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SODIUM DEPLETION DECREASES HEPATIC METABOLISM OF VASOACTIVE INTESTINAL PEPTIDE IN THE RABBIT

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SUMMARY

1. Reports that a greater natriuresis occurs after gastric rather than intravenous sodium loads suggest that a gastric sodium monitor exists which releases a humoral natriuretic factor. As vasoactive intestinal peptide (VIP) is natriuretic, it might act as this mediator. To determine whether it is released from the gut in response to sodium we measured VIP levels in portal and systemic plasma of anaesthetized rabbits after a gastric sodium load. Levels of VIP in systemic plasma were also measured in conscious rabbits after gastric and portal sodium loads to determine the contributions of anaesthesia or increased sodium concentration in the portal tract to any observed rise in systemic VIP levels.

2. In the anaesthetized rabbit study portal and systemic VIP levels had both increased significantly from control values by 5 min after the sodium load in the low salt diet group ($P < 0{\cdot}025$, portal; $P < 0{\cdot}05$, systemic). By 10 min the levels in systemic and portal plasma were equal.

3. In the conscious rabbits an increase in systemic VIP levels was observed in the group on a low salt diet after a gastric but not a portal sodium load.

4. We conclude that VIP is released in response to gastric sodium loads in rabbits on low salt diets and that hepatic metabolism of VIP is reduced in this group.

INTRODUCTION

To effect a natriuresis, the gastrointestinal or portal sodium monitor proposed by Lennane and confirmed by Carey (Lennane, Peart, Carey & Shaw 1975*a*; Lennane, Carey, Goodwin & Peart, 1975*b*; Carey, Smith & Ortt, 1976; Carey, 1978) would need to release a humoral or neural mediator. Vasoactive intestinal peptide (VIP), which has been reported to be released in response to lumenal instillation of saline in the canine ileum (Ebeid, Escourrou, Soeters, Murray & Fisher, 1977*a*) and is natriuretic in the rat (Rosa, Silva, Staff & Epstein, 1985) and rabbit (Dimaline, Peart & Unwin, 1983; Duggan & Macdonald, 1987), could fulfill this role. However other studies in which portal and systemic venous levels of VIP were measured simultaneously in response to a multiplicity of stimuli (Shaffalitzky de Muckadell, Fahrenkrug & Holst, 1977; Ebeid, Murray, Soeters & Fisher, 1977*b*; Bitar, Said, Weir, Saffouri & Makhlouf, 1980; Chijiiwa, Misawa & Ibayashi, 1986) show that significant

metabolism occurs in the liver and so the concentration of VIP reaching the kidney might be insufficient to effect a natriuresis.

As the differences in sodium excretion in response to gastric and intravenous saline were only observed in sodium-depleted rabbits and man (R. J. Lennane, personal communication), it is possible that VIP metabolism is altered by changes in body sodium status. To investigate this possibility, portal and systemic venous levels of VIP were measured following a gastric sodium load in male New Zealand White rabbits on diets with a normal or low sodium content.

To confirm that any effects observed were not the result of changes in hepatic blood flow or metabolism induced by anaesthesia, gastric sodium loads were given to the rabbits when conscious and VIP levels measured in systemic plasma. VIP levels were also measured in response to an intraportal sodium load in the conscious rabbits to determine whether changes in portal sodium concentrations might contribute to any increase in systemic VIP levels.

To determine whether the increase in VIP levels observed in the rabbits on a low salt diet in response to a gastric sodium load reflected a response to a non-specific stimulus such as volume or osmolality or was specific for sodium chloride, plasma concentrations of VIP were measured after intragastric instillation of 5·95% urea.

