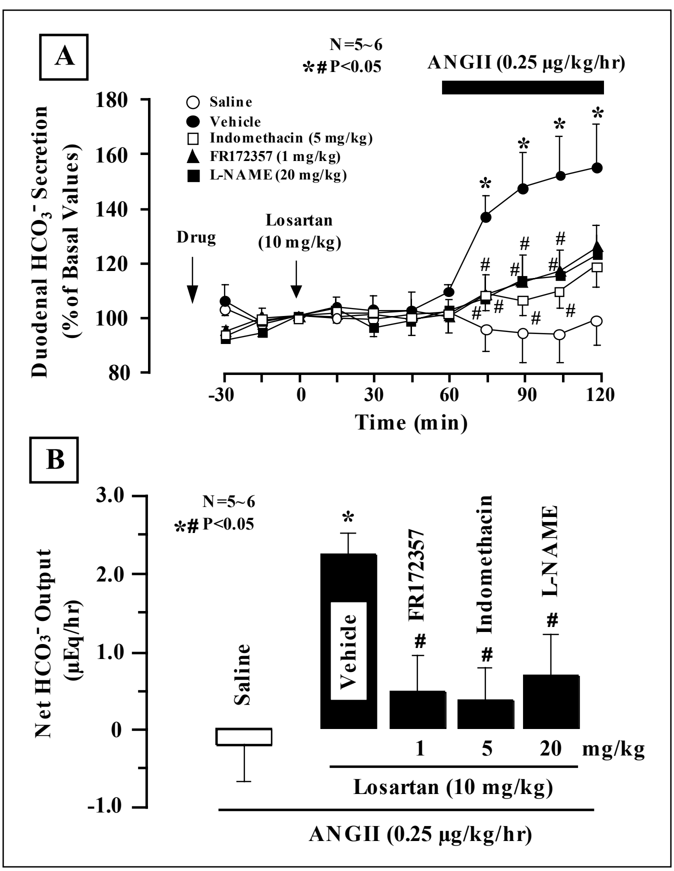

Fig. 5. Effect of various agents on duodenal HCO_3^- stimulatory action of losartan in anesthetized rats, in the presence of ANG II. The secretion of HCO_3^- was stimulated by i.v. administration of losartan at 10 mg/kg in the presence of ANG II infused i.v. (0.25 μg/kg/hr). Indomethacin (5 mg/kg) or L-NAME (20 mg/kg) was given s.c. 1 or 3 hr, respectively, before losartan, while FR-172357 (1 mg/kg) was given i.v. 15 min before. Values are presented as the % of basal values in HCO_3^- secretion and represent the mean±SE of values determined every 15 min from 5~6 rats. Fig. B shows the net HCO_3^- output for 1 hr after the onset of ANGII infusion and are presented as the mean±SE for 5~6 rats. Significant difference at P<0.05; * from saline; # from vehicle.

Table 4. Effect of Various Agents on Changes in Arterial Blood Pressure Induced by Losartan plus Angiotensin II in Anesthetized Rats

Drugs	No. of Rats	Blood Pressure (mmHg)		
		Before	30 min after	Decrease (%)
Saline	5	74.4±5.0	73.8±5.4	0.8
Losartan (10 mg/kg) + ANG II 0.25 μg/kg/hr				
Vehicle	5	83.0±2.8	58.5±2.4*	29.5
Indomethacin	5	80.5±4.0	49.0±9.4*	39.1
FR172357	4	81.0±4.4	64.0±3.2*	20.9
L-NAME	5	121.4±4.8#	84.2±7.1*#	30.6

All values are presented as the mean±SE for 5 rats per group. Blood pressure was continuously measured via the femoral artery with a pressure transducer and amplifier system. Losartan (10 mg/kg) was given i.v., followed by an i.v. infusion of ANG II (0.25 μg/kg/hr) from 1 hr later. Indomethacin (5 mg/kg) or L-NAME (20 mg/kg) was given s.c. 1 or 3 hr, respectively, before losartan, while FR172357 (1 mg/kg) was given i.v. 15 min before. Significant difference at $P<0.05$; * from Before in the corresponding group; # from vehicle.

