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Esophageal white sponge nevus associated with severe dysphagia and odynophagia

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ACE Inhibition by Enalaprilate Stimulates Duodenal Mucosal Alkaline Secretion via a Bradykinin Pathway in the Rat

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The effects of enalaprilate on duodenal mucosal alkaline secretion (*in situ* titration) and mean arterial blood pressure were investigated in chloralose-anesthetized male rats. A bolus injection of enalaprilate (0.7 mg/kg intravenously) increased alkaline secretion by about 60%, and this response was resistant to guanethidine (5 mg/kg intravenously), splanchnicotomy, and vagotomy. Furthermore, angiotensin II infusion (0.25–2.5 μg/kg/hr intravenously) following the administration of enalaprilate failed to influence this response. Bradykinin (10^{-6}–10^{-4} M) applied topically to the serosal surface of the duodenal segment under study increased dose-dependently the duodenal mucosal alkaline secretion, an effect that could be blocked by the selective bradykinin receptor subtype-2 antagonist HOE140 (100 nmol/kg intravenously). HOE140 also antagonized the response to enalaprilate. These data suggest that enalaprilate increases duodenal mucosal alkaline secretion via a local bradykinin pathway involving receptors of the bradykinin receptor subtype-2 antagonist, rather than by blockade of endogenous angiotensin II or by central autonomic neural regulation.

KEY WORDS: angiotensin; bicarbonate; bradykinin; enalaprilate; HOE140; intestinal secretion; sympathetic nervous system.

Alkaline secretion by the duodenal surface epithelium is probably very important for mucosal protection against luminal acid (1). The alkalinity is due to epithelial secretion of bicarbonate, which neutralizes gastric hydrogen ions approaching the mucosal surface. Bicarbonate transport by the duodenal mucosa is up-regulated by vagal activity (2–4), inhibited by the splanchnic nerves (5–8), and can be influenced by several drugs and hormones as well (1). We were recently able to demonstrate that angiotensin II (Ang II) acts in concert with the sympathetic neural inhibition of duodenal mucosal alkaline secretion in the rat (Johansson et al, in preparation). It was also observed that administration of enalaprilate, an inhibitor of angiotensin converting enzyme (ACE), increased alkaline secretion.

The aim of the present study was to further investigate this stimulatory effect of enalaprilate on duodenal mucosal alkaline secretion, with particular regard to extrinsic neural mechanisms and the possible bradykinin accumulation induced by the ACE inhibitor.

MATERIALS AND METHODS

Experiments were performed on male Sprague-Dawley rats, weighing 250–400 g. Before surgery the animals were

deprived of food overnight but had free access to drinking water. Anesthesia was induced with methohexital 75 mg/kg intraperitoneally and a tracheal cannula was inserted to ensure free airways. The right femoral vein and sometimes the left jugular vein were cannulated for drug administration. The right femoral artery was cannulated and connected to a pressure transducer (Statham PD23 Dc, Hato Rey, Puerto Rico) for measurement of arterial pressure by means of a Grass polygraph (model 7D, Grass Instruments). Anesthesia was maintained with chloralose 50 mg/kg given as an intravenous bolus injection and followed by a continuous infusion at a rate of 25 mg/kg/hr. A slow isotonic intraarterial infusion (1 ml/hr) of 0.03 M NaHCO$_3$ containing 1.7% glucose (w/v) was given throughout the experiments to prevent dehydration and acidosis due to the surgical trauma. Body temperature was maintained at 38°C with a heating pad and lamp, both controlled by a thermostat equipment.

Abdominal Surgery. A slight modification of the technique described by Flemström et al (9) was used. The abdomen was opened by a midline incision and a 1.5-cm segment of the duodenum, with its proximal end about 1 cm distal to the pylorus, was cannulated *in situ* between two glass tubes connected to a water-jacketed (38°C) reservoir containing isotonic saline. The saline was recirculated through the duodenal segment by means of a gas lift of room air. The airflow (400 ml/min) was monitored continuously to ensure a similar rate of perfusate circulation in all experiments. Alkaline secretion (HCO$_3^-$) into the luminal perfusate was titrated to pH 7.4 by infusion of 0.02 M HCl (made isotonic with saline) under automatic control by a microcomputer (Luxor-ABC 80, Motala, Sweden) operating as pH-stat. The common bile duct was catheterized 5 mm proximal to the papilla of Vater in order to drain bile and pancreatic secretion, which were not of interest in this study.

