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Gastric acid secretion after blockade of angiotensin AT_1 receptors in the Na^+-depleted rat

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Abstract

This study tested the hypothesis that angiotensin II acting through the angiotensin AT_1 receptor plays an important role in the control of gastric acid secretion. Basal gastric acid secretion and gastric blood flow were lower in Na^+-depleted animals, in which the renin-angiotensin system was activated, than in animals maintained on a normal Na^+ diet. Intravenous infusion of pentagastrin at 0.6 μg/kg/min increased gastric acid secretion to a greater extent in normal Na^+ than in Na^+-depleted animals. In addition to stimulating gastric acid secretion, pentagastrin increased gastric blood flow by proportionally the same amount in both normal and low Na^+ animals. However, because basal gastric blood flow was considerably reduced in Na^+-depleted animals, the increase produced by pentagastrin extended only to the levels observed in non-pentagastrin-treated normal Na^+ animals. Lower gastric blood flow in response to pentagastrin may explain the smaller increase in gastric acid secretion observed in Na^+-depleted animals. In Na^+-depleted animals, the selective angiotensin AT_1 receptor antagonist losartan did not affect basal gastric acid secretion or gastric blood flow, suggesting the involvement of mechanisms other than angiotensin II. Following blockade of angiotensin AT_1 receptors, pentagastrin significantly increased gastric blood flow in Na^+-depleted animals to levels observed with infusion of the pentapeptide in normal Na^+ animals. The results suggest that the decrease in pentagastrin-stimulated acid secretion in Na^+-depleted animals is mediated by angiotensin II acting through the angiotensin AT_1 receptor, most probably through vascular mechanisms.

Keywords: Stomach; Angiotensin II; Gastric hemodynamics; Pentagastrin; Angiotensin AT_1 receptor; Renin-angiotensin system

1. Introduction

Gastric acid secretion is controlled primarily by neuronal and hormonal mechanisms following the intake of food (Schusdziarra, 1993; Lloyd, 1994). However, it has recently been shown that gastric acid secretion is profoundly influenced by alterations in extracellular fluid volume. For example, both in man and in several animal species, hemorrhage is associated with reduced basal and pentagastrin-stimulated gastric acid secretion (Hall et al., 1976; Szabo et al., 1979; Svanes et al., 1981; Leung et al., 1986). Decreased gastric acid secretion is also a characteristic of dehydration in fish (Holstein, 1979). The mechanisms responsible for the decrease in acid secretion following extracellular volume depletion have not been clearly elucidated. However, since gastric acid output is critically dependent upon an adequate blood supply, decreased gastric blood flow has been suggested as the mechanism whereby hemorrhage leads to reduced acid secretion (Leung et al., 1986; Svanes et al., 1981).

Extracellular volume depletion produced either by hemorrhage or by dehydration leads to activation of the sympathetic nervous system and to the release or formation of vasoactive hormones including angiotensin II. Angiotensin II has been shown to decrease gastric acid secretion in fish but its role in the control of gastric acid secretion in mammalian species remains essentially unknown (Holstein and Brigel, 1981). Therefore, in the following studies, the hypothesis that endogenous angiotensin II acting through the angiotensin AT_1 receptor contributes to the reduction of gastric acid secretion following extracellular volume was tested. This objective was achieved by measuring, in Na^+-depleted animals, changes in basal and penta-

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gastrin-stimulated gastric acid secretion after blockade of the renin-angiotensin system with the selective angiotensin AT_1 receptor antagonist losartan (Wong and Timmermans, 1995). Exogenously administered angiotensin II is a potent regulator of gastric blood flow (Wood et al., 1994). Therefore, in the following experiments gastric blood flow was measured to determine whether any change in acid output produced by blockade of angiotensin AT_1 receptors could be explained by vascular mechanisms.

2. Materials and methods

2.1. Animals

These studies were conducted with male Sprague-Dawley rats weighing between 250 and 300 g (Tif:RAIf[SPF], Ciba-Geigy, Sissein, Switzerland). The rats were maintained in a room with a 12 h light/dark cycle (6 a.m.–6 p.m. light) at a temperature of 20–24°C. All animals were given tap water to drink and were maintained either upon a regular diet (NAFAG; Gossau, Switzerland) or a diet deficient in Na^+ (< 4 mmol/kg Na^+; NAFAG). The rats were maintained on either diet for at least 7 days before use in the following protocols. During the 24 h period prior to experiment, all animals were deprived of food, but allowed free access to tap water. To prevent the ingestion of sawdust and faeces during this period, the animals were housed individually in wire bottomed cages.