NO as well as bradykinin. To further investigate the interaction between these mediators, we examined the effect of indomethacin, L-NAME and FR172357 on the HCO_3^- stimulatory action of PGE_2, bradykinin and the NO donor NOR-3.

The secretion of HCO_3^- was significantly increased by PGE_2 (1 mg/kg) and bradykinin (30 μg/kg) given i.v. or NOR-3 (10^{-3} M) applied topically to the loop, the ΔHCO_3^- output being 3.8±0.5 μEq/hr, 1.1±0.2 μEq/hr and 1.6±0.2 μEq/hr, respectively (*Fig. 6A* and *6B*). The stimulatory effect of PGE_2 was affected by prior administration of neither indomethacin (5 mg/kg, s.c), L-NAME (20 mg/kg, s.c.) nor FR172357 (1 mg/kg, i.v.) (not shown). By contrast, the response to NOR-3 was significantly mitigated by indomethacin but not FR172357, while the response to bradykinin was totally attenuated by the B2 antagonist FR172357 and also significantly mitigated by both indomethacin and L-NAME, the inhibition being 68.3% and 54.5%, respectively.

Effects of Enalapril and Losartan plus ANGII on Mucosal PGE_2 Contents

Mucosal PGE_2 levels in the normal rat duodenum were 17.4±1.2 ng/g tissue. Intravenous administration of enalapril (1 mg/kg, i.v.) significantly stimulated PG biosynthesis to increase the mucosal PGE_2 content to about 1.7 fold the control level, the value being 35.6±5.3 ng/g tissue (*Fig. 7*). Similar results were obtained following the treatment with ANGII (0.25 μg/kg/hr) plus losartan (10 mg/kg). The PG biosynthetic response induced by enalapril or losartan plus ANGII was significantly prevented by prior administration of indomethacin (5 mg/kg, s.c) or

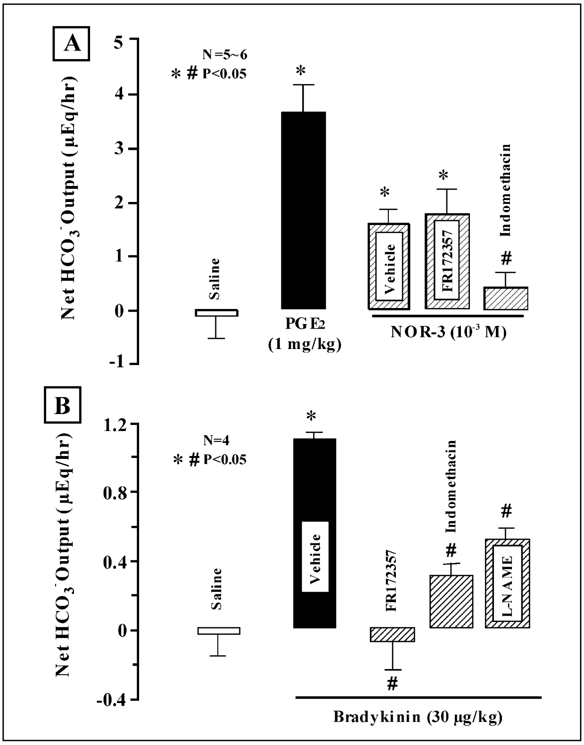

Fig. 6. HCO_3^- stimulatory action of PGE_2 and NOR-3 (A) as well as bradykinin (B) in anesthetized rat duodenums. PGE_2 (1 mg/kg) or bradykinin (30 μg/kg) was given i.v. as a single injection, while NOR-3 (10^{-3} M) was applied to the mucosa for 10 min. Indomethacin (5 mg/kg) was given s.c. 1 hr before NOR-3 or bradykinin, L-NAME (20 mg/kg) was given s.c. 3 hr before bradykinin, and FR172357 (1 mg/kg) was given i.v. 15 min before NOR-3 or bradykinin. Data show the net HCO_3^- output for 1 hr after each treatment and are presented as the mean±SE for 5~6 rats. Significant difference at P<0.05; * from saline; # from vehicle.