Nerve Preparation. The splanchnic nerves were bilaterally dissected and sectioned well proximal to the celiac and suprarenal ganglia in one group. The vagal nerves were carefully dissected and sectioned bilaterally at the sublaryngeal level in another group.

Experimental Protocol. After surgery, the animals were left undisturbed for approximately 1 hr, after which basal duodenal mucosal alkaline secretion and mean arterial pressure were monitored during a 30-min control period as the mean value of every 5 min. The animals were then given enalaprilate 0.7 mg/kg intravenously, and alkaline secretion and mean arterial blood pressure were recorded during the following hour. This protocol was used in control, splanchnicotomized, vagotomized, and guanethidine-pretreated animals. In two groups, the administration of enalaprilate was followed by intravenous infusion of Ang II in two dose intervals. In another group a bolus of the bradykinin subtype-2 (BK-2) receptor antagonist HOE140 was given intravenously 5 min prior to the administration of enalaprilate. Bradykinin was applied topically in a number of experiments. The abdominal wound was arranged to allow access to the duodenal segment and bradykinin, dissolved in isotonic saline, was applied locally to the serosal surface. This treatment was then repeated with stepwise increased concentrations every 20 min. Pads soaked in bradykinin solution covered the segment during the course of the
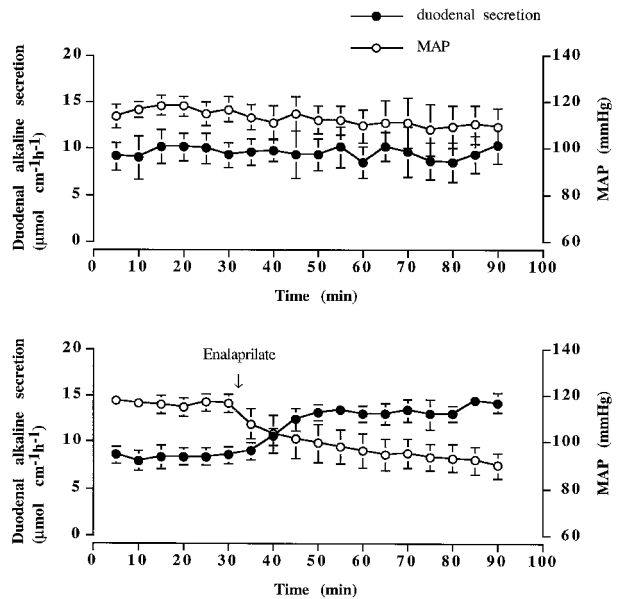
Fig 1. Effects of enalaprilate (0.7 mg/kg) on duodenal mucosal alkaline secretion and mean arterial pressure (MAP) compared to a control group. Data shown are the mean values ± SEM during the 5-min periods.

experiment. In the control situation, pads soaked in isotonic saline were utilized.

Drugs. The following drugs were used: methohexital sodium (Brietal, Lilly Inc., Indianapolis, Indiana), α-chloralose (Société Chimique Pointet Girard S.A., Villneuve la Garenne, France), guanethidinesulphate (Ismelin, Ciba-Geigy, Basle, Switzerland), enalaprilate (Renitec, Merck Sharp & Dohme, Whitehouse Station, New Jersey), bradykinin (Sigma Chemicals, St. Louis, Missouri), and HOE140 (Hoechst AG., Frankfurt, Germany).

Statistics. The values 30 min before (basal condition) the administration of enalaprilate were compared with those during a period of similar duration 30 min after giving the compound (stimulatory values). Differences in data within the groups were tested for significance using Student's *t*-test for paired samples or ANOVA for repeated measurements and the Scheffe's *post hoc* test. Comparisons between groups were made using of one way ANOVA and a *t* test. $P < 0.05$ was considered significant.

