THE INFLUENCE OF GASTROINTESTINAL PEPTIDES ON RENAL FUNCTION, WITH PARTICULAR REFERENCE TO SODIUM AND WATER EXCRETION

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by

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ABSTRACT

The renal effects of 12 "gut-brain" peptides have been studied: Gastrin (as pentagastrin), Cholecystokinin (CCK8), Glucagon, Vasoactive intestinal polypeptide (VIP), Secretin, Neurotensin (NT), Substance P (SP), Motilin, Met-enkephalin, Pancreatic polypeptide, Somatostatin and Bombesin. Experiments were performed in conscious rabbits and man.

The original premise, based on evidence for a gastrointestinal or portal sodium monitor, was that one or a combination of these peptides might be involved in the renal adaptive response to changes in dietary composition; they could act through altered circulating levels in response to food, or local release from renal nerves (VIP, NT and SP).

The major observations were:

i. Pentagastrin exerted a vasopressin-like action in the rabbit and produced natriuresis in man, with effects resembling the K^+-sparing diuretics, triamterene and amiloride.

ii. In the rabbit, CCK8 decreased plasma calcium concentration and was unusual in producing vasodilatation and suppression of plasma renin. In man, its effects were similar to pentagastrin.

iii. VIP increased heart rate and stimulated renin release in both rabbit and man, but increased electrolyte excretion in the former and reduced it in the latter. The renin response was attenuated by indomethacin, but unaffected by propranolol, suggesting prostaglandin dependence. The tachycardia was abolished by propranolol, indicating beta-adrenoreceptor mediation.

iv. NT caused antinatriuresis in the rabbit, but primarily reduced

chloride excretion in man. These effects occurred at post-prandial NT plasma levels as reported in man.

The physiological and clinical relevance of these results are discussed. Apart from NT, changes occurred at supra-physiological plasma concentrations, but seem important because they were dose-related; some peptides are released locally from renal nerves and elevated plasma levels are found in disease states associated with abnormal renal function.

It is concluded that the renal actions of these peptides may be important in health and disease, and are worthy of further study.

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Collation, analysis and presentation of all data obtained, were the work of the author.

PREFACE

In the Introduction to this thesis, particular reference is made to what is known of the regulation of renal sodium excretion, the recognition of the gastrointestinal tract as an endocrine organ and the ubiquitous nature of the gut peptides: Their functions are largely unknown and their actions incompletely characterised. This is followed by descriptions of the experimental protocols used in studying the conscious rabbit and man, and a discussion of potential problems with data interpretation. Subsequent chapters consist of a brief introductory description of each peptide and its actions, followed by the experimental results and their potential physiological, pharmacological and pathological significance. The results in man are presented and discussed in the light of the observations in the conscious rabbit. Each chapter is followed by a summary. The final chapter is a general discussion and overview of the work, together with comments on possible future areas of interest.

RELEVANT PUBLISHED WORK.

Some of the work presented in this thesis has appeared in the following publications (listed by chapter):

GORDON, D., PEART, W.S., RODDIS, S.A. & UNWIN, R.J. (1981). Renal function during pentagastrin infusion in conscious rabbits. J. Physiol. 310, 40-41P.

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CALAM, J., PEART, W.S., SINGH, J. & UNWIN, R.J. (1982). Renal function during vasoactive intestinal polypeptide (VIP) infusion in man. J. Physiol. 332, 96-97P.

CALAM, J., DIMALINE, R., PEART, W.S., SINGH, J. & UNWIN, R.J. (1983). Effects of vasoactive intestinal polypeptide (VIP) on renal function in man. J. Physiol. (In press).

CALAM, J., DIMALINE, R., PEART, W.S. & UNWIN, R.J. (1982). Vasoactive intestinal polypeptide (VIP) and renin release in the conscious rabbit. J. Physiol. 329, 66P.

CALAM, J., DIMALINE, R., PEART, W.S. & UNWIN, R.J. (1983). Studies on the renin response to vasoactive intestinal polypeptide (VIP) in the conscious rabbit. Br. J. Pharmacol. 80, 13-15.

BLOOM, S.R., PEART, W.S. & UNWIN, R.J. (1982). Neurotensin and renal function in the conscious rabbit. J. Physiol. 332, 95-96P.

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CHAPTER 1

INTRODUCTION

(REGULATION OF RENAL SODIUM EXCRETION)

Regulation of renal sodium excretion.

"One fact is certain, the kidney knows how to regulate sodium excretion and we do not." (Orloff & Burg, 1971).

Over 30 years ago, Homer Smith (1951a) stated that "Changes in sodium excretion may be brought about in two ways:- (1) by changes in the distal load of sodium effected by changes in the rate of filtration; (2) by changes in the rate of (distal?) reabsorption under hormonal control. The maintenance of salt and water balance probably involves both factors.".

Normally, the kidney is able to vary sodium output between values of over 1,000 and less than 1 mmol per day, without significant fluctuations in the filtered load (Swales, 1975a). It appears that sodium balance or related factor, such as extracellular fluid volume, governs renal sodium excretion, but the actual mechanism is not clear.

In the intact animal, it is impossible to divorce the control of salt and water excretion from that of extracellular fluid volume. Thus, control may be considered in terms of afferent and efferent limbs. Factors involved in each limb are listed in Table 1.1. The efferent factors are better understood and will be considered first.

The Efferent Limb:

Peritubular Physical Factors

The rate of fluid absorption from the proximal tubule and possibly loop of Henle is likely to be influenced by changes in renal arterial, venous and ureteral hydrostatic pressures, together with haematocrit and plasma protein changes and their effects on colloid oncotic pressure within peritubular capillaries (Earley & Schrier,

Afferent limb:

Central volume receptors - venous

- arterial

Cerebrospinal fluid receptors

Portal vein/hepatic (splanchnic) receptors

Efferent limb:

Physical factors (peritubular)

Glomerular filtration rate

Hormones - aldosterone

- natriuretic hormone(s)

Renal nerves

Table 1.1. Afferent and efferent mechanisms in the control of extracellular fluid volume.

1973). Passage through the glomerulus and filtration, results in an increased plasma protein concentration within the peritubular capillaries which favours passive tubular reabsorption. Although there is now evidence that absolute peritubular oncotic and hydrostatic pressures, rather than the pressure difference across tubular epithelium, modulate tubular reabsorption (Earley & Schrier, 1973). Changes in filtration fraction (the ratio of glomerular filtration rate to renal plasma flow) are associated with parallel changes in peritubular plasma oncotic pressure and thereby alterations in tubular handling of sodium and water (Windhager, Lewy & Spitzer, 1969). It is now believed that such changes in peritubular capillary hydrostatic and oncotic pressures may not affect the proximal tubule in all circumstances, but are likely to be important influences in the loop of Henle. There is little evidence that these factors alter distal tubular function (Knox, Mertz, Burnett & Haramati, 1983).

Glomerular Filtration and Tubular Flow

As the filtered load of sodium (product of glomerular filtration rate and plasma sodium concentration) is so large (approx. 1,000 mmol hr^{-1}), it is clear that minor changes in glomerular filtration rate will require significant changes in tubular reabsorption to compensate. This relative stability of sodium excretion in the face of these changes is known as glomerulo-tubular balance (Orloff & Burg, 1971; Reineck & Stein, 1978). Proximal tubular reabsorption of sodium depends on flow rate rather than luminal concentration, as the latter does not change unless altered by a nonresorbable solute such as mannitol (Giebisch & Windhager, 1964). It is therefore

closely related to glomerular filtration rate (Brenner, Bennett & Berliner, 1968). The mechanism of glomerulo-tubular balance in the proximal tubule probably involves the so-called "physical factors" discussed earlier and reabsorption of organic solutes such as glucose and amino acids which are linked to sodium reabsorption (Burg, Patlak, Green & Villey, 1976). In contrast, distal tubular reabsorption of sodium is directly related to luminal concentration rather than flow rate (Good & Wright, 1979).

Glomerular filtration is linked with tubular reabsorption by another mechanism known as tubulo-glomerular feedback (Wright, 1981a). This governs filtration rate according to the composition of distal tubular fluid. The stimulus at the macula densa (point of contact between distal tubule and respective glomerulus) may involve changes in luminal chloride concentration (Wright, 1981b; Briggs, 1981): a rise in concentration leading to a fall in glomerular filtration. This negative feedback is thought to occur through local production of angiotensin-II, resulting in afferent arteriole vasoconstriction and a reduction in glomerular filtration (Blantz, Steiner & Tucker, 1981).