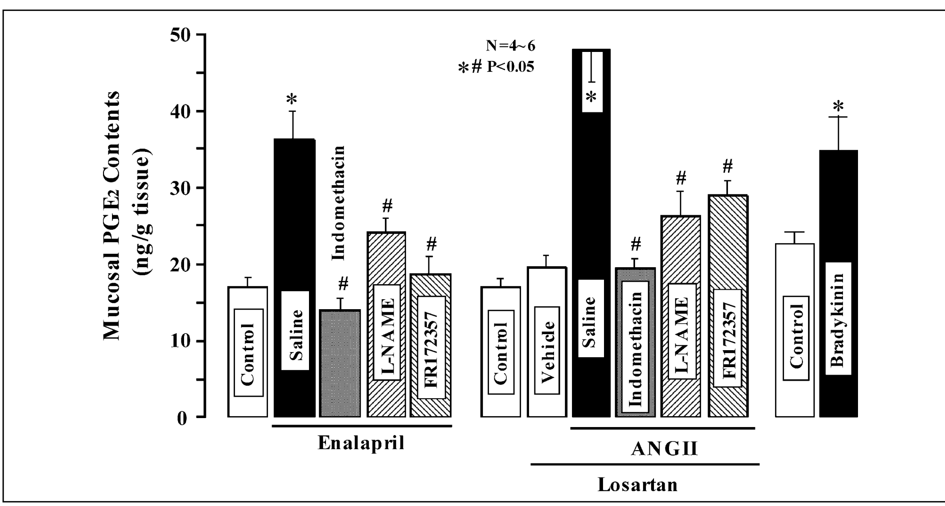

Fig. 7. Effects of enalapril, losartan plus angiotensin II and bradykinin on mucosal PGE_2 contents in anesthetized rat duodenums. Enalapril (1 mg/kg) or bradykinin (30 μg/kg) was given i.v. as a single injection while losartan (10 mg/kg) was given i.v. in the presence of ANG II infused i.v. (0.25 μg/kg/hr). Indomethacin (5 mg/kg) or L-NAME (20 mg/kg) was given s.c. 1 or 3 hr, respectively, before enalapril or losartan, while FR172357 (1 mg/kg) was given i.v. 15 min before. Data are presented as the mean±SE for 4~6 rats. Significant difference at P<0.05; * from control; # from saline.

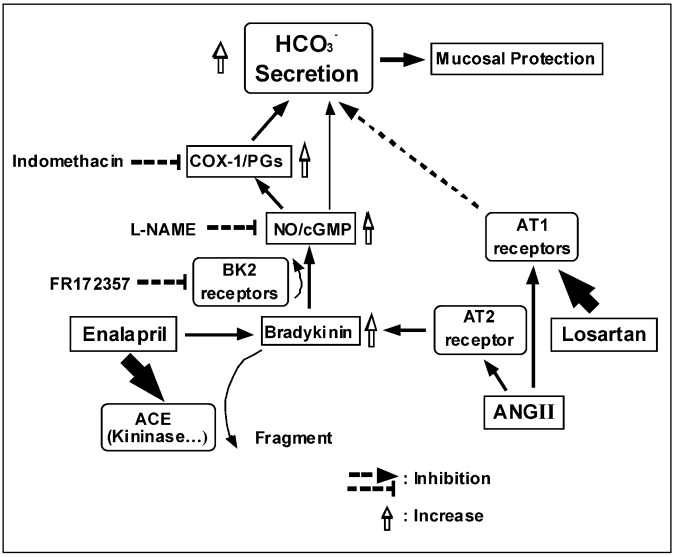

Fig. 8. Summary for the regulatory pathways involved in the HCO_3^- stimulatory effects of enalapril and losartan in the duodenum. Both enalapril (ACE inhibitor) and losartan (AT1 antagonist in the presence of ANGII) increase duodenal HCO_3^- secretion via a common pathway, involving bradykinin, NO and PGs. Bradykinin releases NO locally, which in turns stimulates HCO_3^- secretion mediated by PGs.