2.2. Measurement of gastric acid secretion

Following thiobarbiturate anesthesia (100 mg/kg) the rats were placed in dorsal recumbency upon a heated surgical table maintained at 37°C. A tracheotomy was performed and the right femoral vein cannulated with polyethylene tubing (0.58 mm i.d. × 0.96 mm o.d.; Portex, Hythe, Kent, UK). After making a midline abdominal incision a small hole was made in the forestomach, opposite the mesentery, and a catheter inserted into the lumen. The stomach catheter consisted of 2 concentric tubes. The outer tube (8 mm o.d. × 6 mm i.d.) was 6.5 cm long and made of Silastic. The inner tube (2 mm o.d. × 1 mm i.d.), which was glued to the inner wall of the outer tube, was 30 cm long and made of polyethylene (Portex). At the end of the catheter which entered the stomach, the smaller tube projected 2 cm from the lumen of the larger tube. Once in the stomach lumen, the catheter was tied in place and the abdominal incision closed. Both the femoral venous catheter and the free end of the small tube of the stomach catheter were connected to separate infusion pumps (Precidor, Infors, Basel, Switzerland). One pump delivered isotonic saline to perfuse the stomach at the rate of 0.5 ml/min throughout the experimental period, while the other delivered pentagastrin or the isotonic saline vehicle intravenously at 20 μl/min.

2.3. Experimental procedures

Immediately following completion of the surgical procedures, the stomach lumen was perfused with 20 ml isotonic saline over a 5 min time period to remove remnant food particles. Immediately following this 'washing' procedure, the stomach was perfused with isotonic saline (0.5 ml/min) for the remainder of the experiment. Following a 45 min equilibration period, the perfusate was collected from the stomach catheter at 5 min intervals into 20 ml plastic beakers. After the collection of 3 control samples, an infusion of pentagastrin (0.6 μg/kg/min) or the isotonic saline vehicle was given. Preliminary studies established than an infusion rate of 0.6 μg/kg/min was the minimum necessary to produce essentially maximal gastric acid secretion. The pentagastrin infusion continued for the duration of the following 60 min period, during which time the collection of lumenal samples continued at 5 min intervals. In those experiments in which losartan (0.1, 1.0 or 10 mg/kg) or the isotonic saline vehicle (1 ml/kg) was administered, the drug was injected through a 27 gauge needle directly into the lumen of the left femoral vein at the end of the last 5 min control collection period. Previous studies have shown that an intravenous dose of 10 mg/kg losartan produces a rapid and essentially complete blockade of angiotensin AT_1 receptors in the rat (Wong and Timmermans, 1995).

2.4. Measurement of gastric acid

1 ml aliquots of each sample of the gastric perfusate were assayed for total acid by titrating to pH 7.0 against 0.005 M NaOH using an automatic titration system (ETS 822 –End Point Titration System, Radiometer, Copenhagen, Denmark). Total acid secretion over each 5 min period was determined by multiplying the calculated hydrogen ion concentration by the total volume of each sample.

2.5. Measurement of gastric blood flow

Gastric blood flow was measured using the radioactive microsphere technique using different groups of animals to those used for determining gastric acid secretion. Rats were anesthetized and catheters implanted into the left ventricle, left jugular vein and the left femoral artery. The jugular and femoral catheters were constructed of polyethylene tubing (0.58 mm

i.d. × 0.96 mm o.d.; Portex). The left ventricular catheter consisted of a single piece of polyethylene tubing (0.40 mm i.d. × 0.80 mm o.d.; Portex). One end of the ventricular catheter was connected initially to a blood pressure transducer (P231D, Gould Statham, Oxnard, CA, USA) and the other end inserted into the left carotid artery. During insertion of the catheter into the heart, the blood pressure tracing (Servomed, Hellige, Freiburg, Germany) was observed. Correct placement of the catheter was determined by noting the typical widening of pulse pressure, a characteristic of ventricular entry.

2.6. Experimental procedures

Prior to use the stock solutions of microspheres were vortexed, sonicated for 10 min, vortexed again and examined by light microscopy to ensure even dispersal of the microspheres. The femoral arterial catheter was connected to a pressure transducer (Gould). After determining blood pressure, the femoral catheter was attached to a withdrawal pump (Perfusor, Braun Melsungen, Melsungen, Germany) and blood withdrawn at a rate of 0.5–0.6 ml/min. After 10 s of blood withdrawal, and over the next 30 s period, approximately 100000 microspheres were slowly injected into the left ventricle and the catheter flushed with 0.3 ml isotonic saline. Blood collection continued for a further 20 s after injection of the microspheres. Either drug (pentagastrin or losartan) or the isotonic saline vehicle was administered via the jugular catheter and 30 min later gastric blood flow was measured using the above techniques.

At the end of the experiment, tissues were taken for analysis. After removal from the animal at the gastroduodenal and gastro-esophageal junctions, the stomach was cleared of adherent fat, opened along the midline and flushed with isotonic saline to remove contaminating food particles. Each tissue sample was wet-blotted by a standardized procedure and counted for radioactivity in a gamma well scintillation counter (model 2250, Tracor Analytic, USA). Using the methods described above, each tissue always had more than 400 entrapped microspheres. The left ventricular catheter and the syringe used for injecting the microspheres were counted for residual radioactivity. Experiments were included for data analysis if less than a 15% difference existed in the blood flow to paired organs, indicative of adequate microsphere mixing.