RESULTS

Effects of Enalaprilate (N = 5) Compared to Controls (N = 6). Intravenous administration of enalaprilate (0.7 mg/kg) significantly raised duodenal mucosal alkaline secretion to a level about 60% above baseline and reduced mean arterial pressure by about 20 mm Hg (Figure 1).
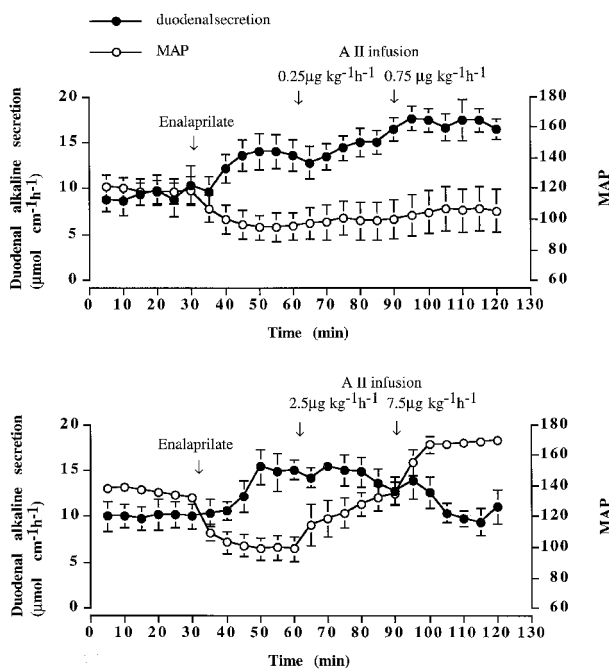
Effects of Splanchnicotomy, Vagotomy, or Guanethidine. After acute bilateral sectioning of the splanchnic nerves (N = 6) or the cervical vagi (N = 6), enalaprilate significantly increased duodenal mucosal
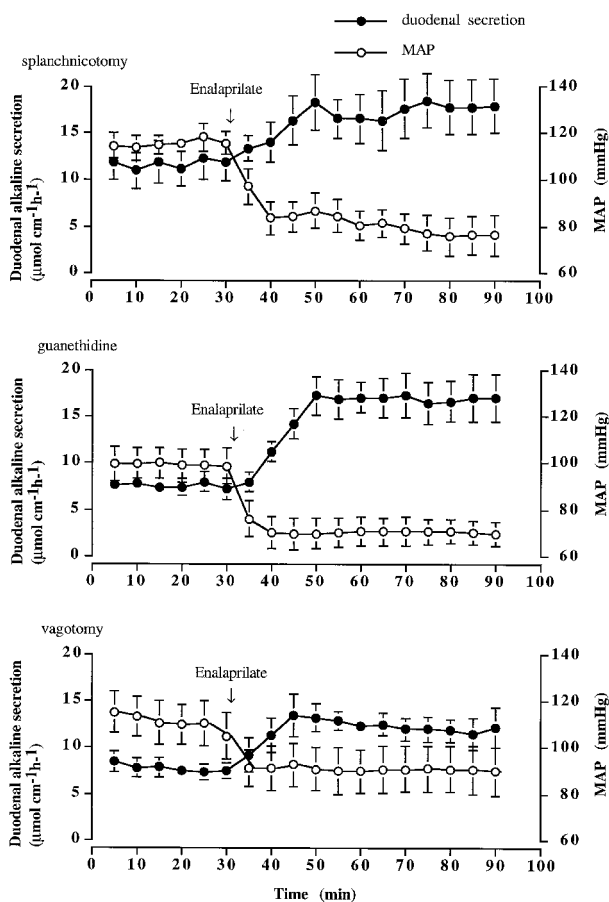
Fig 2. Effects of enalaprilate (0.7 mg/kg) on duodenal mucosal alkaline secretion and mean arterial blood pressure (MAP) in groups with acute bilateral splanchnicotomy, guanethidine pretreatment (5 mg/kg), and acute bilateral vagotomy, respectively. Data shown are the mean values ± SEM during the 5-min periods.

