Vasoactive intestinal peptide down-regulates the intrahepatic renin–angiotensin system in the anaesthetized rat

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ABSTRACT

Gastric sodium loading results in an increase in the portal venous concentration of vasoactive intestinal peptide (VIP) and down-regulation of both the intrahepatic and circulating renin–angiotensin systems. In the present study we sought to determine whether an increase in the concentration of VIP in the portal circulation might act to down-regulate the intrahepatic and/or circulating renin–angiotensin systems. Male Sprague–Dawley rats were infused intra-portal with haemaccel vehicle or VIP in haemaccel for 60 min. Livers were harvested and blood was sampled. Angiotensin-converting enzyme (ACE) activity and angiotensinogen, angiotensin I, angiotensin II and renin concentrations were measured. VIP infusion decreased hepatic ACE activity ($P < 0.05$), the hepatic angiotensinogen concentration ($P < 0.001$) and the hepatic angiotensin I concentration ($P < 0.05$). The plasma angiotensinogen concentration and serum ACE activity were also decreased by intraportal VIP infusion ($P < 0.05$ for each). Plasma renin, angiotensin I and angiotensin II concentrations were unchanged by VIP infusion. We conclude that an increase in the portal venous VIP concentration down-regulates the intrahepatic renin–angiotensin system. These changes are similar to those reported after gastric sodium loading, and we suggest, therefore, that the increase in portal venous VIP that occurs after gastric sodium is the means by which the gastric sodium sensor signals the liver to effect these changes in the renin–angiotensin system.

INTRODUCTION

The concept of an upper intestinal or portal monitor of sodium intake was proposed by Lennane and co-workers [1,2], based upon the demonstration of a greater and more rapid natriuresis after a gastric than an intravenous sodium load. Although the initial studies were performed in humans and rabbits, the existence of the gastric sodium monitor has subsequently been confirmed in other species [3–6]. The gastric or upper intestinal sodium monitor is thought to consist of a sodium sensor located in the stomach or upper intestine, a mediation system responsible for signal transduction to the kidney, and a renal effector mechanism. The nature of the intestinal sodium sensor and the renal effector mechanisms have not been elucidated; however, the components of the mediation system have been delineated in part. Administration of a gastric sodium load results in release of the gut-derived natriuretic peptide, vasoactive intestinal peptide (VIP), into the portal circulation [7]. In contrast with other stimuli which release VIP into the portal circulation [8–12], administration of a gastric sodium load also

Key words: gastric sodium monitor, renin–angiotensin system, vasoactive intestinal peptide.

Abbreviations: ACE, angiotensin-converting enzyme; ANG II (etc.), angiotensin II (etc.); VIP, vasoactive intestinal peptide.

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decreases catabolism of VIP by the liver \[13\] and lung \[14\], thereby permitting VIP to reach the kidney and effect a natriuresis.

Simultaneously with release of VIP from the intestine and an increase in both portal and systemic VIP concentrations, there is a decrease in the concentration of the sodium-conserving peptide angiotensin II (ANG II) \[3\]. This decrease in ANG II does not result from a change in the renin concentration \[3,15,16\]. Instead, the decrease in circulating ANG II appears to result from a decrease in the synthesis and secretion of angiotensinogen by the liver \[16\], as well as from a decrease in hepatic angiotensin-converting enzyme (ACE) activity \[16\].

As well as changes in these humoral mediators, the mediation system for the gastric sodium monitor has some neural components. Sodium ingestion causes an increase in afferent hepatic nerve activity and a decrease in renal nerve activity \[6\]. Hepatic denervation completely prevents the natriuresis in response to gastric sodium loading \[6\], while renal denervation decreases the natriuresis, but does not prevent it entirely.

The changes in the intrahepatic renin–angiotensin system that we have reported previously \[15,16\] must be induced by a signal arising from the gastric or upper intestinal sodium sensor. From the data currently available, the possible mediators are a change in afferent hepatic nerve traffic or the increase in the concentration of VIP in portal venous blood reaching the liver. In the present study we have investigated the effects of an increase in the portal venous concentration of VIP on the intrahepatic renin–angiotensin system to determine whether this might be the stimulus.

**METHODS**

**Animals**

Male Sprague–Dawley rats weighing 250–300 g were placed on a low-sodium diet (0.008 % NaCl; Janos Chemicals, Forbes, NSW, Australia) for 7 days. During this period, the rats were permitted access to diet and distilled drinking water *ad libitum*. On the day of experiment, the rats were randomly allocated to one of three groups: baseline control group, vehicle infusion, and VIP infusion (*n* = 8 rats per group).