Hormones

A recent monograph and earlier review (Brenner & Stein, 1979; Katz & Lindheimer, 1977) have extensively discussed the actions of various hormones, including the sex hormones, on renal function. Of the hormones listed in Table 1.2, only parathyroid hormone and aldosterone have well established effects on renal sodium handling. Parathyroid hormone (or parathormone) inhibits proximal tubular reabsorption of sodium and water through an effect mediated by cyclic adenosine monophosphate (Dousa, 1979). It is believed that earlier

Aldosterone

Angiotensin

Parathyroid hormone

Calcitonin

Thyroid hormones

Growth hormone

Prolactin

Oestrogens

Progesterone

Glucagon

Insulin

Catecholamines

Prostaglandins

Kallikrein-kinin system

Table 1.2. Hormones reported to influence renal function (based on Katz & Lindheimer, 1977; Brenner & Stein, 1979).

reports of increased glomerular filtration rate and renal vasodilatation were due to vasoactive contaminants of parathyroid extracts (Slatopolsky, Hruska, Martin & Freitag, 1979), although there has been a recent report of synthetic parathormone producing coronary vasodilatation (Crass & Pang, 1980). Aldosterone increases sodium reabsorption in the distal tubule and collecting duct by increasing permeability and adenosine triphosphate (ATP)-dependent Na^+/K^+ exchange (Feldman, Funder & Edelman, 1972; Mulrow & Forman, 1972).

Renal Nerves

The sympathetic nervous system may influence renal salt excretion in 3 ways: extrarenally by changes in systemic haemodynamics, intrarenally by changes in renal haemodynamics and directly by alteration of tubular sodium transport (Schrier & de Wardener, 1971a,b). Noradrenergic nerve endings have been identified in contact with proximal and distal tubules (Barajas, 1978). It has proved difficult to differentiate experimentally between direct tubular effects and those due to systemic and renal haemodynamic changes. In man and dogs, the transplanted kidney appears to function normally (Swales, 1975b; Gottschalk, 1979). Although the evidence for a direct tubular effect is conflicting, the balance seems to favour such an action during efferent renal nerve stimulation (Gottschalk, 1979).

The Afferent Limb:

Volume Receptors

Only one afferent mechanism is fairly well established experimentally and that is the presence of central (venous) low

pressure volume receptors. Their exact location is unknown, but on histological grounds they are thought to exist within the atria and pulmonary interstitium (Paintal, 1973; Gauer, 1978). Moreover, the largest portion of the blood volume is contained within the distensible venous circulation and is therefore well suited to this role. Their existence was originally suggested by McCance (1936) and their action well demonstrated by the work of Epstein (1978) involving water immersion which redistributed blood volume from periphery to thorax. This volume stimulus produces a marked salt and water diuresis.

Evidence for their location within the arterial circulation comes from the effects of sudden closure of arterio-venous fistulae in animals and man (Epstein, Post, McDowell, 1953; Holman, 1962; Davis, Urquhart, Higgins, Rubin & Hartroft, 1964). The resulting increase in salt and water excretion is apparently independent of changes in glomerular filtration rate and renal blood flow (Epstein et al., 1953).

Cerebral Receptors

The existence and role of such receptors in the control of sodium excretion is very difficult to ascertain. Despite evidence that raising cerebrospinal fluid sodium concentration in a variety of species increases sodium excretion (Andersson, Dallman & Olsson, 1969; Dorn, Levine, Kaley & Rothballer, 1969; Chiu & Sawyer, 1974) and that infusion of hypertonic saline into the carotid arteries produces the same (Thornborough, Passo & Rothballer, 1973), these manoeuvres are so gross and unphysiological (often associated with increases in glomerular filtration rate and blood pressure) that further experimental work is warranted before an adequate assessment

of their significance can be made. However, some recent studies in sheep do lend indirect support to the belief that cerebral receptors may sense physiological changes in sodium status. This work involved first confirming the natriuretic response to intraventricular (third ventricle) injection of saline and then demonstrating attenuation, and even abolition of the response, by producing sodium loss through a parotid fistula. Loss of only 54-75 mmol of sodium in the hour preceding injection clearly blunted the natriuretic response, suggesting that a sodium deficit of as little as 50-70 mmol can have a physiological effect (Denton, 1982).

Splanchnic Receptors

Since Haberich (1968) proposed the existence of liver or portal osmoreceptors over a decade ago, the subject has attracted a great deal of experimental attention with conflicting results (Sawchenko & Friedman, 1979). The hypothesis was based on the observation that a larger diuretic response to intravenously infused water was obtained in rats following portal vein infusion compared with systemic (femoral) vein infusion. Stimulation of hepatic baroreceptors, which are also believed to exist, was thought unlikely as flow rates were well within the range over which portal flow does not influence urine output. Similar results were obtained when sodium free glucose and mannitol solutions were used as osmotic stimuli suggesting true osmoreception. Alongside this grew the belief that sodium receptors rather than osmoreceptors existed (Sawchenko & Friedman, 1979). A brisk natriuresis was reported in response to portal vein infusion of hypertonic saline compared with femoral vein infusion (Daly, Roe & Horrocks, 1967; Passo, Thornborough & Rothballer, 1973). This was thought to indicate an hepatic site for production of de Wardener's

natriuretic hormone (Daly et al., 1967; de Wardener, 1977). Circumstantial evidence for antidiuretic hormone release in response to a hyperosmolar stimulus was also found (Sawchenko & Friedman, 1979). These receptors were thought to act through vagal afferents as increased nervous activity was found in response to sodium stimulation and section of the hepatic branch of the vagus nerve abolished the response (Passo et al., 1973; Horner Andrews & Orbach, 1974).

Others have not been able to confirm the existence of such receptors and many explanations have been put forward for this, including differing rates of infusion, minor differences in protocol and species variation (Sawchenko & Friedman, 1979). It may be relevant that experiments in support of sodium receptors were performed in anaesthetised animals and a recent study in conscious dogs failed to find any difference in the renal response to portal and caval saline infusions (Kapteina, Motz, Schwartz-Porsche & Gauer, 1978).

More recently, attention has been turned to the existence of a gastrointestinal sodium monitor, after the observation in the conscious rabbit and man, that an equivalent saline load given intravenously or orally, was more rapidly excreted after oral administration (Lennane, Peart, Carey & Shaw, 1975; Lennane, Carey, Goodwin & Peart, 1975). Once again, evidence is conflicting, with almost as many publications for and against the existence of such a receptor or monitor (Table 1.3). These conflicting observations cannot be satisfactorily explained by differences in protocol alone. However, these studies have been concerned with the renal response to a sodium load; few have addressed themselves to the mechanism of

IN SUPPORT OF	AGAINST OR INCONCLUSIVE
Lennane, Peart, Carey & Shaw, 1975	Gordon & Peart, 1979
Lennane, Carey, Goodwin & Peart, 1975	Hanson, McLane-Vega, Childers, Gleason & Schneider, 1980
Carey, Smith & Ortt, 1976	Obika, Fitzgerald, Gleason, Zucker & Schneider, 1981
Carey, 1978	

Table 1.3. Authors of reports for and against the existence of a gastrointestinal sodium monitor.

antinatriuresis following sodium deprivation and even among these, there is no clear consensus (Anderson & Linas, 1978). The initial rapid fall in renal sodium excretion cannot be explained by changes in plasma electrolytes, osmolality, protein concentration, renin and aldosterone, extracellular fluid volume or glomerular filtration rate (Anderson & Linas, 1978; Gordon, James, Peart & Wilson, 1978; Moss, Gordon, Forsling, Peart, James & Roddis, 1981). In relation to the differential natriuresis discussed above, Carey (1978) has suggested that a natriuretic gastrointestinal hormone such as glucagon may play a role. The corollary may also apply, which is that one or a combination of gut peptides may be part of the signal influencing renal sodium excretion in response to dietary changes such as salt restriction, leading to antinatriuresis.

The integration of the factors (afferent and efferent) involved in renal sodium handling is not understood. The possibility of an entero-renal axis is attractive, for a gut peptide may act as both signal and effector, by virtue of intrinsic renal actions (direct tubular) or systemic effects involving haemodynamic changes and reflex sympathetic responses.

Gut and Brain Neuropeptides

The discovery of secretin (Bayliss & Starling, 1902) at the turn of the century, challenged the Pavlovian dogma that digestive glands were solely controlled by the nervous system and established the concept of "chemical messengers" (hormones) involved in the function of many organs. Thereafter, progress in gut endocrinology was slow. Design of valid experiments was difficult because of alteration in function produced by necessary and often extensive surgical procedures and inadequate extraction and purification of the active

substance and because of failure to recognise the widely distributed endocrine cells. In 1953, Feyrter described a system of "clear cells" scattered throughout the gastrointestinal tract secreting substances which acted locally and termed "paracrine". In 1968, Pearse suggested that these cells were derived from neuroectoderm and could be classified together under their common cytochemical characteristics which he entitled APUD (amine precursor uptake and decarboxylation). While this concept has proved helpful by unifying the heterogeneous gut endocrine system, and providing a stimulus to much fruitful neuroendocrine research, it is clear that not all endocrine cells are APUD and that many are of endodermal origin (Andrew, 1982). The criterion now for grouping together these various cell types should perhaps be the production of so-called "regulatory peptides".

Since 1974, the subject has come full circle and there is now much evidence to indicate that the gut endocrine system and brain are closely linked (Powell & Skrabanek, 1979; Bloom, 1980). Several biologically active peptides are present in both gut endocrine cells and central and peripheral neurones (Larsson, 1980; Dockray & Gregory, 1980; Hökfelt, Johansson, Ljungdahl, Lundberg & Schultzberg, 1980). This suggests that the same molecule may function in unrelated systems such as a circulating gut hormone or central neurotransmitter. Table I.4 lists neuropeptides found in both gut and brain.