2.7. Measurement of extracellular fluid volume

Extracellular fluid volume was measured as the inulin space. Following anesthesia, each animal was placed on a heated table and the right common carotid artery and trachea cannulated as described above. Through a midline abdominal incision, both kidneys were removed and the wound tightly closed. The right femoral vein was exposed and 0.5 ml of a solution of inulin in isotonic saline (250 mg/ml) was injected directly into the lumen. One hour later, 1 ml of blood was collected from the carotid cannula. The blood was centrifuged at 4°C for 10 min and the plasma frozen at −20°C prior to the assay of inulin. The quantity of inulin in the plasma was determined spectrophotometrically (Schreiner, 1950) and extracellular fluid volume calculated from standard formulae.

2.8. Measurement of plasma angiotensin II concentrations

Animals were anesthetized with a mixture of 1.5% gaseous halothane (Hoechst-Pharma, Zurich, Switzerland) in oxygen and placed onto a heating table as described above. A midline abdominal incision was made and the abdominal aorta 2 cm below the kidneys exposed. A 22 gauge needle attached to an ice-cold plastic syringe was inserted into the lumen of the aorta and 5 ml of blood removed. Immediately after collection, the blood was placed into ice-cold EDTA-coated tubes containing 50 μl of an inhibitor solution designed to prevent the breakdown of angiotensin II (Wood et al., 1989). The blood was centrifuged at 4°C for 10 min and, the plasma snap frozen and stored at −80°C. Plasma angiotensin II levels were determined by previously described methods but without high pressure liquid chromatography separation (Nussberger et al., 1985). Angiotensin II was extracted from plasma onto phenyl-silica gel cartridges. After elution, the quantity of angiotensin II was determined by radioimmunoassay (sensitivity, 1 fmol/ml).

2.9. Na^+ depletion

To enhance the degree of Na^+ depletion induced by the low Na^+ diet, on the 2 days immediately prior to use, the animals were housed alone and received single subcutaneous injections of the diuretic furosemide (5 mg/kg).

2.10. Microspheres and drugs

Nentrac microspheres (New England Nuclear, Boston, MA, USA) 15.5 ± 0.1 μm in size and labelled either with ^{113}Sn (specific activity 524.3 MBq/g) or ^{85}Sr (specific activity 284.2 MBq/g) were used in these investigations. The microspheres were suspended in 0.9% saline and 0.05% Tween 89 solution. Furosemide (Lasix, 20 mg/ml) was obtained from Hoechst-Pharma (Zurich, Switzerland). The selective angiotensin AT_1 receptor antagonist losartan (2-butyl-4-chloro-1-[[2'-(1*H*-tetrazol-5-yl)[1,1'-biphenyl]-4-yl]methyl]-1*H*-imida-

zole-5-methanol) was synthesized by Ciba-Geigy. Pentagastrin (Peptavlon) was obtained from ICI Pharmaceuticals (Macclesfield, UK). The thiobarbiturates thiobutabarbital and thiopental were obtained from Byk Gulden (Konstanz, Germany) and Abbott Laboratories (Cham, Switzerland) respectively.

2.11. Data and statistical analysis

Gastric blood flow and gastric vascular resistance were calculated by standard formulae. Statistical analysis was by unpaired *t*-tests or by analysis of variance followed by the least significant difference procedure. Where parameters were followed as a function of time, the data were analyzed using multivariate analysis of variance for repeated measures using the significance of the Wilkes' lambda in place of the *F* value (Armitage and Berry, 1987).

3. Results

3.1. Na^+ status on basal (non-pentagastrin-stimulated) gastric acid secretion (Fig. 1)

The results demonstrate that in the resting stomach, gastric acid secretion in each time period was significantly lower in Na^+-depleted animals than in animals maintained on a normal Na^+ diet. Gastric acid secretion in animals maintained on a normal Na^+ diet averaged 2.5 μmol/5 min. In Na^+-depleted animals, acid secretion averaged 0.8 μmol/5 min –a value 67% below those recorded in normal Na^+ animals.

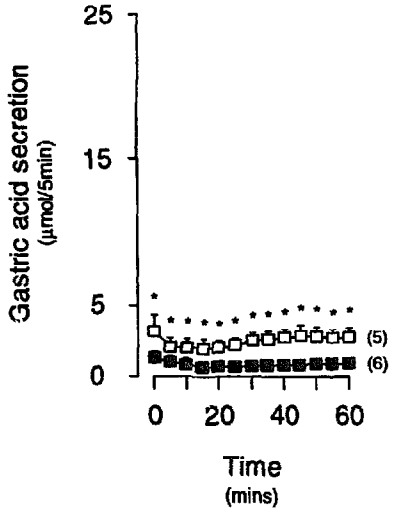

Fig. 1. Na^+ status on basal acid secretion. Normal Na^+ animals: □; low Na^+ animals: ■. Results expressed as mean+S.E. In some cases the S.E. bars are smaller than the symbols. Numbers of experiments in parentheses. Statistics by multivariate analysis of variance for repeated measures comparing gastric acid secretion between normal Na^+ and Na^+-depleted animals as a function of time. * $P < 0.05$ between normal Na^+ and low Na^+ animals at each time point by the least significant difference test.