**Experimental protocol**

The rats were anaesthetized using gaseous general anaesthesia: halothane (2.5%) delivered in nitrous oxide (70%) and oxygen (30%) via a non-rebreathing mask. The abdomen was opened via a mid-line incision through the linea alba. The portal vein was located and cannulated using a 22-gauge silastic cannula. After a 30 min resting equilibration period, during which haemaccel was infused through the portal cannula, arterial blood was sampled and livers were harvested and immediately snap-frozen for the baseline control group. In the vehicle control group, infusion of haemaccel (Hoechst Marion Roussel, Sydney, Australia) was commenced at 0.017 ml/min. In the VIP infusion group, VIP in haemaccel was infused at 0.017 ml/min, and the concentration of VIP in the infusion was adjusted so as to achieve a VIP infusion rate of 10 pmol min⁻¹ kg⁻¹. Haemaccel was used as the infusion vehicle because previous studies have demonstrated that loss of VIP from infusate on to glass or plastic was minimized under these conditions \[13,14\]. The dose of VIP was based on previous studies which demonstrated that the resulting plasma VIP concentrations were similar to those achieved after gastric sodium loading \[13,14\]. After 60 min of vehicle or VIP infusion, arterial blood was sampled and livers were harvested and immediately snap-frozen in liquid nitrogen.

**Serum and hepatic ACE activity**

Each liver was pulverized using a stainless steel tissue grinder and anvil pre-cooled with liquid nitrogen. The pulverized tissue was then homogenized in phosphate buffer (0.5 mol l⁻¹ K₂HPO₄ and 1.5 mol l⁻¹ NaCl, pH 8.4) using an Omni 2000 (Omni International) homogenizer at speed 3 for 60 s. The homogenate was divided into aliquots and stored at −20 °C until assayed.

For serum ACE activity, 10 μl of serum was incubated with 240 μl of hippuryl-histidyl-leucine (5 mM) at 37 °C for 15 min. For estimation of ACE activity in liver, 100 μl of homogenate was incubated with 240 μl of hippuryl-histidyl-leucine for 60 min. In each case the reaction was terminated by addition of NaOH. The histidyl-leucine generated was estimated fluorimetrically following incubation with 0.2 % o-phthalaldehyde in methanol for 10 min. Fluorescence was determined by excitation/ emission (350/500 nm) fluorometry using an Hitachi F2000 fluorometer. The amount of histidyl-leucine generated was derived from comparison with a standard curve \[16\].

**Angiotensinogen concentrations in plasma and liver**

For determination of the hepatic angiotensinogen concentration, 2–3 g of tissue was pulverized as above and homogenized in phosphosaline buffer containing 1 μg ml⁻¹ perindoprilate (Servier Laboratories, Melbourne, Australia), 20 mmol l⁻¹ EDTA (Sigma Chemical Co., St. Louis, MO, U.S.A.) and 8 % PMSF (Sigma). The inhibitor mixture was designed to prevent degradation of ANG I formed during the incubation step. The homogenate was incubated with exogenous renin and the ANG I formed was extracted as described previously \[16\].
For determination of the plasma angiotensinogen concentration, 250 µl of plasma was incubated with exogenous renin and extracted as above [16].

For assay, samples were reconstituted in barbitone buffer and ANG I was determined by radioimmunoassay [16].

**ANG I concentrations in plasma and liver**

For determination of ANG I in plasma and liver, 250 µl of plasma or liver homogenate was extracted on a C18 Sep-Pak column, as above. After blowing the extract down to dryness and reconstitution in barbitone buffer, the ANG I concentration was determined by radioimmunoassay.

**Plasma renin concentration**

The plasma renin concentration was determined by radioimmunoassay of ANG I generated after incubation of 250 µl of plasma with an excess of exogenous renin substrate for 2 h at 37 °C, as described previously [16].

**Plasma ANG II concentration**

For determination of the plasma ANG II concentration, blood was collected into pre-cooled syringes containing 0.3 M EDTA (0.05 ml/ml of blood) and 0.2 M 2,3-dimercaptopropanol (0.03 ml/ml of blood). After centrifugation, plasma was extracted on a C18 Sep-Pak cartridge using 2 ml of acetonitrile/distilled water/acetic acid (74:24:4, by vol.). The eluates were then blown down to dryness under nitrogen and stored at −20 °C until assay. The samples were reconstituted in barbitone buffer and assayed as described previously [3,16]. The polyclonal antibody used in this assay cross-reacts with ANG III, but has negligible cross-reactivity with ANG I under assay conditions [3,16].

**Statistical methods**

Differences between groups for each parameter were assessed using analysis of variance. When significant differences were found, individual comparisons were made by LSD test for planned comparisons (CSS-Statistica). P values of < 0.05 were accepted as significant.