Fig 3. Effects of enalaprilate (0.7 mg/kg) and of angiotensin II infusion on duodenal mucosal alkaline secretion and mean arterial blood pressure (MAP). The administration of enalaprilate was followed by Ang II infusion intravenously at a rate of either 0.25 µg/kg/hr for 0.5 hr and 0.75 µg/kg/hr for another 30 min (upper panel) or 2.5 µg/kg/hr for 0.5 hr and 7.5 µg/kg/hr for another 30 min (lower panel). Data shown are the mean values ± SEM during the 5-min periods.

alkaline secretion and reduced mean arterial pressure ($P < 0.05$) (Figure 2, upper and lower panel). The enalaprilate-induced responses were virtually of the same order of magnitude as in the control group. Furthermore, similar responses to enalaprilate were obtained in the presence of the adrenolytic compound guanethidine (5 mg/kg intravenously prior to laparotomy, $N = 6$) (Figure 2, middle panel).

Effects of Ang II Substitution ($N = 5 + 5$). Exogenous Ang II was infused to elucidate whether the enalaprilate-induced secretion was due to lowered levels of endogenous angiotensins. Thirty minutes after the administration of enalaprilate, Ang II was infused at a rate of 0.25 µg/kg/hr over 30 min, after which the infusion rate was increased threefold to 0.75 µg/kg/hr. During the infusion period, there was no significant change in mean arterial pressure or duodenal mucosal alkaline output (Figure 3 upper

panel). A similar protocol was used in a second series of experiments, but the infusion rates were 10-fold higher, ie, 2.5 µg/kg/hr followed by 7.5 µg/kg/hr. Both these infusion rates influenced mean arterial pressure. During Ang II-infusion of 2.5 µg/kg/hr mean arterial pressure returned to the baseline value observed prior to enaprilate, whereas secretion was not significantly influenced. During Ang II-infusion of 7.5 µg/kg/hr mean arterial pressure increased to about 40 mm Hg above baseline. Furthermore, the duodenal mucosal alkaline secretion decreased ($P < 0.05$) towards baseline (Figure 3, lower panel).

Effects of Bradykinin ($N = 6 + 5$). Bradykinin was applied topically to the serosal surface of the duodenum in increasing concentrations (10^{-6} M, 10^{-5} M, and 10^{-4} M during four consecutive 20-min periods). Such serosal administration of bradykinin dose-dependently increased duodenal mucosal alkaline secretion (Figure 4), whereas mean arterial pressure remained largely stable (not shown in figure). The bradykinin-induced secretory increments were diminished in the presence of the BK-2-receptor antagonist HOE140 (100 nmol/kg, intravenously bolus injec-
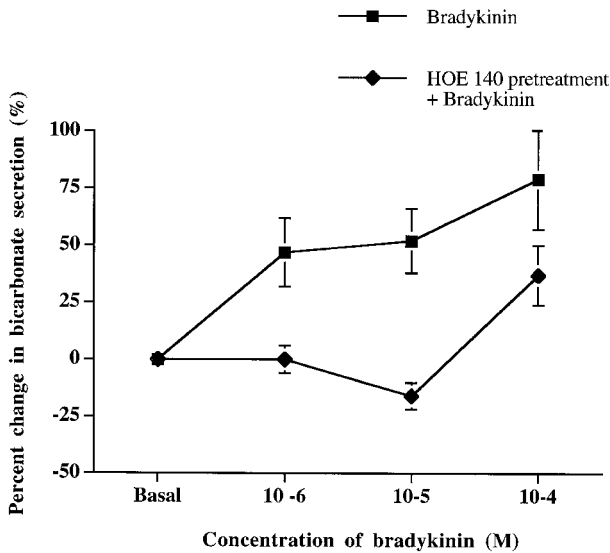
Fig 4. Effects of bradykinin, applied topically to the duodenal serosa, on duodenal alkaline secretion. Bradykinin was administered during four consecutive 20-min periods in stepwise increased concentrations (10^{-6} M, 10^{-5} M, and 10^{-4} M). Data shown are the mean percent increase from baseline to peak value within each concentration interval. Note that the presence of the BK-2 antagonist HOE 140 (100 nmol/kg, intravenously) inhibits the secretory increments induced by bradykinin *per se*.
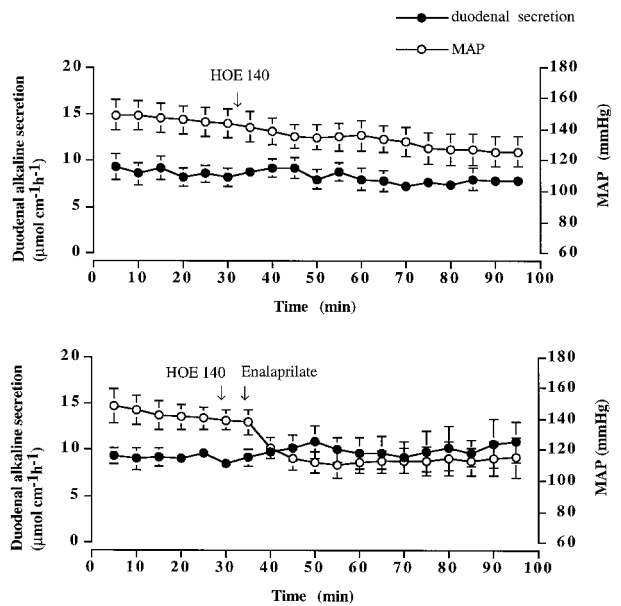