3.2. Na^+ status on pentagastrin-stimulated acid secretion (Fig. 2)

Intravenous infusion of the isotonic saline vehicle had no significant effect on the change in basal gastric acid secretion occurring over time in either normal Na^+ or Na^+-depleted animals (left panel). In contrast, a maximum stimulatory infusion rate of pentagastrin (0.6 μg/kg/min) increased gastric acid secretion significantly above baseline values (right panel). Infusion of pentagastrin produced a significantly smaller increase in acid secretion in Na^+-depleted than in normal Na^+animals. Gastric acid secretion in animals maintained on a normal Na^+ diet averaged 15.9 μmol/5 min. In Na^+-depleted animals, acid secretion averaged 9.5 μmol/5 min –a value 41% below those recorded in normal Na^+ animals.

3.3. Na^+ status on extracellular fluid volume and plasma angiotensin II levels (Fig. 3)

Compared to animals in normal Na^+ balance, extracellular fluid volume (the inulin space) was 15% lower and plasma angiotensin II concentrations 551% higher in Na^+-depleted animals. The differences in both parameters were statistically significant.

3.4. Losartan on basal and pentagastrin-stimulated gastric acid secretion in Na^+-depleted animals (Figs. 4 and 5)

In Na^+-depleted animals, blockade of angiotensin AT_1 receptors with losartan produced a dose-dependent decrease in mean arterial pressure (Fig. 4) and a dose-dependent increase in gastric acid secretion in response to the intravenous infusion of 0.6 μg/kg/min pentagastrin (Fig. 5A). The increase in acid secretion reached statistical significance from control values following injection of losartan at either 1 and 10 mg/kg. Following administration of 10 mg/kg losartan, a dose which results in complete blockade of the renin-angiotensin system, gastric acid secretion was restored close to the values obtained in pentagastrin-infused normal Na^+ animals. Gastric acid secretion in response to pentagastrin averaged 14.3 μmol/5 min in Na^+-depleted animals treated with 10 mg/kg losartan and 15.9 μmol/5 min in vehicle-treated normal Na^+ animals. Within the analysis of variance these 2 values were not significantly different. Administration of losartan at a dose of 10 mg/kg did not affect basal (non-pentagastrin-stimulated) acid secretion in Na^+-depleted animals (Fig. 5B).

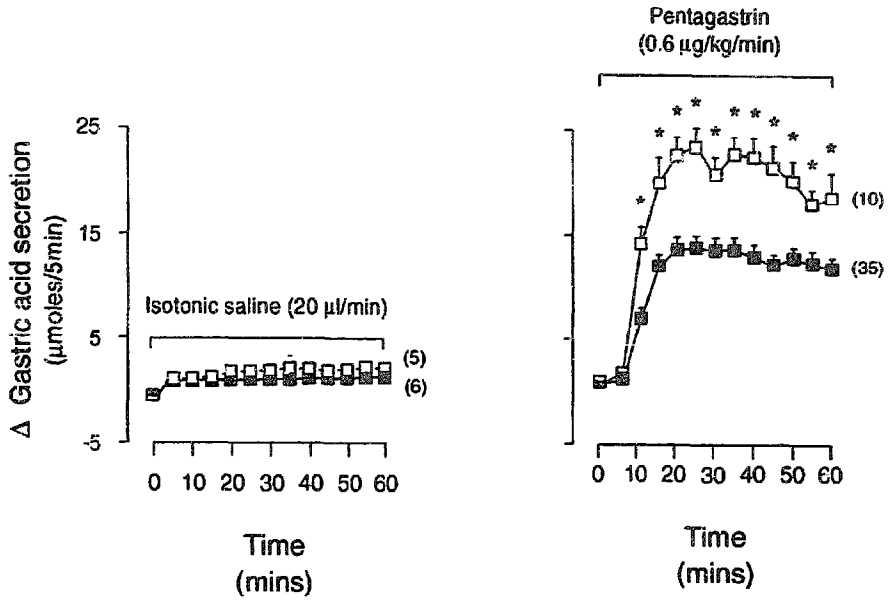

Fig. 2. Na^+ status on pentagastrin-stimulated gastric acid secretion. Normal Na^+ animals: □; low Na^+ animals: ■. Results expressed as mean + S.E. In some cases the S.E. bars are smaller than the symbols. Numbers of experiments in parentheses. Baseline determinations of acid output were made and then either saline or differing doses of pentagastrin were infused for the duration of the experimental period. Results presented are the absolute changes from baseline values. Statistics by multivariate analysis of variance for repeated measures comparing gastric acid secretion between normal Na^+ and Na^+-depleted animals for each dose of pentagastrin as a function of time. * $P < 0.05$ between normal Na^+ and low Na^+ animals at each time point by the least significant difference test.