**RESULTS**

**Serum and hepatic ACE activity**

Serum ACE activity was unchanged by vehicle infusion (Figure 1, upper panel), whereas VIP infusion resulted in a decrease in ACE activity. This decrease was significant compared with the control (P < 0.05).

The level of ACE activity in the liver decreased significantly in response to VIP infusion. There was no significant change compared with the baseline level of hepatic ACE activity (2.9 ± 0.3 nmol min⁻¹ g⁻¹ tissue; mean ± S.E.M.; n = 8 rats) after infusion of haemaccel vehicle (3.05 ± 0.36 nmol min⁻¹ g⁻¹; Figure 1, lower panel). VIP infusion resulted in a significant decrease in hepatic ACE activity (3.05 ± 0.36 nmol min⁻¹ g⁻¹; Figure 1, lower panel). This decrease in plasma angiotensinogen concentration (9.0 ± 0.8 nmol l⁻¹) was significant when compared with both the baseline level (P < 0.05) and the level of ACE activity after vehicle control infusion (P < 0.05).

**Angiotensinogen concentrations in plasma and liver**

Haemaccel vehicle infusion resulted in a non-significant increase in the plasma concentration of angiotensinogen (19.2 ± 1.8 nmol l⁻¹; mean ± S.E.M.; n = 8 rats) compared with that in controls (15.8 ± 2.4 nmol l⁻¹; Figure 2, lower panel). Portal infusion of VIP resulted in a significant decrease in the plasma angiotensinogen concentration (15.8 ± 2.4 nmol l⁻¹; Figure 2, lower panel). This decrease in plasma angiotensinogen was significant when compared with control (P < 0.05) and haemaccel vehicle-infused (P < 0.001) rats.

The concentration of angiotensinogen in the liver was unchanged after haemaccel vehicle infusion (11.08 ± 0.24 pmol g⁻¹ tissue; mean ± S.E.M.; n = 8 rats) compared with the baseline hepatic concentration of angiotensinogen (11.01 ± 0.27 pmol g⁻¹; Figure 3, upper panel). In contrast, portal venous infusion of VIP caused a significant decrease in the hepatic angiotensinogen concentration (9.0 ± 0.8 pmol g⁻¹; Figure 3, upper panel).
Plasma renin concentration

There was a significant increase in the plasma renin concentration, from $11.1 \pm 1.5$ ng·h$^{-1}$·ml$^{-1}$ (mean ± S.E.M. for $n = 8$ rats) in the control group to $7.07 \pm 0.35$ pmol·g$^{-1}$. This was significantly lower than both the baseline concentration of angiotensinogen ($P < 0.001$) and that achieved after vehicle control infusion ($P < 0.001$).

**ANG I concentrations in plasma and liver**

Following haemaccel vehicle infusion, there was a non-significant increase in the plasma ANG I concentration ($5.0 \pm 0.6$ nmol·l$^{-1}$; mean ± S.E.M.; $n = 8$ rats) compared with controls ($3.5 \pm 0.5$ nmol·l$^{-1}$). Infusion of VIP did not cause any significant change in the plasma concentration of ANG I when compared with vehicle infusion (Figure 4, upper panel).

There was a non-significant increase in the hepatic concentration of ANG I in the haemaccel vehicle group ($4.43 \pm 0.74$ pmol·g$^{-1}$ tissue; mean ± S.E.M.; $n = 8$ rats) compared with controls ($3.78 \pm 0.56$ pmol·g$^{-1}$). Portal venous infusion of VIP resulted in a significant decrease in the concentration of ANG I in the liver ($2.66 \pm 0.49$ pmol·g$^{-1}$; $P < 0.05$ (Figure 3, lower panel).

Plasma renin concentration

There was a significant increase in the plasma renin concentration, from $11.1 \pm 1.5$ ng·h$^{-1}$·ml$^{-1}$ (mean ± S.E.M. for $n = 8$ rats) in the control group to $7.07 \pm 0.35$ pmol·g$^{-1}$. This was significantly lower than both the baseline concentration of angiotensinogen ($P < 0.001$) and that achieved after vehicle control infusion ($P < 0.001$).
addition, portal VIP infusion caused a decrease in the hepatic ACE activity that we have reported after gastric sodium loading [15,16]. In addition, portal VIP infusion caused a decrease in hepatic renin–angiotensin system. The present study suggests that this increase in the portal venous concentration of VIP is the intermediary mechanism whereby sodium ingestion down-regulates the intrahepatic renin–angiotensin system. The greater and more rapid natriuresis that results from a gastric sodium load compared with an intravenous sodium load was found to be prevented by hepatic denervation [6]. This was interpreted to mean that sodium ingestion causes a change in hepatic nerve activity and that this change in neural traffic is integral to the functioning of the mediation system for the gastric sodium monitor. Thus one possible means by which the gastric or upper intestinal sodium sensor signals the liver to effect these changes in the intrahepatic renin–angiotensin system may be via changes in hepatic nerve activity.