The role of many of these peptides is not fully understood even in relation to gastrointestinal function, and the actions of at least one well established gut hormone, namely cholecystokinin, are currently being re-defined in terms of its various molecular forms

Adrenocorticotrophic hormone (ACTH)

Bombesin

Cholecystokinin octapeptide (CCK8)

Beta-endorphin

Gastrin

Glucagon

Insulin

Leucine (leu-) enkephalin

Methionine (met-) enkephalin

Motilin

*Neurotensin

Pancreatic polypeptide

Secretin

*Somatostatin

*Substance P

*Vasoactive intestinal polypeptide (VIP)

Table 1.4. Putative neuropeptide transmitters found in gut and brain (based on articles by Powell & Skrabanek, 1979; Hökfelt, Johansson, Ljungdahl, Lundberg & Schultzberg, 1980; Brownstein, 1980; Roberts, Crow & Polak, 1981; Kelly, 1982). *Immunoreactivity against these peptides has been found within renal nerve fibres (Hökfelt, Schultzberg, Elde, Nilsson, Terenius, Said & Goldstein, 1978; Forssman, Hock & Metz, 1982).

(c.f. gastrin) and distribution (Rehfeld, 1980). Advances have been made in gastrointestinal physiology, but have only served to reveal the complexities of control mechanisms and the innumerable interactions between these peptides. Although at a cellular level the actions of a given peptide in gut and brain may be identical, it is possible that their effects are quite different i.e., development has been in parallel and independent rather than necessarily indicating a gut-brain interaction. That such an interaction does exist is suggested by the observations that intraperitoneal and intravenous injection of some of these peptides can produce satiety (Koopmans, 1981). They include cholecystokinin, pancreatic polypeptide, bombesin, substance P and neurotensin. The validity of these observations is difficult to assess, as many extraneous influences may inhibit feeding in conscious animals. These peptides are not thought to cross the blood-brain barrier, but to indirectly influence central nervous function by effects on peripheral sensory nerves, such as the vagus (Smith, Jerome, Cushin, Eterno & Simansky, 1981).

The kidney is involved in the metabolism of several gut peptides, including cholecystokinin, gastric inhibitory peptide, glucagon, pancreatic polypeptide and substance P (Go, 1978; Lefebvre, Luyckx & Nizet, 1974; Boden, Master & Owen, 1978; Campbell & Ward, 1979). This could be important in health and disease, as the kidney may be a specific target for such peptides.

OBJECTIVES OF THIS THESIS.

The possibility of "communication" between the gut and kidneys is an appealing concept. Thus, considering all the circumstantial evidence cited, but in the absence of specific clues from what is already known of these neuropeptides, a systematic study of the haemodynamic and renal effects of 12 gastrointestinal peptides was undertaken (Table 1.5). Such a series of experiments would also characterise further the actions of these peptides and could reveal a mechanism for the renal response to changes in dietary composition.

As the overall effect of these peptides may be to produce changes in behaviour, thus perhaps affecting nature and quantity of food eaten and activity, experiments were performed in conscious animals. Renal function is significantly disturbed by anaesthesia which makes interpretation of results and extrapolation to normal physiology difficult (Swales, 1975b; Gottschalk, 1979). Rabbits were used because the small size of the rabbit facilitates handling and preparation for repeated studies. Also, litter-mates can be studied which minimises inter-rabbit variations in response and adequate statistical analysis may be performed on repeated observations in a small number of animals. Different rabbits for each peptide study were chosen rather than trained conscious dogs because of the evidence that renal responses in conscious dogs may be more readily conditioned (Corson, O'Leary Corson, Dykman, Peters, Reese & Seager, 1962; Corson, O'Leary Corson & England, 1963) and therefore fail to reflect true renal actions, if any, of infused substances. Where significant effects have been observed in the conscious rabbit, further studies have been conducted in man.

Table 1.5 (legend overleaf).

Peptide	No.of aa	Mol.wt.	Distribution Brain	GN	GE	Probable mode of action	Amino acid sequence similarities with
Gastrin (human)	34	3839					
	17	2098	+-		+	H	Cholecystokinin
Cholecystokinin (porcine)	39	4675					
	33	3918					
	8	1143					
	4	612	+	+	+	H, NT	Gastrin
Secretin (porcine)	27	3056	+-		+	H	Glucagon,VIP,GIP & PHI
Glucagon (pancreatic)	29	3550	+	+	+	H, NT	Secretin,VIP,GIP & PHI
VIP (porcine)	28	3326	+	+	+	NT	Glucagon,Secretin,GIP & PHI
Neurotensin (bovine)	13	1673	+	+	+	H, NT	Amphibian xenopsin
Substance P (bovine)	11	1348	+	+	+	NT	Bombesin
Bombesin (amphibian)	14	1620	+	+	+	NT, ? P	Substance P
Enkephalin (-met,-leu)	5	574	+	+	+	NT, ? H	Opioid family
Pancreatic polypeptide (bovine)	36	1348	+-	+	+	H	NPY and PYY
Motilin (porcine)	22	2700	+-		+	H	Not known
Somatostatin (bovine)	14	1640	+	+	+	H, NT, P	Fish urotensin II

Table 1.5.

Properties and distribution of the gut regulatory peptides studied. Species in brackets refer to number of amino acids and molecular weight. Abbreviations are as follows: GN, gut nerves; GE, gut endocrine cells; H, hormonal; NT, neurotransmitter; P, paracrine; VIP, vasoactive intestinal polypeptide; GIP, gastric inhibitory peptide; PHI, peptide histidine isoleucine; NPY and PYY, neuropeptide Y and peptide YY; +- indicates unconfirmed reports (adapted from Bloom & Polak, 1980; Polak & Bloom, 1983).

CHAPTER 2

GENERAL METHODS

General Methods.

Animal Experiments (studies in conscious rabbits)

Animal experiments were performed on male Sandy Half-lop rabbits (body wt. 3-5 kg). For each peptide, studies involved 4 or 5 rabbits. Where possible litter-mates were studied. Few rabbits were used to examine more than one peptide and these were only re-studied after a minimum period of three months rest.

For all peptides, each rabbit underwent 4 separate infusions at intervals of seven days. Experimental order was determined by a randomised Latin square (4 rabbits) or random block (5 rabbits) design (Armitage, 1977a, 1977b).

Experimental Procedure

The rabbits were allowed free access to food (RHM, Labsure Animal Diets) and water until 3 hours before each experiment. A marginal ear vein and central artery were cannulated with heparinised and tapered polyethylene cannulae (PP60) under lignocaine local anaesthesia. The bladder was catheterised per urethram with a size 10 Fr self-retaining (3 ml balloon) Foley catheter lubricated with lignocaine local anaesthetic gel (Figs. 2.1 and 2.2).

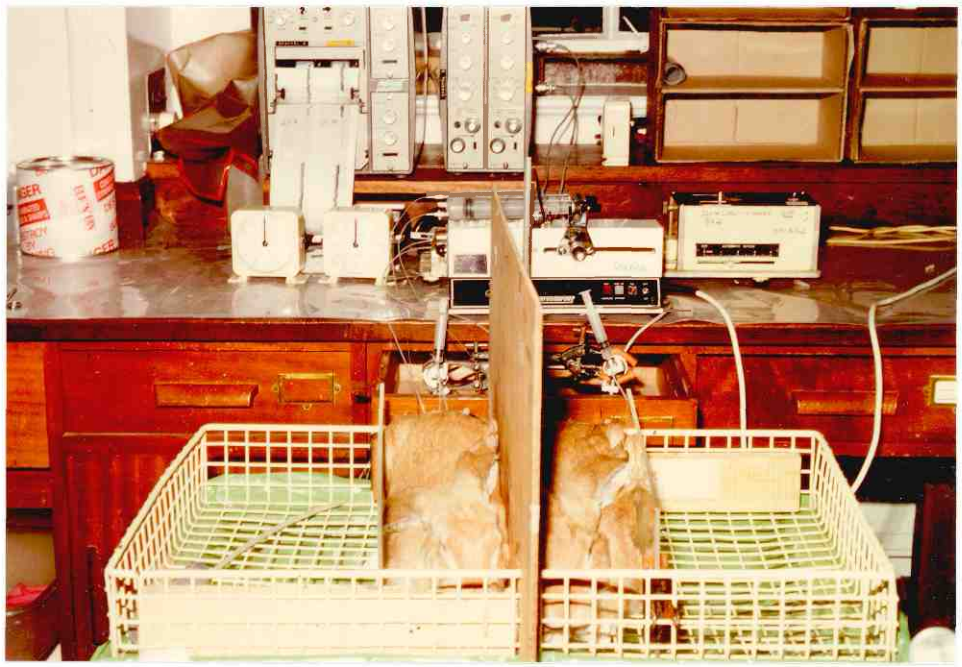
After cannulation, the rabbit rested quietly on a metal grid beneath which urine could be collected. To prevent turning, the animal was lightly restrained between 2 vertical partitions (height 16 cm) approximately 25 cm apart. One hour elapsed before the first blood sample (P_0) was taken from the arterial cannula, which was then connected to a pressure transducer (Bell & Howell). An intravenous fluid load of 25 ml kg^{-1} of 0.28 M glucose (isotonic) was given over 20 minutes. This was followed by a continuous infusion of





Fig. 2.1.