3.5. Na^+ status on basal (non-pentagastrin-stimulated) gastric blood flow (Fig. 6)

Compared to normal Na^+ animals gastric blood flow, measured 30 min after starting the measurement of gastric acid secretion, was significantly lower (by 0.5 ml/min) and gastric vascular resistance significantly higher (by 34 mm Hg/ml/min) in Na^+-depleted animals. There was no significant difference in mean arterial pressure between Na^+-depleted and normal Na^+ animals.

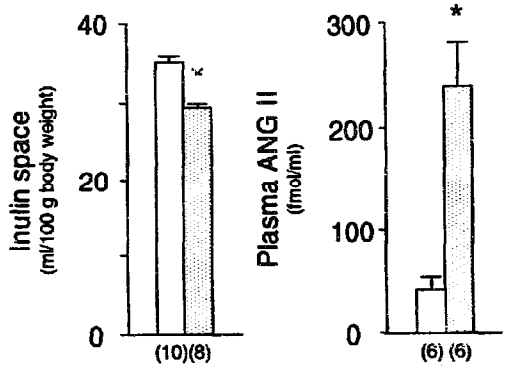

Fig. 3. Na^+ status on extracellular fluid volume (inulin space) and plasma angiotensin II concentrations. Normal Na^+ animals: open columns; low Na^+ animals: stippled columns. Results expressed as mean+S.E. Numbers of experiments in parentheses. Statistics by unpaired *t*-tests which compare values of inulin space and plasma angiotensin II concentrations between normal Na^+ and Na^+-depleted animals. * $P < 0.05$.

3.6. Na^+ status on pentagastrin-stimulated gastric blood flow (Fig. 6)

Thirty minutes into the intravenous infusion of 0.6 µg/kg/min pentagastrin, gastric blood flow was stimulated to a similar extent in both normal Na^+ (0.71 ml/min) and Na^+-depleted (0.64 ml/min) animals. However, because basal values were low in Na^+-de-

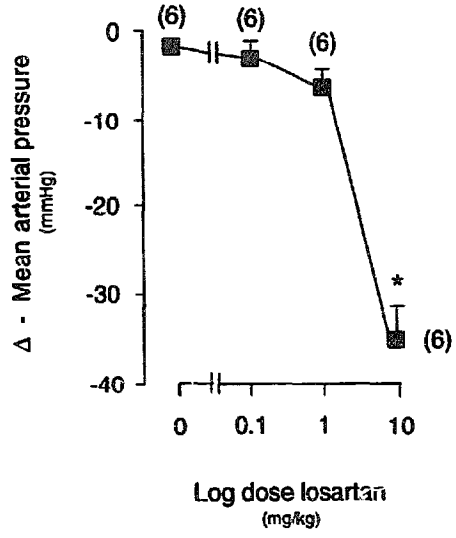

Fig. 4. Losartan on mean arterial pressure in Na^+-depleted animals. Results expressed as mean+SE. Numbers of animals used at each dose are shown in parentheses. Statistics by analysis of variance followed by the least significant difference procedure which compares the fall in blood pressure produced by each dose of losartan with control values.

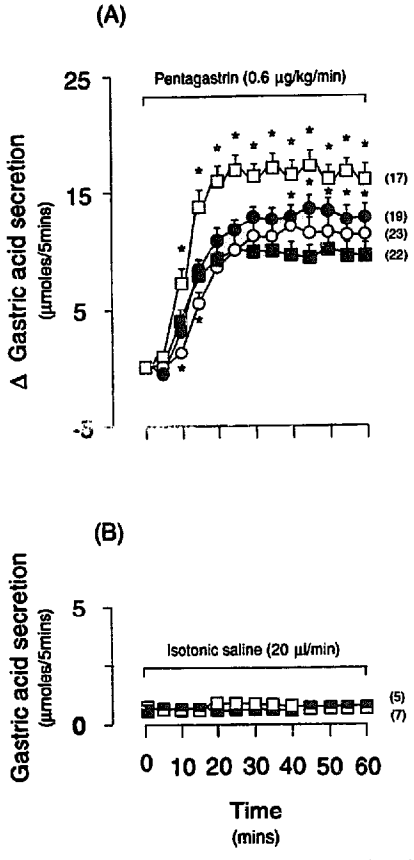

Fig. 5. Losartan on basal and pentagastrin-stimulated gastric acid secretion in Na^+-depleted animals. Saline vehicle: ■; losartan 0.1 mg/kg: ○; losartan 1.0 mg/kg: ●; losartan 10.0 mg/kg: □. (A) Each animal received an infusion of 0.6 µg/kg/min pentagastrin from time 0 to 60 min. Differing doses of losartan were given i.v. 5 min prior to the infusion of pentagastrin. Results presented are the changes from baseline values. (B) Each animal received an infusion of isotonic saline from time 0 to 60 min. Losartan (10 mg/kg) was given i.v. 5 min prior to the infusion of saline. Results presented are absolute values. All data expressed as mean+S.E. Numbers in parentheses. Statistics by multivariate analysis of variance for repeated measures which compare acid secretion between control animals and each dose of losartan as a function of time. * $P < 0.05$ between control and losartan-treated animals at each time point by the least significant difference test.