L-NAME (20 mg/kg). Likewise, FR172357 (1 mg/kg, i.v.) also significantly reduced the increase in PGE_2 production in response to enalapril or losartan plus ANGII, the inhibition being 87.9% and 89.1%, respectively. Bradykinin also significantly increased PGE_2 content in the duodenum, and this effect was totally prevented by either FR172357, L-NAME or indomethacin (not shown).

DISCUSSION

The present study confirmed the previous findings by others (7, 8, 18) that both an ACE inhibitor and an AT1 antagonist (in the presence of ANG II) increased the secretion of HCO_3^- in the rat duodenum via a common pathway, involving bradykinin B2 receptors. We further demonstrated that the HCO_3^- stimulatory action of these treatments was significantly mitigated by inhibition of PG biosynthesis or blockade of NO production, suggesting the involvement of endogenous PGs and NO. In the present study, we also observed that bradykinin increased duodenal HCO_3^- secretion mediated by PGs and NO, the process totally dependent on activation of B2 receptors. It is assumed that bradykinin/B2 receptors play a pivotal role in the modulation of duodenal HCO_3^- secretion by the renin-angiotensin system. Bradykinin augments NO production locally via B2 receptors, which in turn stimulates PG production by increasing COX-1 activity (13, 19) and thereby results in an increase of HCO_3^- secretion in the duodenum.

ANGII plays an important role in the cardiovascular, electrolyte, and fluid homeostasis, and has been implicated as a causative factor in the development of hypertension (20). ACE inhibitors and nonpeptide antagonists of the AT1 receptor have been successfully introduced in the treatment of hypertension and other cardiovascular diseases. Aneman *et al.* (6) first reported that the renin-angiotensin system plays a role in supporting mesenteric perfusion and organ function in hypovolemic shock. They showed that hypovolemia caused a reduction of duodenal HCO_3^- secretion as well as mesenteric oxygenation, and these responses were prevented by the ACE inhibitor enalaprilate. Since no such effects were observed on pretreatment with guanethidine to block the sympathetic neuronal influence, they concluded that the renin-angiotensin system may play a role in the integrated duodenal response to hypovelemic shock, including the epithelial function. Chen *et al.* (7) reported that ANG II did reduce the secretion of HCO_3^- in the rat duodenum, while the ACE inhibitor even by itself increased the secretion. It is known that ACE inhibitors not only interfere with the renin-angiotensin system by inhibiting the generation of ANGII but also potentiate the effect of bradykinin by inhibiting its degradation (9). Indeed, it was demonstrated that the HCO_3^- stimulatory action of enalaprilate was blocked by the selective bradykinin B2 receptor antagonist HOE140. On the other hand, AT1 antagonists such as losartan interfere with the renin-angiotensin system via a specific blockade of AT1 receptors which mediate most of the known actions of ANGII (21). Johansson *et al* (8) reported that ANGII in the presence of losartan increased HCO_3^- secretion by activating AT2 receptors located in the duodenal mucosa. It was also reported that the AT2 receptor-stimulated HCO_3^- secretion is mediated via bradykinin B2 receptors located in the duodenal epithelium (8). Consistent with these results, we observed in the present study that the stimulatory effects of enalapril as well as losartan plus ANGII were totally blocked by a selective bradykinin B2 receptor antagonist, FR172357, confirming the involvement of a common pathway mediated by bradykinin B2 receptors. These results were also supported by the finding that a single i.v. injection of bradykinin increased duodenal HCO_3^- secretion, and this response was antagonized by prior administration of FR172357. The changes in duodenal HCO_3^- secretion following administration of enalapril or losartan plus ANGII were accompanied by a decrease in arterial blood pressure. It is possible that such HCO_3^- responses are brought about by the mechanisms related to blood pressure changes, involving neural reflex. However, the hypotensive effects of these treatments were not significantly affected by FR172357 at the dose that antagonized the HCO_3^- stimulatory action, suggesting a dissociation of these two effects.