METHODS

Gastrostomy insertion

Twelve male New Zealand White rabbits were anaesthetized with 100 μg fentanyl (Janssen Pharmeceutica, French's Forest, Australia) intramuscularly and 2·5% halothane delivered in oxygen at a flow rate of 3 l min^{-1} via a non-rebreathing mask. The abdominal cavity was entered by a mid-line incision through the linea alba. A purse-string suture (4/0 silk) was placed in a non-vascular area of the body of the stomach, the gastrostomy tube was inserted through an incision in its centre and the suture closed leaving the larger Silastic segment in the gastric lumen. The distal end was then brought out onto the dorsum of the neck behind the ears via a subcutaneous tunnel and the abdomen closed.

One week after the operation the rabbits were randomly assigned to low salt (0·008%) or normal salt diets (2·2%, Labsure Animal Diets). During a 7 day equilibration period, the six rabbits in each group were placed in metabolic cages to allow urine collection and were allowed free access to their designated diets and distilled water *ad libitum*. Their food intake was recorded and their weights monitored every 2 days.

Experimental details

On the day of the experiment the rabbits were again anaesthetized and the abdominal cavity entered via a lower mid-line incision through the linea alba. A segment of the inferior mesenteric vein draining the right colon was isolated and ligatures placed around it. The catheter was inserted through the lumen of an introducing needle and advanced until its tip lay in the proximal portal vein. The introducing needle was withdrawn and the ligatures tied to maintain the catheter position. The muscle layer was then closed and a cannula inserted into an ear artery. The halothane was reduced to 2·0% in oxygen at 3 l min^{-1} and an interval of 1 h allowed before commencing sampling. Blood was sampled simultaneously from the ear artery and portal vein before administering a sodium chloride load (1·5 mmol kg^{-1} as 0·513 M-NaCl solution) via the gastrostomy and at 5, 10 and 30 min after. Blood was collected into pre-cooled tubes containing 50 units of sodium heparin and 100 units of Trasylol (a protinin, Bayer, Leverkusen, FDR) per millilitre, centrifuged immediately, frozen and stored at −20 °C until assayed.

Following completion of this study the distal end of the catheter was brought out onto the dorsum of the neck via a subcutaneous tunnel and the skin closed. The rabbits were then allowed a recovery period of 1 week before being returned to their designated diets. During the ensuing 7 days re-

equilibration period, their food intakes were monitored, their weights recorded and their urine collected for determination of sodium excretion.

For the studies in conscious rabbits, only systemic blood was sampled as it was not possible to obtain an adequate sample volume from the portal vein in conscious upright animals and discomfort was evident with sampling in these circumstances. Thus, on the day of the experiment, an ear artery cannula was inserted using lignocaine local anaesthesia and the rabbits were placed in restraint cages. They were fasted for 2 h, then a sodium load identical to that given in the previous protocol was administered into the gastrostomy tube or portal vein catheter (in random order). Blood was sampled from the ear artery cannula before the sodium and at 15 min, 30 min and 2 h afterwards for determination of VIP levels by radioimmunoassay. Following another 7 day re-equilibration period on their appropriate diet the rabbits received the sodium load by the alternate route.