pleted animals, pentagastrin stimulated gastric blood flow only to the levels observed in the resting stomach of normal Na^+ animals. The changes in gastric vascular resistance mirrored those of gastric blood flow in these studies. Pentagastrin infusion did not alter mean arterial blood pressure in either normal Na^+ or Na^+-depleted animals.

3.7. Losartan on basal and pentagastrin-stimulated gastric hemodynamics in Na^+-depleted animals (Fig. 6)

Thirty minutes after injection of 10 mg/kg losartan, a time corresponding to the maximal change in gastric acid secretion, mean arterial pressure fell by 32.7 mm Hg to a value significantly below those recorded in control animals. Despite the significant falls in blood pressure, neither gastric blood flow nor gastric vascular resistance changed significantly. Intravenous infusion of 0.6 µg/kg/min pentagastrin significantly increased gastric blood flow and reduced gastric vascular resistance without significantly changing mean arterial pressure. When losartan was given at the same time as 0.6 µg/kg/min pentagastrin, blood pressure fell by 27.8 mm Hg, a fall similar to that observed in the experiments performed with losartan alone. However, both the increase in gastric blood flow and the decrease in gastric vascular resistance were significantly greater after blockade of angiotensin AT_1 receptors in these experiments than observed with infusion of 0.6 µg/kg/min pentagastrin alone. Indeed, similar to the changes in gastric acid secretion described above, gastric blood flow in response to pentagastrin reached the values obtained with infusion of pentagastrin in normal Na^+ animals.

4. Discussion

These studies tested the hypothesis that angiotensin II, acting through the angiotensin AT_1 receptor, plays an important role in producing the decrease in gastric acid secretion that occurs following extracellular volume depletion. Gastric blood flow was also measured in these studies to determine whether the change in gastric secretion produced by angiotensin AT_1 receptor blockade had a vascular component.

4.1. Na^+ status, angiotensin II and gastric acid secretion

Dehydration and hemorrhage are both associated with reduced basal and pentagastrin-stimulated gastric acid secretion (Hall et al., 1976; Szabo et al., 1979; Svanes et al., 1981; Leung et al., 1986; Holstein, 1979). The results of the present studies demonstrate that Na^+ depletion also leads to a reduction in both basal and pentagastrin-stimulated gastric acid secretion. Although hemorrhage, dehydration and Na^+ depletion are dissimilar animal models, all three share decreased extracellular fluid volume as a common characteristic. Extracellular volume depletion is a potent stimulus for the release of a number of vasoactive hormones including angiotensin II. In the present studies, Na^+ depletion led to a significant reduction in extracellular fluid volume and to a significant increase in plasma angiotensin II concentrations. To determine whether angiotensin II was responsible for the fall in basal and pentagastrin-stimulated gastric acid secretion, in Na^+-depleted animals, the renin-angiotensin system was blocked with losartan. Losartan specifically blocks the angiotensin AT_1 receptor which has been shown to mediate the majority of the actions of angiotensin II (Wong and Timmermans, 1995).