In addition to inducing changes in hepatic nerve activity, gastric sodium loading causes a very rapid increase in VIP levels in the portal circulation [7]. Sodium ingestion also causes a decrease in both the hepatic [13] and pulmonary [14] clearance of VIP from the circulation, so that the concentration of VIP in the systemic plasma increases. This increased level of a natriuretic peptide [17] combined with a decrease in the concentration of an anti-natriuretic peptide (ANG II) [3] may also contribute to the natriuresis that follows sodium ingestion. In addition to a humoral role in the natriuretic response to sodium ingestion, the increase in portal venous VIP concentration may act as a signal to induce down-regulation of the intrahepatic renin–angiotensin system. The present study suggests that this increase in the portal venous concentration of VIP is the intermediary mechanism whereby sodium ingestion down-regulates the intrahepatic renin–angiotensin system.

Infusion of VIP into the portal vein significantly decreased hepatic ACE activity. This decrease was similar in magnitude to the decrease in hepatic ACE activity that we have reported after gastric sodium loading [15,16]. In addition, portal VIP infusion caused a decrease in the concentration of angiotensinogen in the liver. The magnitude of this decrease in the hepatic concentration of angiotensinogen was also similar to that which we observed after administration of a gastric sodium load [16]. In addition, infusion of VIP resulted in a significant decrease in the plasma concentration of angiotensinogen. A decrease in the plasma angiotensinogen concentration was also observed following gastric sodium loading [16]. Thus it would appear that the increase in VIP concentration in the portal circulation engendered by gastric sodium acts to down-regulate hepatic ACE activity, as well as the plasma and hepatic concentrations of angiotensinogen. Angiotensinogen synthesis is known to be regulated by plasma ANG II in a positive-feedback manner, with increasing concentrations of ANG II causing an increase in hepatic angiotensinogen mRNA and angiotensinogen [18,19], the mechanism being that ANG II stabilizes angiotensinogen mRNA [20,21]. The effect of portal VIP infusion on hepatic and plasma angiotensinogen levels therefore appears to be a direct regulatory effect of VIP on angiotensinogen synthesis, as plasma ANG II was unchanged by VIP infusion.

The effects of portaly infused VIP on other elements of the circulating renin–angiotensin system, however, differed from those that occur after gastric sodium administration. Portally infused VIP had no effect on the plasma renin concentration, although vehicle infusion did increase plasma renin significantly above baseline levels. This contrasts with the effects of gastric sodium loading and systemic infusion of VIP, which have both been found to increase the plasma renin concentration [16,22–26]. An increase in the portal concentration of VIP was associated with an increase in the plasma concentration of ANG I. This contrasts with the effects of administration of a gastric sodium load, which resulted in a decrease in the circulating ANG I concentration [16]. The difference in these effects on ANG I may be explained by differing effects on ACE activity: VIP infusion caused a significant decrease in ACE activity, whereas gastric sodium administration did not change serum ACE activity. Plasma ANG II was unchanged by portal VIP infusion. This again contrasts with the effect of gastric sodium administration in the rabbit [3] and the rat [16]. In both of these species, gastric sodium administration decreased plasma ANG II levels. However, in studies in which VIP was infused systemically rather than intraportally, there was an increase in the circulating plasma concentration of ANG II [27]. This increase was found to be due to the combined effects of a decrease in the metabolism of ANG II and an increase in the synthesis/secretion of ANG II [27]. Thus this lack of a change in systemic ANG II concentrations may reflect opposing effects of VIP infusion. The decreases in hepatic and plasma angiotensinogen levels, resulting from an increased VIP concentration in the portal circulation, should act to decrease ANG II, as would the decrease in...
ACE activity, while the increase in renin we observed over baseline would act to increase ANG II.

Thus we conclude that an increase in the portal VIP concentration down-regulates the intrahepatic renin–angiotensin system and some, but not all, elements of the circulating renin–angiotensin system. We suggest, therefore, that VIP released as a result of gastric sodium administration may act as the signal to the liver to decrease ACE activity and the synthesis of angiotensinogen and ANG I.

ACKNOWLEDGMENTS

We acknowledge the support of the National Health and Medical Research Council of Australia and the Ingham’s Medical Research Foundation for this project.

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Received 8 November 1999/20 March 2000; accepted 11 April 2000