Photographs showing the prepared and cannulated central ear artery and marginal vein of one rabbit and the self-retaining urinary catheter in position.



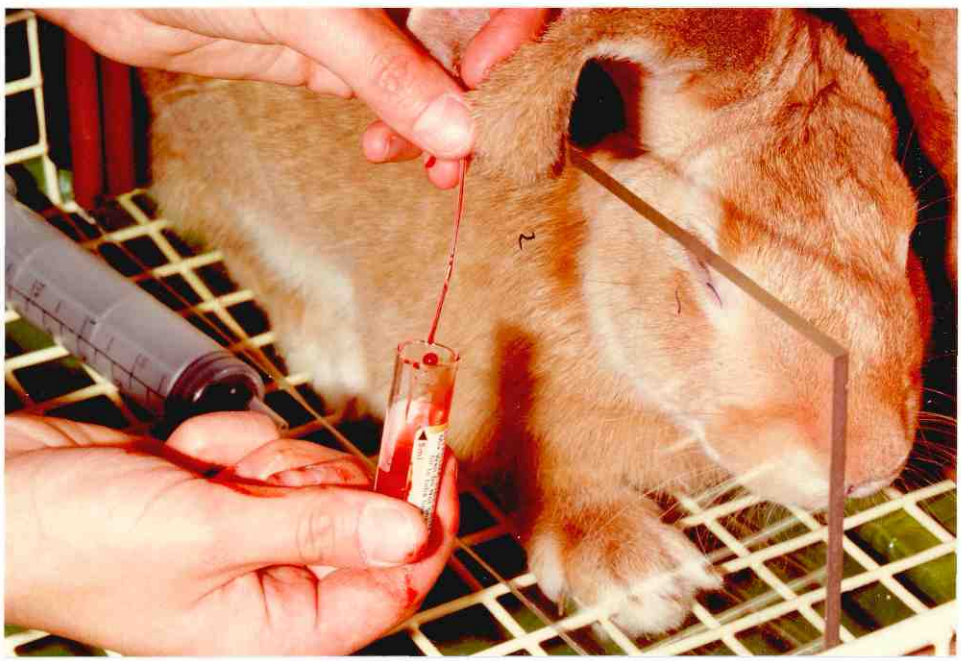


Fig. 2.2

Photographs of 2 rabbits during an infusion and of one rabbit being bled via its arterial cannula.

0.57 ml min^{-1}, to promote urine flow. A loading dose of the radioactive compounds (^{51}Cr) ethylene diaminetetra-acetate (EDTA; 74 kBq) and (^{125}I) sodium p-aminohippurate (Hippuran, 148 kBq) in 0.5 ml 0.154 M saline (physiological), was administered via the venous cannula. This was followed by a continuous infusion of EDTA (0.74 kBq min^{-1}) and Hippuran (1.48 kBq min^{-1}) in 0.28 M glucose.

According to the pre-determined order, each rabbit received the peptide at either of 3 different rates of infusion, or an infusion of the vehicle alone (control), intravenously over 115 minutes. The infusion rates were chosen in an effort to span the range from physiological, through pharmacological to toxicological and based on previously published work. Each dose of peptide was stored frozen in 0.05 M acetic acid solution diluted to the appropriate concentration immediately prior to use, unless otherwise stated. The vehicle consisted of 0.1 g 100 ml^{-1} rabbit albumin and 0.01 M acetic acid in 0.28 M glucose, pH ca. 5.0.

Twenty minutes were allowed for the EDTA and Hippuran to reach plateau concentrations in plasma before the bladder was washed out with 10 ml deionised water and the first timed urine collection begun. Eight urine collections were made, each of 20 minutes duration, with intervals of 10 minutes. Arterial blood samples were taken at the mid-point of each collection period. Systemic blood pressure and heart rate were recorded continuously by a Devices pen recorder (Model M2, frequency response > 100 Hz). The bladder was flushed with 5 ml deionised water just before the end of each collection and 10 ml at the end of each interval; all interval-urine was discarded. Infusion of peptide or vehicle alone, was begun 5 minutes before the start of the third urine collection until the end

of the sixth urine collection. A single blood sample was taken between the fifth and sixth collections for estimation of the plasma concentration of the peptide under study (see Fig. 2.3). These samples were taken into pre-chilled tubes containing aprotinin (400 *KIU/ml) and EDTA (1 mg/ml), immediately centrifuged at 4°C and the plasma stored at -20°C until thawed for assay.

Human Experiments (studies in normal volunteers)

Each project was approved by the local Ethics Committee and 6 healthy volunteers (usually male) aged 22 to 35 years gave informed consent. For 3 days before each study, subjects abstained from alcohol, smoking and vigorous exercise and ate a diet containing 2100 Calories, 150 mmol sodium (as sodium chloride) and 2.5 litres of water per day, unless otherwise stated. They then fasted overnight and before 08.30 hrs on the day of study, a peripheral vein in each arm was cannulated with either a 16 or 18 gauge Teflon cannula (Abbocath, Abbott). The 16 gauge cannula was used to take blood samples, and the 18 gauge cannula for infusions. During each study, subjects stood to pass urine every 30 minutes, but were otherwise recumbent. Between 08.00 and 08.30 hrs, an oral water load of 10 ml kg^{-1} was given. Thereafter, subjects drank water at a rate equal to their urine flow during the preceding 30 minutes. Sodium para-amino hippurate (PAH) diluted with normal saline (120 mg ml^{-1}) was given intravenously as a bolus of 800 mg at 09.00 hrs, followed by a continuous infusion of PAH (26.7 mg min^{-1}). The peptide under study was infused between 11.00 and 12.30 hrs in a vehicle consisting of

*Kallikrein Inactivator Units

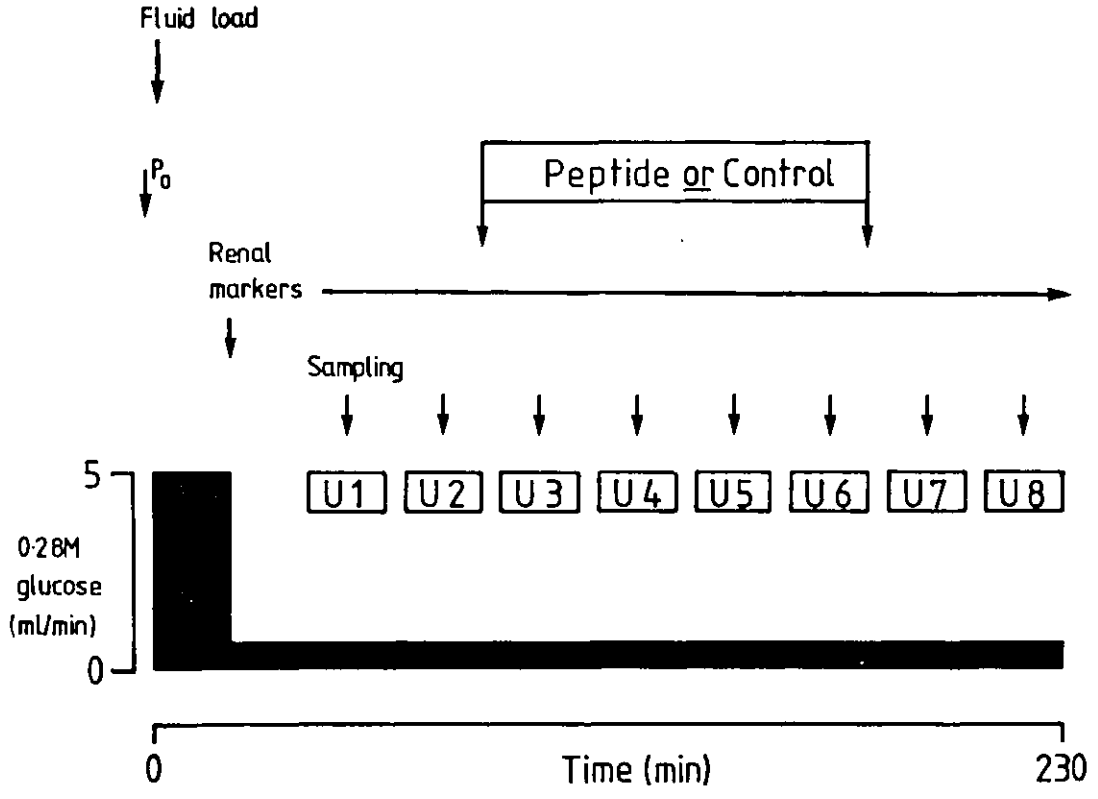


Fig. 2.3.

Diagram of rabbit infusion protocol as described in the text.