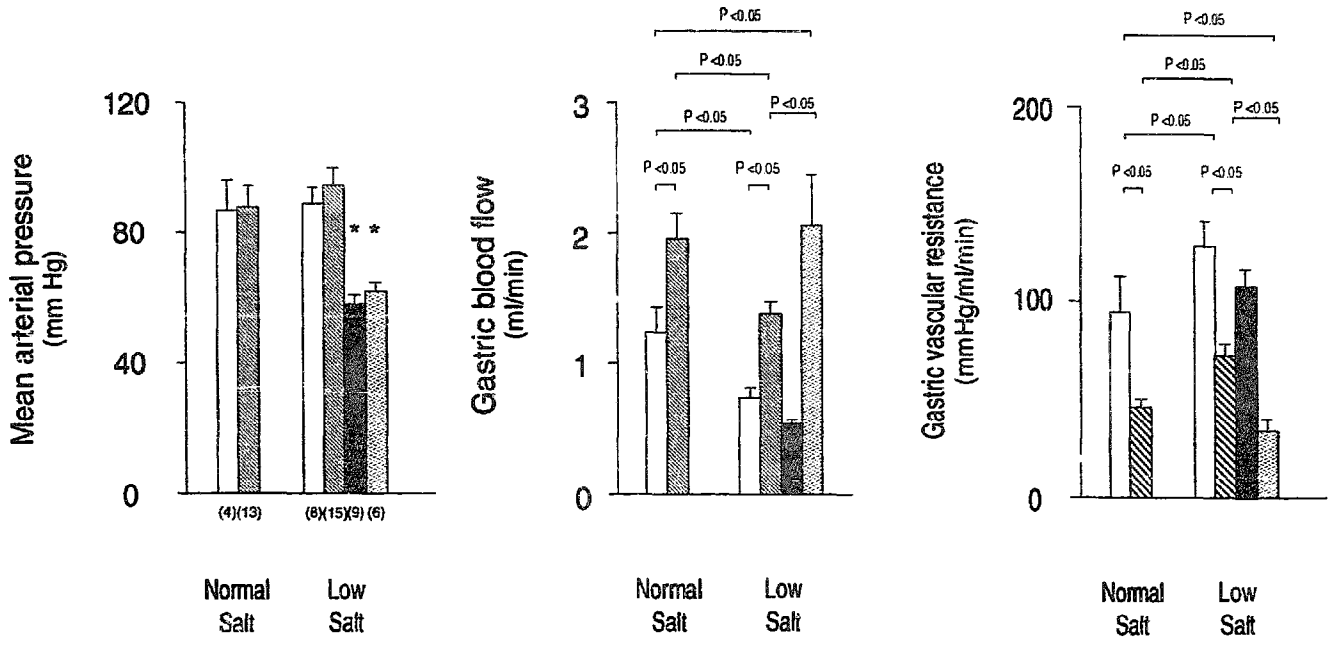

Fig. 6. Losartan and pentagastrin on gastric hemodynamics in normal Na^+ and Na^+-depleted animals. Controls: open columns, pentagastrin (0.6 μg/kg/min): hatched columns, losartan (10 mg/kg): black columns; losartan (10 mg/kg) + pentagastrin (0.6 μg/kg/min): stippled columns. Results expressed as mean ± S.E. Numbers in parentheses, which are the same for all parameters. Statistics by analysis of variance which compares the change in each parameter with each other. All shown significances by the least significant difference procedure. * $P < 0.05$ to all other bar graphs of blood pressure without losartan.

4.2. Blockade of angiotensin AT_1 receptors in the resting stomach

Gastric acid secretion is critically dependent upon the adequate delivery of nutrients and oxygen to the parietal cells. At the low rates of gastric blood flow encountered in these studies, parietal cell function is so dependent upon an adequate oxygen supply that even small reductions in blood flow can markedly affect acid secretion (Kauffman, 1982; Holm and Perry, 1988). Na^+ depletion, in addition to lowering gastric acid secretion, also resulted in a significant lowering of resting gastric blood flow and an increase in gastric vascular resistance compared to animals in normal Na^+ balance. Decreased vascular perfusion of the stomach may therefore explain the fall in basal gastric acid secretion observed in Na^+-depleted animals. Decreased gastric blood flow is also believed to be the mechanism by which basal gastric acid secretion falls after hemorrhage (Kauffman, 1982; Leung et al., 1986).

The factors responsible for the decrease in resting gastric blood flow following extracellular volume depletion have not been clearly elucidated. In the present studies, angiotensin AT_1 receptor blockade lowered blood pressure in Na^+-depleted animals. Although blockade of endogenous angiotensin II lowered blood pressure indicating peripheral vasodilation, the gastric vasculature did not dilate following angiotensin AT_1 blockade in animals which had not received pentagastrin. These observations appear to provide evidence against the involvement of angiotensin II in the control of basal gastric acid secretion and gastric hemodynamics in the resting stomach. Several investigators have shown the gastric vasculature to be very sensitive to the constrictor actions of angiotensin II (Wood et al., 1994; Bailey et al., 1987). Thus it is surprising that an increase in gastric blood flow and a decrease in gastric vascular resistance did not occur in response to blockade of angiotensin AT_1 receptors with losartan. An explanation for the failure of angiotensin II blockade to influence the gastric vasculature circulation in this study may be the observation that norepinephrine, vasopressin and angiotensin II are each believed to individually exert a maximal vasoconstrictor effect on the mesenteric vasculature in the volume-depleted state. Blockade of only one vasoconstrictor therefore has little effect on mesenteric hemodynamics, since the vasculature remains fully constricted by the other two factors (McNeill et al., 1970, 1976). Thus, the results of the present studies, while demonstrating that blockade of angiotensin AT_1 receptors does not alter basal gastric blood flow following Na^+ depletion, do not rule out a vasoconstrictor effect of endogenous angiotensin II on the gastric vasculature.