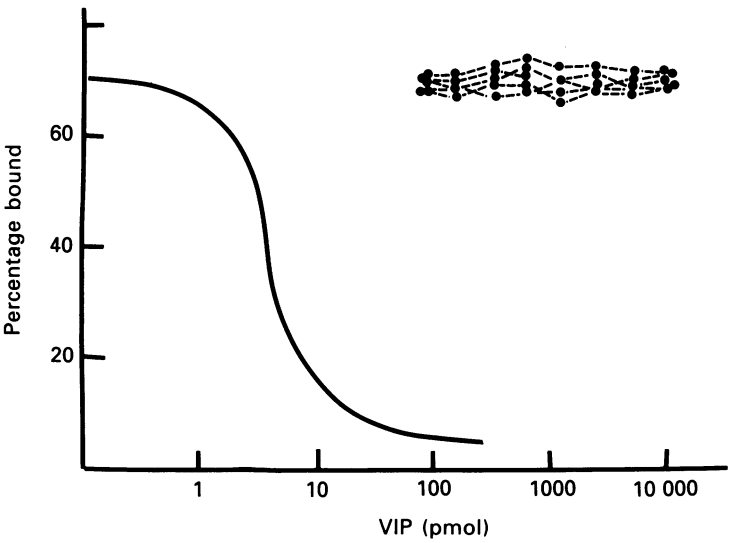

Fig. 1. Standard curve for vasoactive intestinal peptide (VIP) antiserum showing the lack of displacement of the radio-ligand [^{125}I]VIP by the structurally related peptides glucagon, secretin and gastric inhibitory peptide as well as the non-related peptides somatostatin and pancreatic polypeptide (dashed curves to right).

To determine whether the volume or osmolality of the sodium chloride solution rather than the specific ionic constituents were responsible for changes in plasma VIP concentrations observed following intragastric repletion, rabbits were re-equilibrated on their low salt diet and the gastric loading experiments repeated substituting 5.95% urea solution. This solution is isosmotic with the sodium chloride solution used and provides the same volume stimulus.

Radioimmunoassay

Antibodies were raised in guinea-pigs to VIP (Penninsula Laboratories) conjugated to rabbit serum albumin by carbodiimide condensation. The conjugate (equivalent to 20 nanomoles per guinea-pig) was emulsified in complete Freund's adjuvant (Diffco Laboratories, Detroit, USA) and administered by multiple intradermal injections under general anaesthesia for the primary inoculation. For subsequent inoculations, the conjugate was emulsified with incomplete Freund's adjuvant and administered by intraperitoneal injection at intervals of 6 weeks. Sera were obtained by cardiac puncture under general anaesthesia 10 days post-innoculation. Pooled antisera were used at a dilution of 1 : 12000 for the present study. The specificity of the antiserum was established using structurally related peptides (glucagon, gastric inhibitory peptide and secretin) as well as the non-related ones (somatostatin and pancreatic polypeptide) to inhibit [^{125}I]VIP (New England Nuclear) binding to the antiserum (see Fig. 1). Assay tubes were set up in duplicate, each with

200 μl unknown plasma or standard in 0·1 M-phosphate buffer (pH 7·6) containing 0·2% bovine serum albumin, 0·1% Triton X-100 (Ajax Chemicals, Sydney, Australia) and 0·01% sodium azide, 100 μl antibody and 50 μl [^{125}I]VIP (7000 c.p.m. (50 μl)$^{-1}$). After a 48 h incubation at 4 °C antibody-bound [^{125}I]VIP was separated by addition of 200 μl of 6% charcoal (Norit GSX) suspension followed by centrifugation at 3000 r.p.m. for 20 min. Under these conditions the detection limit of the assay (defined as that value able to be differentiated from the reagent blank with 95% confidence) was 5 pmol (l of plasma)$^{-1}$. The interassay coefficient of variation was 17·6% (eight replicate estimations) and the intra-assay coefficient of variation was 14·9% (eight estimations).

Statistical Methods

For the anaesthetized rabbit experiments VIP concentrations in portal and systemic plasma at 5, 10 and 30 min after sodium load were compared with their respective initial values using a paired t test, with P values of less than 0·05 being considered significant. In addition systemic and portal VIP concentrations at each time point were also compared by paired t test. Concentrations of VIP at 15, 30 and 120 min after the gastric or portal sodium or urea loads in the conscious rabbits were compared with their control values using a paired t test with P values of less than 0·05 being considered significant.

RESULTS

On the low sodium diet the mean food intake ($\pm$S.E.M.) was 98·72 $\pm$ 10·41 g day^{-1}, statistically indistinguishable from 92·09 $\pm$ 16·29 g day^{-1} for rabbits on the normal diet. The mean sodium intakes were thus 0·34 mmol day^{-1} for the low salt diet and 8·81 mmol day^{-1} on the normal diet. Whilst on the low salt diet the rabbits did not lose weight and at all stages of the experiments their weights were similar to those of rabbits on a normal salt diet, thus comparable sodium loads were given in each dietary group.