50:50 v/v Haemaccel (degraded gelatine 35 g l^{-1}, average molecular weight 35,000) and 0.28 M glucose at a rate of 13.33 ml hr^{-1}. This vehicle, without peptide, was infused at the same rate during control periods between 10.00 and 11.00 hrs and between 12.30 and 13.30 hrs. At the mid-point of each 30 minute urine collection period, blood was withdrawn and the heart rate and blood pressure recorded by an automatic self-inflating sphygmomanometer (Dinamapp, Criticon, Florida, U.S.A.). When required, facial skin temperature was measured in ^{o}C using a thermocouple (Bright Laboratories, Brighton) placed 2 cm. lateral to the left palprebral fissure. The protocol is outlined in Figure 2.4.

Laboratory analyses

Sodium and potassium concentrations in plasma and urine were measured by flame photometry (Corning Flame Photometer, *C.V. < 1%, n = 20), chloride by a potentiometric method (Corning Chloride Analyser, C.V. < 2%, n = 20) and osmolality cryoscopically (Advanced Osmometer, C.V. ca. 1%, n = 20). Plasma glucose was estimated using the oxygen rate method of Kadish, Litle and Sternberg (1968) (Alpha Laboratories, C.V. ca. 1% at 10 mmol l^{-1}, n = 40). Urine pH was recorded at room temperature with a Russell pH glass electrode. Calcium and phosphate in plasma and urine were measured colorimetrically by the autoanalyser methods of Ripoll (1976) and Robinson, Roughan and Wagstaff (1971) respectively. Urine samples were acidified to pH < 2 with concentrated nitric acid before estimation of calcium. C.Vs. based on Wellcontrol quality control

*C.V. is intra-assay coefficient of variation

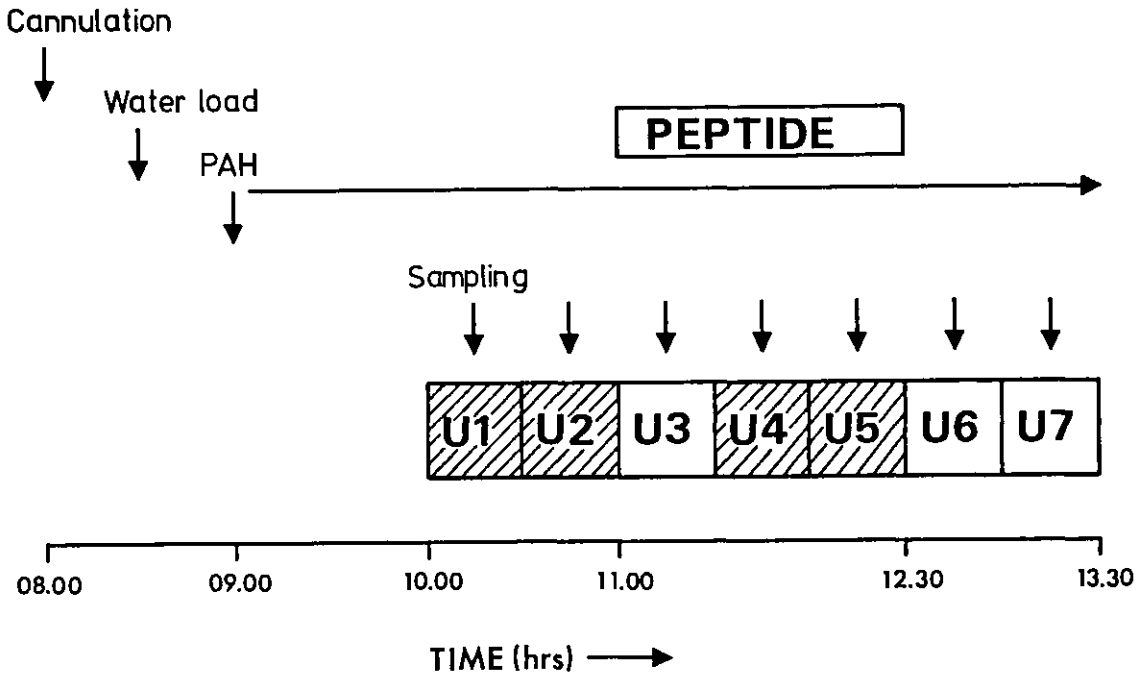


Fig. 2.4.

Diagram of human infusion protocol as described in the text. In the case of pentagastrin, an extra urine collection was made during infusion, but as in the other peptide studies, only the observations made during the last 2 collections were compared with those made during the 2 basal collections (hatched).

sera (Wellcomtrol One, 2.3 - 2.6 and 1.3 - 1.9 mmol l^{-1} for calcium and phosphate respectively; 2.4%, n = 28 and 8.4%, n = 76 respectively).

Arterial plasma solids (PS), used as a measure of plasma protein concentration (O'Connor, 1962) were determined by drying weighed plasma samples to constant weight at 120°C (C.V. ca. 1%, n = 20) and subtracting the weight of crystalloids, calculated from the corresponding plasma sodium concentration. Haematocrit was estimated as the mean of 2 measurements with a microhaematocrit centrifuge (Hawksley). In the rabbit studies, radioactivity of 0.5 ml samples of arterial plasma and urine were counted with a Packard Auto-Gamma counter. Glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) were calculated from the clearances of EDTA and Hippuran respectively. In the human studies, creatinine was measured colorimetrically as creatinine alkaline picrate (Beckman Creatinine Analyser 2, C.V. ca. 3%, n = 20) and PAH by the single channel autoanalyser method described by Harvey & Brothers (1962) (C.V. 2.1%, n = 15 and 0.7%, n = 23 for low and high standards respectively). ERPF and GFR were estimated from PAH and endogenous creatinine clearances respectively. Plasma renin activity was measured by radioimmunoassay of angiotensin-I generated from its endogenous substrate (Sever, Peart, Davies, Tunbridge & Gordon, 1979). Aldosterone was measured by radioimmunoassay. Plasma peptide concentrations were measured by radioimmunoassay and details will be given in a later section. Filtration fraction (FF) was calculated as GFR/ERPF. In the human studies, renal blood flow (RBF) was calculated from ERPF and haematocrit, assuming renal PAH extraction of 90%, thus:

$$RBF = \frac{ERPF \times (100/90)}{1 - \text{haematocrit}}$$

The concentration of colloids in the plasma of the efferent glomerular arteriole (post-glomerular plasma solids: PGPS) was calculated as PS/(1-FF) (Bresler, 1956). Fractional excretion (FE_x) was calculated from each filtered load (F_x, product of GFR and plasma concentration) and corresponding renal excretion rate (U_xV), thus:

$$FE_x = \frac{[1 - (F_x - U_xV)]}{F_x} \times 100$$

Sixty and 80% of plasma calcium and phosphate respectively, were assumed to be filterable at the glomerulus. Mean arterial blood pressure was calculated as 2/3 diastolic pressure plus 1/3 systolic pressure.

Data analysis

Rabbit studies:

The data were analysed by a 2 or 3-way analysis of variance (random block or Latin square designs respectively) with weight and time as covariates; $P < 0.05$ was considered significant. A fixed effects model was applied (Colquhoun, 1971a) using all data and computations performed with the GLIM3 statistical programme (Generalised Linear Interactive Modelling: Baker & Nelder, 1978) on the Digital pDp 11 computer. In the case of significant differences, comparisons with the control were made using Dunnett's (1964) multiple comparison test. Before analysis, Bartlett's test (Snedecor & Cochran, 1967) was used to assess homogeneity of variance for each

variable and logarithmic transformations applied where appropriate. In these cases, geometric mean values and approximate standard error of mean (S.E. of mean) are given. All transformed variables are indicated in tables and figures. The results presented are based on observations made during the fourth, fifth and sixth urine collections, when a steady state had been reached during peptide or vehicle infusion (Fig. 2.3).

Human studies:

A priori, it was intended to compare observations made during the basal collection periods 1 and 2, with those obtained during collections 4 and 5 (or 5 and 6 in the case of pentagastrin infusions; see diagram of protocol, Fig. 2.4) when a steady state had been achieved during peptide infusion. This data was analysed by Student's paired t-test, performed as an analysis of variance (Colquhoun, 1971b), using the Statistical Package for Social Sciences (Nie, Hull, Jenkins, Steinbrenner & Bent, 1975); $P < 0.05$ was considered significant. Data presented are based on observations made during collections 1 and 2 ($n = 12$) and during collections 4 and 5 ($n = 12$). Results are expressed as mean and standard deviation (S.D.). As with the rabbit data, Bartlett's test was applied before analysis and logarithmic transformations performed as necessary.

Modification of general methods as applied to individual peptides

1. Pentagastrin (Peptavlon, ICI)

Four rabbits each received infusions of pentagastrin at rates of 0.1, 1.0 and 10 μg kg^{-1} min^{-1}. Infusion order was determined according to a random block design.

When the effect of pentagastrin infusion on renal handling of water was realised, some plasma samples were used for arginine vasopressin estimation. This necessitated assay of samples originally taken for measurement of plasma solids, but which had been taken under suitable conditions i.e., pre-chilled commercial tubes containing lithium heparin. This is the reason for the small number of observations for arginine vasopressin and plasma solids in this study (Chapter 3.1).

Plasma arginine vasopressin concentrations were measured by radioimmunoassay according to the method of Lightman & Forsling (1980).

Data were analysed by 2-way analysis of variance with weight and time as covariates.