The most important finding of the present study is that the stimulatory action of both enalapril and losartan plus ANGII was significantly attenuated by prior administration of not only indomethacin to suppress the production of PG but also L-NAME to block NO production as well. Several studies demonstrated that NO is an important physiological mediator of the renin-angiotensin system via AT2 receptors (14, 15). ANGII increased cGMP in the kidney, and this response was blocked by the AT2 antagonist PD123319 but not by the AT1 antagonist losartan (14). Gohlke *et al.* (9, 22) reported that the ANGII-induced increase in aortic cGMP was abolished by the NO synthase inhibitor L-NAME as well as the bradykinin B2 receptor antagonist icatibant, suggesting the involvement of both NO and bradykinin in the AT2 receptor-dependent actions of ANGII. Furthermore, since NO stimulates the secretion of HCO_3^- in the rat duodenum through up-regulation of PGE_2 production (10, 11), it is possible that endogenous PGs play a role in the response of HCO_3^- induced by activation of AT2 or B2 receptors. Yet, there are very few studies concerning these points. Esert *et al.* (18) reported that the HCO_3^- stimulatory action of losartan in the presence of ANGII was not affected by L-NAME and excluded the involvement of endogenous NO in the AT2 receptor-stimulated HCO_3^- secretion in the rat. In the present study, however, we found that the HCO_3^- responses to enalapril as well as losartan plus ANGII were both significantly curtailed by prior administration of L-NAME or indomethacin. The reason for these different results remains unknown, yet we observed that the HCO_3^- response to bradykinin was inhibited not only by FR172357 but also by indomethacin and L-NAME as well, strongly indicating the involvement of NO and PGs in the bradykinin B2 receptor-mediated action. It should be noted that these two agents did not affect the changes in blood pressure caused by enalapril and losartan, again suggesting no relationship between the HCO_3^- and blood pressure responses induced by these treatments. Certainly, duodenal HCO_3^- secretion was stimulated by exogenously administered PGE_2 and the NO donor NOR-3, and the latter effect was attenuated by indomethacin as well as the selective B2 receptor antagonist FR172357. All these results suggest that enalapril, while inhibiting ANGII production, increases bradykinin levels through inhibition of its degradation, which then stimulates the secretion of HCO_3^- by activation of B2 receptors and mediated by endogenous NO and PGs (*Fig. 8*). On the other hand, losartan in the presence of ANGII increases the secretion of HCO_3^- by unmasking the stimulation by the action of AT2 receptors via bradykinin B2 receptors, which is subsequently mediated by both NO and PGs, similar to the HCO_3^- response to the ACE inhibitor.

Does enalapril or losartan (in the presence of ANGII) really increase PG production in the duodenal mucosa? We have previously reported that the NO donor NOR-3 increased PGE_2 production in the rat duodenum *in vivo* and the isolated bullfrog duodenum *in vitro* (12, 13). In the present study, bradykinin also significantly increased mucosal PGE_2 contents, and this response was significantly blocked by FR172357 and L-NAME as well as indomethacin (not shown). Likewise, duodenal PGE_2 content was increased by both enalapril and losartan plus ANGII in an indomethacin-, L-NAME- and FR172357-inhibitable manner. These results suggest that bradykinin releases NO locally, which in turns stimulates HCO_3^- secretion mediated by PGs, and strongly support the finding that the activation of bradykinin B2 receptors induced by the ACE inhibitor and the AT1 antagonist (in the presence of ANGII) increases the secretion of HCO_3^- in the duodenum mediated by endogenous NO and PGs.

Given the present study, it is concluded that both the ACE inhibitor and AT1 antagonist (in the presence of ANGII) increased duodenal HCO_3^- secretion via a common pathway, involving bradykinin, NO and PGs. The latter two substances play an important role in maintaining the mucosal integrity of the gastroduodenal mucosa (23, 24), supporting the beneficial influence of the ACE inhibitor or AT1 antagonist on the mucosal defensive mechanism. A recent study even showed that blockade of AT1 receptors or inhibition of ACE reversed the negative effect of chronic sensory denervation on gastric ulcer healing by means of increasing the gastric mucosal blood flow (25). Furthermore, since the AT2 receptors are reportedly up-regulated by ANGII (26), it is assumed that the stimulatory effect of both the ACE inhibitor and the AT1 antagonist is more pronounced in patients with hypertension, where bradykinin/B2 receptors would also be up-regulated. Thus, these treatments may have a favorable influence on the mucosal defense in the duodenum against acid injury.

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