Response to gastric sodium loads in anaesthetized rabbits

Normal sodium diet

There was no significant change in rabbit weights, with a mean of 2·55 $\pm$ 0·08 kg at the commencement of the diet and 2·55 $\pm$ 0·02 kg on the experimental day. Sodium balance was attained 3 days before the experiment. (Fig. 2, lower panel).

Portal concentrations of VIP increased non-significantly after administration of the sodium load while the systemic levels initially decreased non-significantly before returning to baseline values (see Fig. 3, lower panel).

Low sodium diet

The rabbits had a mean weight at the commencement of the low salt diet of 2·56 $\pm$ 0·13 kg and a mean weight of 2·66 $\pm$ 0·14 kg on the day of portal catheter insertion and experiment. Sodium balance was achieved 3 days before the study (see Fig. 2, upper panel).

VIP levels increased in both portal and systemic plasma in response to the gastric sodium load. Portal levels rose from 13·25 $\pm$ 4·83 to 22·33 $\pm$ 5·02 pmol l^{-1} 5 min after administration of the sodium ($P < 0·025$), remained elevated at 10 min and had almost returned to baseline by 30 min (see Fig. 3, upper panel). The levels of VIP in systemic plasma had also increased significantly by 5 min, from 9·16 $\pm$ 3·80 to 15·55 $\pm$ 3·70 pmol l^{-1} ($P < 0·05$). By 10 min the levels in portal and systemic plasma were comparable and remained so at 30 min.

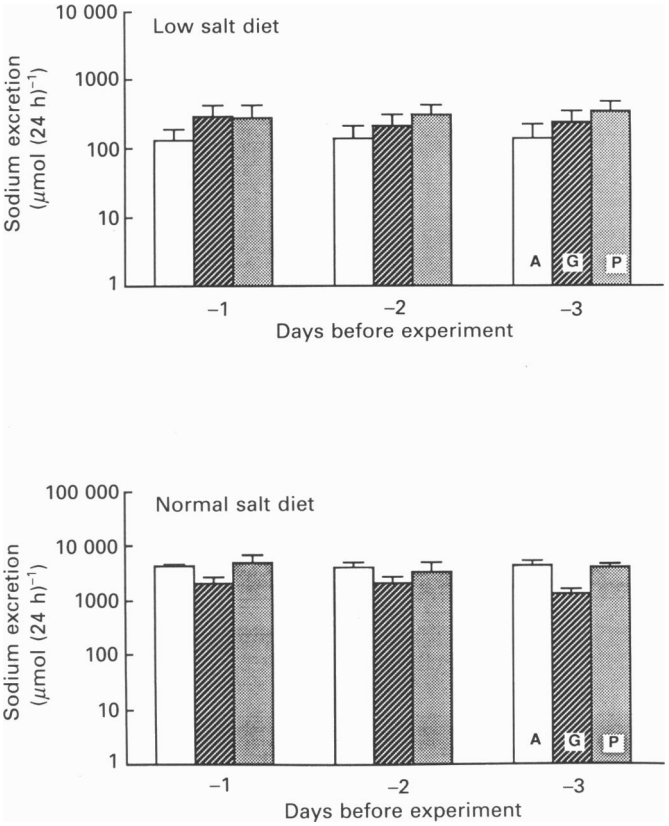

Fig. 2. Twenty-four-hour sodium excretions for the three days before experiment, upper panel for rabbits on the low sodium diet and lower panel for those on a normal diet. A, gastric sodium loading under anaesthesia; G, gastric sodium loading in conscious rabbits; P, portal sodium loading. Bars show S.E.M., $n = 6$.

Response to portal and gastric sodium loading in conscious rabbits

Again the rabbit weights were similar in both dietary groups and the animals had attained sodium balance before each study (see Fig. 2).