2. Sulphated cholecystokinin octapeptide (CCK8)

Four rabbits received infusions of CCK8 (Cambridge Research Biochemicals) at rates of 5, 50 and 450 pmol kg^{-1} min^{-1}.

Each dose of CCK8 was stored frozen in 0.05 M ammonium bicarbonate plus 0.1 g 100 ml^{-1} rabbit albumin, then thawed and diluted to the appropriate concentration immediately prior to use. The vehicle consisted of 0.1 g 100 ml^{-1} rabbit albumin and 0.01 M ammonium bicarbonate in 0.28 M glucose.

Rises in plasma CCK8 concentration during infusions were measured by radioimmunoassay using C-terminal specific CCK/gastrin antibody L48 (Calam, Ellis & Dockray, 1982). Basal immunoreactivity was 90-114 pmol l^{-1} using this assay, but could all be attributed to gastrin, as measured using antibody 1296 which has about 30 times greater cross-reactivity with gastrin than CCK.

3. Pancreatic glucagon (glucagon hydrochloride, Novo)

Five rabbits received glucagon infusion of 1, 10 and 1000 ng kg^{-1} min^{-1} and 2 separate vehicle (control) infusions in a random block design. The vehicle (diluent) included glycerin 23 mg, methyl hydroxybenzoate 1 mg and propyl hydroxybenzoate as preservatives, per mg of glucagon in deionised water. An equivalent amount of diluent alone (without glucagon) was given during control infusions. Further dilutions were made as required with 0.28 M glucose.

Data from the control infusion were pooled and all data analysed by 2-way analysis of variance with weight and time as covariates.

Changes in plasma pancreatic glucagon concentration were measured by radioimmunoassay using a C-terminal specific antibody (Christofides, 1982a).

4. Vasoactive intestinal polypeptide (VIP, synthetic porcine)

Four rabbits received infusions of VIP (Cambridge Research Biochemicals) at rates of 1, 10 and 25 pmol kg^{-1} min^{-1}.

Changes in plasma VIP concentration were measured by radioimmunoassay using an N-terminal specific antibody (Dimaline & Dockray, 1978).

5. Secretin (hog, Kabi Diagnostica)

Five rabbits received infusions of secretin at rates of 5, 50 and

100 mCU (*Clinical Units) kg^{-1} min^{-1}. The vehicle (diluent) included l-cysteine hydrochloride 1 mg and mannitol 20 mg per 75 CU of secretin in 7.5 ml of 0.154 M saline. An equivalent amount of diluent alone (without secretin) was used for control infusions. Further dilutions were made as required.

Data were analysed by 2-way analysis of variance, with weight and time as covariates.

Changes in plasma secretin concentration were measured by radioimmunoassay (Schaffalitzky de Muckadell & Fahrenkrug, 1977).

6. Neurotensin (synthetic bovine)

Four rabbits received infusions of neurotensin (Cambridge Research Biochemicals) at rates of 2, 20 and 200 pmol kg^{-1} min^{-1}.

Changes in plasma neurotensin concentration were measured by radioimmunoassay using antibody directed towards the mid- to C-terminal region of the neurotensin molecule (Blackburn, 1982).

7. Substance P (synthetic bovine)

Four rabbits received infusions of substance P (Cambridge Research Biochemicals) at rates of 2, 20 and 200 pmol kg^{-1} min^{-1}.

Attempts to measure substance levels in rabbit plasma were unsuccessful.

*Activity is > 3000 CU per mg. 1 CU was originally defined as the amount of a secretin preparation required to produce a response in man. The most sensitive bioassay system for secretin is the cat pancreas: 1 Hammersten cat unit being the amount of secretin which produces a pancreatic response of 0.1 ml of 0.1 M bicarbonate when injected intravenously (Häcki, 1980). 1 CU of secretin is identical with 20 Hammersten cat units.

8. Somatostatin-14 (synthetic bovine)

Four rabbits received infusions of somatostatin (Cambridge Research Biochemicals) at rates of 3, 30 and 250 pmol kg^{-1} min^{-1}.

Changes in plasma somatostatin concentration were measured by radioimmunoassay using a C-terminal specific antibody which also reacts fully with somatostatin-28 (O'Shaughnessy, 1982).

Plasma arginine vasopressin levels were determined by radioimmunoassay (Lightman & Forsling, 1980).

9. Pancreatic polypeptide (synthetic human)

Four rabbits received infusions of pancreatic polypeptide (Cambridge Research Biochemicals) at rates of 1, 10 and 25 pmol kg^{-1} min^{-1}.

Changes in plasma pancreatic polypeptide concentration were measured by radioimmunoassay (Adrian, 1982).

10. Bombesin (amphibian)

Four rabbits received infusions of bombesin (courtesy of Professor Gregory, Liverpool) at rates of 0.06, 0.6 and 6 pmol kg^{-1} min^{-1}.

Changes in plasma bombesin concentration were measured by radioimmunoassay using a C-terminal specific antibody (Ghatei, 1982).

11. Motilin (13nle)

Four rabbits received synthetic 13nle-motilin (Fluka Chemicals) at rates of 1, 10 and 100 pmol kg^{-1} min^{-1} .

Changes in plasma motilin concentration were measured by radioimmunoassay using an N-terminal specific antibody (Christofides, 1982b).

12. Methionine (met-) enkephalin

Four rabbits received methionine enkephalin (Fluka Chemicals) at rates of 10, 100 and 1000 ng kg^{-1} min^{-1}.

Changes in plasma met-enkephalin concentration were measured by radioimmunoassay (Clement-Jones, Lowry, Rees & Besser, 1980a).

Each dose of met-enkephalin was stored frozen in 0.5 ml aliquots of 0.154 M saline plus 0.1 g 100 ml^{-1} rabbit albumin, then thawed and diluted to the required concentration with 0.28 M glucose immediately before infusion.

Plasma arginine vasopressin levels were determined by radioimmunoassay (Lightman & Forsling, 1980).

Potential difficulties with data interpretation

The value of obtaining dose-response data in studies of this sort is not only in an effort to distinguish potential physiological from pharmacological effects, but to strengthen the conclusion that an observed change is real rather than a chance occurrence. A dose-dependent change in a variable is far more likely to reflect a real effect of an infused substance than an isolated change at a single dose. However, there are potential drawbacks even with dose-response data. If a substance has a biphasic action e.g., direct renal tubular effects at low doses and additional renal haemodynamic effects at higher doses, two overlapping dose-response curves may exist. It is therefore possible to choose (by chance) doses which "straddle" these two relationships. In which case, the response may not be typical or progressive. Moreover, such a response pattern may indicate any or a combination of the following:

1. A truly biphasic action.
2. Random variation (although less likely if the changes are systematic).
3. Undetectable changes in a related variable.

This problem really applies to changes in renal electrolyte excretion and whether or not they reflect direct renal tubular, or renal haemodynamic effects. Estimation of ERPF and GFR by clearance methods is subject to an error of between 5 and 10% (O'Connor, 1962). Thus a 5% (undetectable) change in GFR may result in a more than 100% increase in renal sodium excretion (O'Connor, 1962). But again, if the change in renal electrolyte excretion is systematic, it is less likely to be due to unrecorded changes in GFR. Moreover, the derived

variable of fractional electrolyte excretion minimises the changes in renal electrolyte excretion due to measured alterations in GFR. This is really only of value in distinguishing between tubular and haemodynamic effects if the changes in GFR and fractional excretion are in opposite directions.

Even with these difficulties of interpretation, studies of this sort can provide useful information concerning the renal effects of infused peptides and can act as a pointer for future work. It should also be remembered that statistical analysis of small samples is such that differences must be large before they are considered statistically significant (Iliff, 1983).

An important difference between the rabbit and human studies described in this thesis is that in the latter, few subjects are prepared to undergo repeated study, particularly if it entails prolonged dietary restrictions and venous cannulation. Renal studies are found to be particularly arduous by many. As a compromise, single infusions are performed, recognising fully the inherent difficulties of data interpretation, the major one being any trend or change with time. In an effort to improve discrimination, "off-on-off" infusions are carried out, with control periods before and after peptide infusion. Only if changes during peptide infusion return towards pre-infusion values after infusion, is it safe to regard such a change as peptide-related. If changes persist, interpretation and significance depends upon prior knowledge and expectation, both dangerous sources of bias. It is best to treat such effects with caution, but not to ignore them.

Finally, any error in the estimate of GFR will invalidate the derived variable of fractional excretion. This is most likely to

occur at low urine flow rates, particularly in the human experiments where incomplete collections and carry-over of urine into the next collection period are possible. At least in the rabbit studies, this problem can be minimised by continuous drainage of urine via a catheter and bladder washouts with deionised water at the end of each collection. In all these studies common sense has to be applied as there is no objective means of discarding suspect observations, or so-called outliers (Armitage, 1978c). In the studies to be reported, clearly abnormal estimates of GFR have been excluded.

CHAPTER 3

PART 1:

RENAL FUNCTION DURING PENTAGASTRIN INFUSION IN CONSCIOUS RABBITS

Renal function during pentagastrin infusion in conscious rabbits.