Fig 5. Effects of HOE 140 (100 nmol/kg, intravenously) on duodenal mucosal alkaline secretion and mean arterial pressure (upper panel). The lower panel shows the effects of enalaprilate (0.7 mg/kg) given 5 min after the administration of HOE 140 (100 nmol/kg) on duodenal mucosal alkaline secretion and mean arterial blood pressure (MAP). Data shown are the mean values ± SEM during the 5-min periods.

tion), administered 5 min before exposure to bradykinin (Figure 4). Administration of HOE 140 alone influenced neither secretion nor mean arterial pressure (Figure 5, upper panel).

Effects of HOE 140 on Enalaprilate-Induced Secretion ($N = 6$). Five minutes before the administration of enalaprilate, the animals received an intravenous bolus injection of HOE 140 (100 nmol/kg). The compound blocked the stimulatory effect of enalaprilate on duodenal mucosal alkaline secretion, but did not influence the decrease in mean arterial pressure compared to the control group 1 (Figure 5, lower panel).

DISCUSSION

Enalaprilate is an effective ACE inhibitor that significantly inhibits the conversion of angiotensin I (Ang I) to angiotensin II (Ang II). The results of the present study show that enalaprilate increases the duodenal mucosal alkaline (HCO_3^-) secretion, when given intravenously in anesthetized rats.

It is well documented that Ang II facilitates sympathoadrenergic inhibition of jejunal net fluid secretion and rat duodenal mucosal alkaline output (5–8, 10–14, Johansson et al, in preparation). It would therefore be reasonable to assume that enalaprilate-induced removal of endogenous Ang II results in a

damped tonic sympathoadrenergic inhibition, the net effect being a slight increase of the mucosal secretory rate. However, the enalaprilate-induced secretory effect was resistant both to splanchnicotomy and to the adrenolytic compound guanethidine. These data argue against the possibility that the secretory effects, in response to the blocked synthesis of Ang II, are due to changes in basal sympathetic nerve activity.