4.3. Blockade of angiotensin AT_1 receptors in the stimulated stomach

The results of the present study demonstrate that Na^+ depletion, in addition to inhibiting basal gastric

acid secretion, also attenuated pentagastrin-stimulated gastric acid secretion. Pentagastrin not only stimulates gastric acid secretion but also induces gastric vasodilation. Pentagastrin-stimulated acid secretion can occur in the absence of an increase in blood flow. However, gastric blood flow becomes limiting at high rates of gastric acid secretion (Leung et al., 1986; Kauffman, 1982). Since the gastric vasculature was more constricted in Na^+-depleted than in normal Na^+ animals, the decrease in acid secretion in response to the highest infusion rate of pentagastrin was most probably due to the reduced delivery of metabolic substrates. A similar mechanism of action has been used to explain the effect of hemorrhage on pentagastrin-stimulated gastric acid secretion (Leung et al., 1986).

Although not affecting basal gastric acid secretion, or that produced by a low dose of pentagastrin, losartan reversed, in dose-dependent manner, the attenuated increase in acid secretion produced by infusion of 0.6 μg/kg/min pentagastrin in Na^+-depleted animals. At a dose of 10 mg/kg, which resulted in an essentially complete block of the renin-angiotensin system, losartan increased pentagastrin-stimulated acid secretion close to the values observed in normal Na^+ animals. The increase in pentagastrin-stimulated gastric acid secretion following blockade of angiotensin AT_1 receptors most probably has a vascular basis since gastric blood flow also increased to the levels observed in normal Na^+ animals infused with the pentapeptide. The increase in gastric blood flow was remarkable given the marked fall in blood pressure, and is reflected in the large fall in gastric vascular resistance observed in these experiments. These results demonstrate that in the actively secreting stomach, angiotensin II, acting through the angiotensin AT_1 receptor, is the primary vasoconstrictor of the gastric circulation following extracellular volume depletion.

An important aspect of this study is the observation that only in the presence of pentagastrin could an effect of endogenous angiotensin II upon the gastric vasculature be demonstrated. Since pentagastrin could produce gastric vasodilation in Na^+-depleted animals, the results imply that the endogenous vasodilators produced in response to pentagastrin must, under conditions of volume depletion, act to attenuate the vasoconstrictor actions of angiotensin II on the gastric circulation. The mechanisms producing gastric vasodilation during pentagastrin infusion involve a complex interplay of circulating, paracrine and autocrine factors. Furthermore, blockade of the renin-angiotensin system in this study lowered blood pressure and must therefore have activated a number of counter-regulatory systems. Thus, the actual mechanisms involved in mediating the interaction between pentagastrin and angiotensin II in these studies is likely to be rather complex. However, the release of prostaglandins and nitric oxide by the secreting stomach are 2 likely candidates to attenuate the vasoconstrictor properties of angiotensin II and are worthy of further study (Wood et al., 1994; Pique et al., 1992). The reason why blockade of angiotensin AT_1 receptors produced an increase in gastric blood flow may also be explained by the above-mentioned hypothesis (McNeill et al., 1970, 1976). Extending this reasoning to the current situation where each endogenous vasoconstrictor is not able to produce maximal vascular constriction, blockade of one, for example angiotensin II, leads to a fall in gastric vascular resistance. This is in contrast to the case of the quiescent stomach where little or no endogenous vasodilator is being produced and vasoconstrictors may each be exerting maximal constriction.

Whatever the actual mechanism or mechanisms involved, the results have important implications for the study of the effects of endogenous angiotensin II on the gastric vasculature, the effect of which appears to differ depending upon the metabolic activity of the secreting stomach.

Previous studies that have investigated a role for endogenous angiotensin II in the control of gastric blood flow have provided equivocal results. In some studies, a role for endogenous angiotensin II in the control of gastric blood flow has been observed while in others it has not (Bulkley et al., 1985; Bailey et al., 1987; MacDonald et al., 1991; Cullen et al., 1994; Wang et al., 1992). The present results suggest that the variant reponse to inhibition of the renin-angiotensin system may be explained by the underlying secretory activity of the stomach which was not characterized in previously reported studies.

Those studies where an effect of blockade of the renin-angiotensin system on gastric hemodynamics has been observed, have largely been confined to the use of angiotensin converting enzyme inhibitors which are known to potentiate the actions of bradykinin, a potent vasodilator substance (Ender et al., 1993; Linz et al., 1995; Bailey et al., 1987; Bulkley et al., 1985). Indeed, kinins may, under certain conditions, contribute significantly to the vascular actions of angiotensin converting enzyme inhibitors (Linz et al., 1995). The results of the present studies using losartan, which does not influence kinin metabolism, provide direct and compelling evidence for an important primary role for angiotensin II in the control of the gastric vasculature following extracellular volume depletion.

In summary, the results demonstrate that analogous to hemorrhage and dehydration, Na^+ depletion also lowers both basal and pentagastrin-stimulated acid secretion. Endogenous angiotensin II does not contribute to the changes in basal (non-stimulated) gastric acid secretion and gastric hemodynamics observed after Na^+ depletion. Angiotensin II does however appear to be responsible for the fall in pentagastrin-stimulated acid

secretion observed after Na^+ depletion. The results have important implications for the understanding of the control of gastric acid secretion and gastric blood flow in volume-deplete states.

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