INTRODUCTION

Much controversy surrounded the early history of gastrin, which began with the observation by Edkins (1905) that intravenous injections of antral mucosa extracts produced acid secretion in cats; he gave the name gastrin to the active principle. The fact that these extracts also contained histamine was the source of much disagreement and confusion; even the results of Komarov (1938) with histamine-free extracts were contested. The matter was eventually settled by Gregory and Tracy (1964) when they isolated gastrin from hog antral mucosa.

Several forms of gastrin are known to exist which differ according to the number of peptide residues. These are G34, G17 and G14, of which G17 is the most potent (on a molar basis) and is the predominant form in tissue (Walsh & Kum Lam, 1980). G34 is thought to be the main circulating form, (G34:G17, 2:1), although levels of both G17 and G34 rise after meals (G34:G17, 1:1; Walsh & Kum Lam, 1980). The C-terminal tetrapeptide of gastrin has all the biological actions of the whole molecule and is shared by cholecystokinin (Gregory, 1974). Both these peptides have been found in central and peripheral neurones, although cholecystokinin (mainly as the octapeptide) is present in greater amounts and is more widely distributed (Rehfeld, Goltermann, Larsson, Emson & Lee, 1979). Although controversial, the C-terminal tetrapeptide of gastrin and cholecystokinin ("tetrin") has recently been reported to exist as a distinct entity within gut endocrine cells and neurones (Rehfeld, 1981).

The haemodynamic changes observed after feeding in dogs (Fronek & Stahlgren, 1968) and in particular the increases in renal blood flow and sodium excretion, have been attributed to changes in circulating levels of gastrointestinal hormones, and more specifically, gastrin (Reinhardt, Kaczmarczyk, Fahrenhorst, Blendinger, Gatzka, Kuhl & Riedel, 1975). These authors (Gatzka, Reinhardt, Fahrenhorst, Kaczmarczyk, Riedel & Kuhl, 1975) have taken this further by infusing pentagastrin (synthetic pentapeptide consisting of the C-terminal tetrapeptide amide of gastrin plus beta-alanine, Fig. 3.1) intravenously in conscious dogs and reported an increase in renal blood flow.

The present study examined the renal effects of intravenously infused pentagastrin in conscious rabbits at doses of 0.1, 1.0 and 10 $\mu g\ kg^{-1}\ min^{-1}$.

RESULTS

Systemic blood pressure and heart rate (Table 3.1)

These variables were largely unaffected by pentagastrin infusion, although mean arterial blood pressure fell by approximately 10 mm Hg during infusion of the low dose (0.1 $\mu g\ kg^{-1}\ min^{-1}$) and heart rate increased by approximately 30 beats min^{-1} during infusion of the middle dose (1.0 $\mu g\ kg^{-1}\ min^{-1}$). No significant changes occurred during infusion of the high dose (10 $\mu g\ kg^{-1}\ min^{-1}$).

Renal haemodynamics (Fig. 3.2)

No significant changes occurred in ERPF, GFR and filtration fraction during pentagastrin infusion.

Pentagastrin

BOC - Ala - Trp - Met - Asp - Phe - NH_2

Mol. wt. 768

Sulphated cholecystokinin octapeptide (CCK8)

Asp - Tyr(SO_3H) - Met - Gly - Trp - Met - Asp - Phe - NH_2

Mol. wt. 1143

Fig. 3.1. Amino acid sequences and molecular weights of the structurally related (C-terminal homology) peptides, pentagastrin and CCK8.

Table 3.1. Effects of pentagastrin on mean arterial blood pressure, heart rate and renal electrolyte excretion.

	Control (vehicle only)	Pentagastrin in $\mu g\ kg^{-1}\ min^{-1}$ 0.1 (low)	1.0 (middle)	10 (high)	n
BP (mm Hg)	84 ± 4	*72 ± 3	84 ± 2	87 ± 4	11
HR (beats min^{-1})	228 ± 12	246 ± 8	*260 ± 10	249 ± 15	11
$^{+}U_{Na}V$ ($\mu mol\ min^{-1}$)	6.6 ± 1.4	6.9 ± 1.5	*3.4 ± 0.7	8.1 ± 1.6	11
$^{+}U_{K}V$ ($\mu mol\ min^{-1}$)	5.0 ± 0.8	7.2 ± 1.2	6.2 ± 1.0	5.8 ± 0.6	11
$^{+}U_{Cl}V$ ($\mu mol\ min^{-1}$)	6.0 ± 0.9	5.8 ± 1.0	4.6 ± 0.8	6.5 ± 0.8	11
$^{+}U_{Ca}V$ ($\mu mol\ min^{-1}$)	1.01 ± 0.31	0.38 ± 0.13	0.64 ± 0.26	0.68 ± 0.21	10

Values are expressed as mean ± S.E. of mean. $^{+}$Transformed variable, in which case the geometric mean and approximate S.E. of mean are given. n = number of observations. * and ** signify $P < 0.05$ and < 0.01 respectively, when compared with the control value by Dunnett's test after analysis of variance. Abbreviations are as follows:- BP, mean arterial blood pressure; HR, heart rate; $U_{Na}V$, $U_{K}V$, etc., renal excretion.

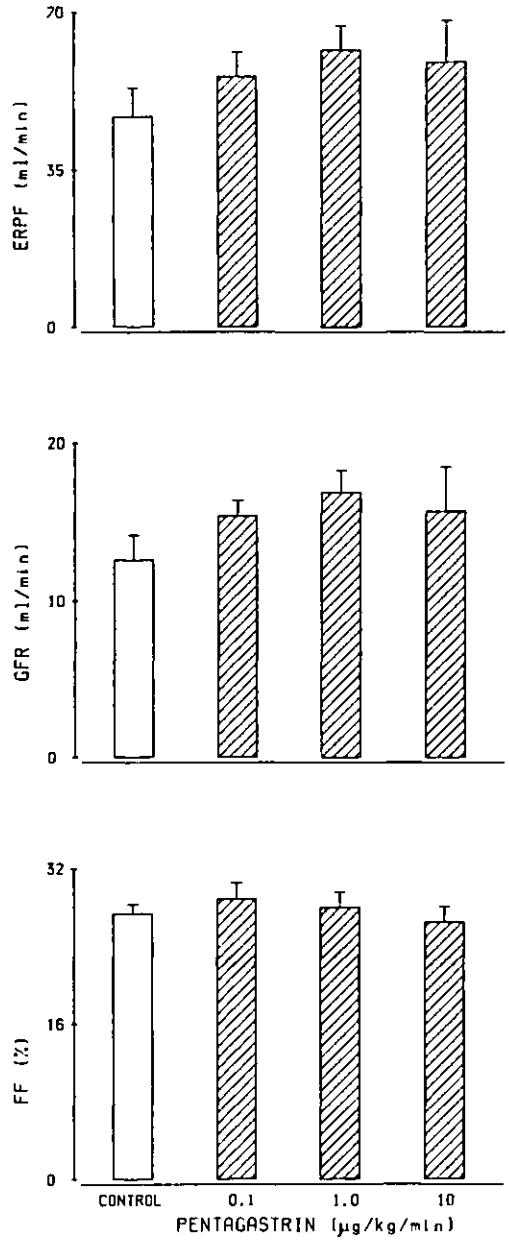


Fig. 3.2.

Effects of increasing doses of pentagastrin on effective renal plasma flow (ERPF), glomerular filtration rate (GFR) and filtration fraction (FF). Results are expressed as mean ± S.E. of mean, n = 11 for each treatment group.

Urine composition (Table. 3.1)

There was no significant alteration in renal electrolyte excretion, although sodium excretion fell during infusion of the middle dose. A dose-related fall in urine flow and free water clearance did occur, but little change in osmolar clearance (Fig. 3.3). These changes began to recover after pentagastrin infusion (Fig. 3.4).

Plasma composition and haematocrit (Table 3.2)

Little change in plasma electrolyte concentration occurred, except for a small reduction in plasma potassium concentration and a slight increase in plasma calcium concentration at the middle dose. Haematocrit, plasma solids and PGPS all appeared to rise slightly, but only a small number of observations were made. Plasma renin activity (PRA) and arginine vasopressin (AVP) concentration did not change significantly at any dose of pentagastrin.

DISCUSSION

Pentagastrin produced significant dose-related changes in urine flow and free water clearance. Osmolar clearance also increased a little, but this change was not statistically significant. These effects are similar to those of vasopressin (Jamison, 1981) and suggest either a direct vasopressin-like action of pentagastrin on the kidney, or stimulation of vasopressin release from the posterior pituitary. The hypothalamus and pituitary are outside the blood-brain barrier and accessible to systemically infused substances (Guyton, 1976a). Moreover, gastrin as G17 has been found in both lobes of the pituitary and may be involved in the regulation of pituitary hormone release (Rehfeld, 1978; Larsson & Rehfeld, 1981).