An alternative explanation for the enalaprilate-induced secretion may be that the compound removes an Ang II-dependent tonic inhibition, acting directly on the duodenal epithelium (15). In the present study, therefore, exogenous Ang II was administered to enalaprilate-treated animals. Such Ang II substitution, in a dose range of 0.25–0.75 µg/kg/hr without pressor effects, was without effect on the duodenal mucosal bicarbonate secretion. Higher doses of Ang II (2.5–7.5 µg/kg/hr) were associated with slight reduction of the enalaprilate-induced duodenal mucosal alkaline secretion. However, pronounced pressor effects were obtained in the latter experiments. Such effects on the cardiovascular system indicate an "unphysiological" dose range, which may result in indirect effects on the mucosal secretion due to restriction of mucosal blood flow. It has been shown during severe conditions (ie, hemorrhagic shock) with

marked reductions in mucosal blood perfusion that duodenal alkaline secretion seems to be blood flow limited (17, 18, Åneman et al, in preparation). It is thus possible that the rate of infusion of Ang II, which was associated with increased arterial pressure (2.5–7.5 µg/kg/hr), decreases duodenal blood flow to an extent influencing the secretion. However, although blood flow was not recorded in the present experiments, it may be considered to play a minor role at the lower Ang II infusion rate (0.25–0.75 µg/kg/hr), as this dose interval was without pressor effects, indicating small or no effects on vascular resistance. Furthermore, this infusion rate is within the range that has been reported to give physiological plasma levels of Ang II (16), and it prolongs inhibition of rat duodenal alkaline secretion in response to activation of the sympathoadrenergic system (Johansson et al, in preparation). Taken together, these data speak against Ang II *per se* being responsible for the increased alkaline secretion observed after enalaprilate.

ACE is a relatively nonspecific dipeptidylcarboxy peptidase, which, in addition to the conversion of Ang I to Ang II, also degrades other peptides, eg, bradykinin, neurotensin, enkephalins, substance P, and luteinizing hormone-releasing hormone (19). Enalaprilate can therefore also cause accumulation of these peptides, some of which (eg, enkephalins) have been reported to stimulate duodenal mucosal alkaline secretion. Interestingly, the BK-2 receptor antagonist HOE140 (20) also inhibited the enalaprilate-induced secretion. That finding favors bradykinin being the mediator of the enalaprilate-stimulated alkaline secretion. Bradykinin, which is closely associated with effects of ACE inhibitors in many systems, evokes mucosal eletrolyte secretion in the guinea pig and rabbit ileum, as well as in the rabbit and rat colon (21–23). Hyperkininemia is also associated with effects on gastrointestinal motility and intestinal mucosal inflammation (24).

In the present study, when applied topically to the serosal surface of the duodenum, bradykinin elicited duodenal mucosal alkaline secretion in a dose-dependent manner. This effect was blocked by HOE140, strongly suggesting the involvement of BK-2 receptors. As mentioned before, many previous studies have demonstrated effects of ACE inhibitors on sympathetic neurotransmission. These effects are ascribed not only to the blockade of Ang II formation, but also to the accumulation of vasoactive kinins, such as bradykinin (25–27). Furthermore, the vagal nerves have a stimulatory effect on duodenal mucosal alkaline secretion (3, 4, 8), and both Ang II (28) and

bradykinin (29) binding sites have been identified in the dorsal motor nucleus of the vagus. The present study shows that the enalaprilate-induced secretory effect was resistant to splanchnicotomy, to guanethidine, and to acute vagotomy. Thus, it appears unlikely that enalaprilate-dependent accumulation of bradykinin influences duodenal alkaline secretion via extrinsic autonomic nerves.

Most probably, enalaprilate increases duodenal mucosal alkaline secretion via a local pathway, either indirectly via the enteric nervous system or directly by acting on the transporting epithelial cells. ACE has been identified in the mucosa of all intestinal regions (31). A particularly high density of ACE is present within the villi of the duodenum and jejunum. Bradykinin binding sites have also been identified within rat intestinal epithelia (15). The existence of epithelial ACE and bradykinin receptors may be related to the role of bradykinin as a stimulator of intestinal secretion. In addition to direct effects on the secretory process, bradykinin, in turn, releases other bioactive compounds with potential to influence mucosal alkaline transport, for example, prostaglandin synthesis (32, 33).

In conclusion, the present study suggests that acute administration of enalaprilate increases the duodenal mucosal alkaline secretion mainly via a local bradykinin pathway, dependent neither on the extrinsic vagal and splanchnic nerves, nor on adrenergic transmission.

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