Normal salt diets

There was no significant change in the level of VIP in systemic plasma after either intraportal or intragastric sodium loading (see Fig. 4; lower panel).

Low salt diet

Plasma VIP levels had increased significantly from the control value of $12{\cdot}95 \pm 1{\cdot}93$ to $53{\cdot}50 \pm 16{\cdot}11$ pmol l^{-1} at 15 min after the gastric sodium load ($P < 0{\cdot}05$). The levels remained elevated 120 min after the load. In contrast there was no significant change in the levels of VIP in systemic plasma after intraportal instillation of sodium (see Fig. 4, upper panel).

Response to urea

No significant change in plasma VIP concentration from the baseline value of $10{\cdot}55 \pm 1{\cdot}41$ pmol l^{-1} was observed following intragastric administration of 5·95% urea solution over the 2 h sampling period.

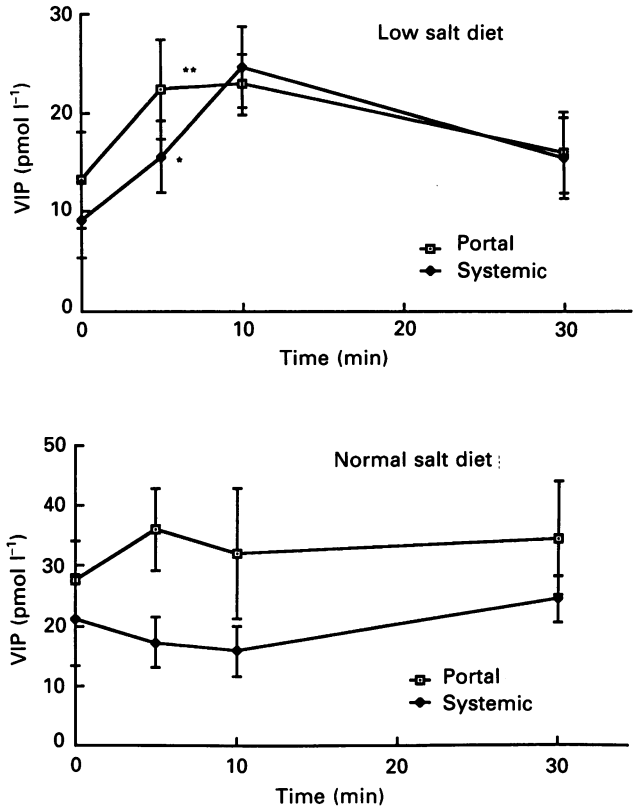

Fig. 3. Concentrations of VIP in portal and systemic plasma at 5, 10 and 30 min after a gastric sodium load in rabbits on a low salt diet (upper panel) and a normal salt diet (lower panel). Bars indicate S.E.M. * indicates a significant increase from control, $*P < 0{\cdot}05$ and $**P < 0{\cdot}025$, $n = 6$).

DISCUSSION

The studies in the anaesthetized rabbits on low sodium diets show that VIP is released into the portal circulation in response to a gastrointestinal sodium load, and VIP may therefore be considered a possible mediator for the gastric sodium monitor proposed by Lennane *et al.* (1975*a*, *b*).

Previous reports of VIP release after stimuli such as the intravenous infusion of calcium (Ebeid *et al.* 1977*b*), oxytocin and neostigmine (Bitar *et al.* 1980), ileal instillation of bile (Chijiiwa *et al.* 1986) and electrical stimulation of the vagus (Shaffalitzky de Muckadell *et al.* 1977) have shown that although VIP concentrations

increased significantly in portal plasma, its levels in systemic plasma did not. This led to suggestions that VIP was extensively metabolized in the liver, which were later confirmed by clearance studies of VIP infused intraportally and intravenously in whole animals (Chayvialle, Rayford & Thomson, 1981) and in the isolated

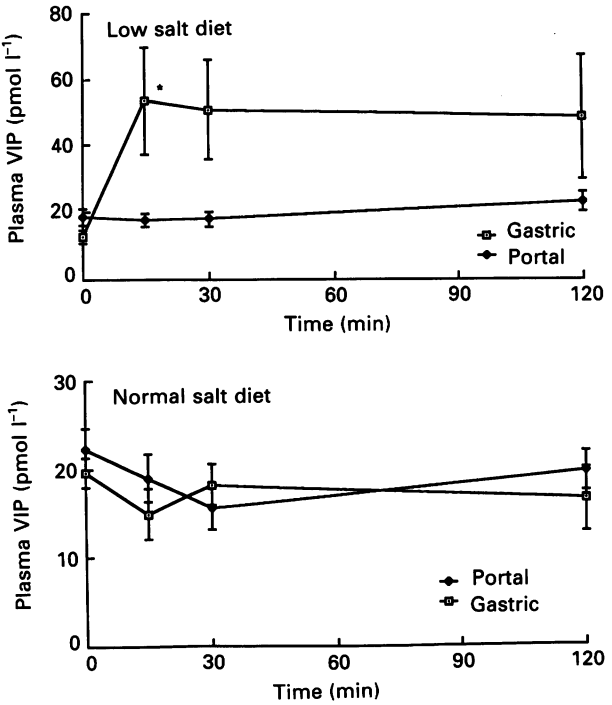

Fig. 4. VIP levels in systemic plasma at 15, 30 and 120 min following intragastric and intraportal sodium loads in rabbits on low salt diets (upper panel) and normal salt diets (lower panel). Bars indicate S.E.M. * indicates a significant increase from control, $P < 0{\cdot}05$, $n = 6$.

perfused liver (Misbin, Wolfe, Morris, Buynitzky & McGuigan, 1982). These studies demonstrated that 70–80% of VIP is removed on passage through the liver. Thus, although VIP is released from the gut into the portal circulation by a number of stimuli, there was no corresponding change in systemic plasma concentration nor were systemic effects observed because of high hepatic clearance of the peptide.

All these studies were performed in sodium-replete animals and VIP concentrations seen in the sodium-replete group of rabbits in our study are consistent with these reports. In contrast, in our rabbits on a low sodium diet, systemic plasma VIP concentrations increased significantly after the sodium load. In addition, these concentrations closely approximated those attained in portal plasma, a situation not predicted by the clearance studies referred to above. These data then suggest that hepatic metabolism of VIP has been reduced by dietary sodium restriction leading to higher systemic plasma VIP concentrations than expected.

The studies in the conscious rabbits confirmed that VIP in systemic plasma increased significantly after gastric sodium load. The increase observed in the

conscious rabbits was more marked than that observed in the anaesthetized rabbit study although the difference was not statistically significant. The reason for this is not apparent from our study but it may reflect a longer period on the low sodium diet. Thus anaesthesia has been excluded as a possible cause for the decrease in hepatic metabolism implied by the study in anaesthetized rabbits which had been on a low sodium diet. Further, the lack of response in systemic levels of VIP to the portal administration of sodium excludes increased portal sodium concentrations as a cause of the increased systemic levels after the gastric sodium load. This increase in VIP concentration appears to be a response specific to sodium chloride since an isosmolar solution of urea produced no such change. Further, the concentrations of VIP achieved in systemic arterial plasma were comparable to those we have previously shown to be natriuretic when VIP was infused directly into the rabbit renal artery (Duggan & Macdonald, 1987).

We conclude that gastric instillation of sodium chloride causes VIP release from the intestine in rabbits maintained on a low sodium diet, a setting in which a decreased hepatic metabolism of VIP is observed. We suggest that these observations provide circumstantial evidence that VIP acts as a humoral mediator for the gastric sodium monitor proposed by Lennane (Lennane *et al.* 1975*a*, *b*).

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