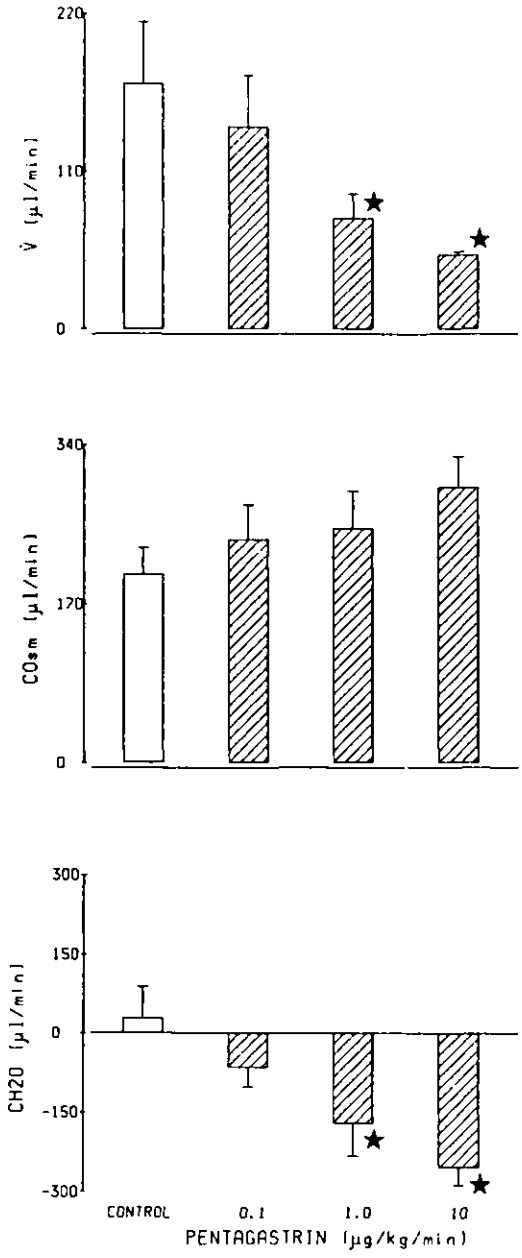


Fig. 3.3.

Effect of increasing doses of pentagastrin on urine flow rate ($\dot{V}$), osmolar clearance (C_{Osm}) and free water clearance (C_{H_2O}). These variables were found to be lognormally distributed and therefore values are given as geometric means ± approximate S.E. of mean, n = 11 for each treatment group. * indicates significant difference ($p < 0.05$) from control value as determined by Dunnett's test after analysis of variance.

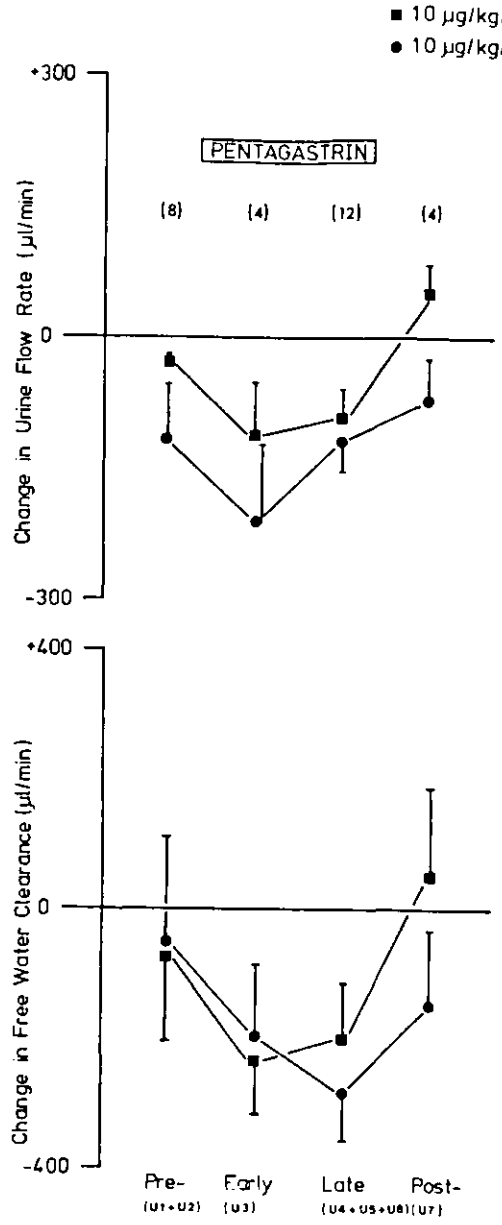


Fig. 3.4.

Effect of the middle and high doses of pentagastrin on urine flow and free water clearance expressed as the difference from the corresponding control value. Each fell during pentagastrin infusion, but began to recover after the infusion ended. These variables were lognormally distributed and therefore results are presented as geometric mean ± approximate standard error. For ease of presentation, infusions are divided into 4 periods and the observations made during each collection pooled as shown in brackets. Numbers in parentheses indicate n of observations for each period.

Table 3.2. Effects of pentagastrin on plasma composition and haematocrit.

	Control (vehicle only)	Pentagastrin in $\mu g\ kg^{-1}\ min^{-1}$ 0.1 (low)	1.0 (middle)	10 (high)	n
P_{Na} ($mmol\ l^{-1}$)	138.3 ± 0.5	140.6 ± 1.0	138.2 ± 0.4	138.3 ± 1.5	11
P_{K} ($mmol\ l^{-1}$)	3.97 ± 0.02	4.06 ± 0.05	*3.62 ± 0.06	3.64 ± 0.10	11
P_{Cl} ($mmol\ l^{-1}$)	98.2 ± 1.6	97.7 ± 1.1	95.7 ± 1.0	97.8 ± 1.0	11
P_{Ca} ($mmol\ l^{-1}$)	2.57 ± 0.14	2.90 ± 0.14	*3.13 ± 0.06	2.96 ± 0.09	7
P_{Osm} ($m\ Osm\ kg^{-1}$)	277 ± 4	290 ± 4	276 ± 3	293 ± 4	11
PS (%)	5.34 ± 0.64	5.70 ± 0.53	7.63 ± 0.73	7.02 ± 0.12	3
Hct (%)	34 ± 1	36 ± 1	35 ± 2	35 ± 1	4
+PGPS (%)	7.43 ± 0.85	8.02 ± 0.86	11.04 ± 1.74	9.60 ± 1.67	3
+PRA ($ng\ ml^{-1}\ hr^{-1}$)	19.9 ± 1.7	25.2 ± 1.2	27.2 ± 2.1	24.2 ± 2.7	7
+AVP ($pmol\ l^{-1}$)	1.25 ± 0.55	1.81 ± 0.36	3.87 ± 1.57	2.98 ± 1.0	5

Values are expressed as mean ± S.E. of mean. +Transformed variable, in which case the geometric mean and approximate S.E. of mean are given. n = number of observations. * and ** signify $P < 0.05$ and < 0.01 respectively, when compared with the control value by Dunnett's test after analysis of variance. Abbreviations are as follows:- P_{Na}, P_{K}, etc., plasma concentrations; PS, plasma solids; Hct, haematocrit; PGPS, post-glomerular plasma solids; PRA, plasma renin activity; AVP, plasma arginine vasopressin concentration.

Noxious stimuli may also release vasopressin (Weitzman, 1979) and, although the rabbits did not appear "stressed" during pentagastrin infusion, this may have been a factor. However, no significant changes in plasma AVP concentration were observed during pentagastrin infusion. This may have been because, as explained earlier, only a small number of samples were assayed for AVP and so the error was probably large. Also, the animals were water-loaded at the start of each experiment, thus lowering basal AVP levels which were at the limit of assay sensitivity (ca. 1.68 pmol l^{-1}). Thus, small but significant changes in circulating AVP may not have been detected. Although only a few estimates of haematocrit, plasma solids and PGPS were obtained, these tended to rise with increasing doses of pentagastrin and such changes may increase salt and water reabsorption (Chapter 1).

Renal electrolyte excretion altered little, except for a fall in sodium excretion during infusion of the middle dose. As discussed in Chapter 2, the significance of isolated changes is uncertain. No significant increase in renal perfusion occurred, although the low dose of pentagastrin infused was approximately 3 times the highest dose used by Gatzka et al. (1975), which produced an increase in renal blood flow in the conscious dog. This may be a dose-dependent effect or due to species differences, including dietary habits and secondary hormonal responses.

Like cholecystokinin (Chapter 4), small doses of pentagastrin (0.025 µg kg^{-1} min^{-1}) have been shown to affect calcium homeostasis through release of calcitonin and parathormone and to produce hypocalcaemia (Cooper, Schwesinger, Mahgoub & Ontjes, 1971; Parthemore, Roos, Parker, Kripke, Avioli & Deftos, 1978; Vantini,

Cominacini, Piubello, Ghidini, Fattovich, Ederle, Benini, Cocchetto, Cavallini, LoCascio & Scruro, 1981). In contrast, plasma calcium concentration rose during pentagastrin infusion in the present study. One explanation may be the secretin response to increased duodenal acidity (Hacki, 1980) and secretin-induced hypercalcaemia (Isenberg, Brickman & Moore, 1973; Chapter 9). Also, if parathormone release is stimulated by pentagastrin, it may be relevant that an interaction between AVP and parathormone has been reported, in which exogenous parathormone enhances the antidiuretic effect of submaximal doses of AVP (Humes, Simmons & Brenner, 1980).

Although the number of observations is small, in spite of continuous infusion of "water" as isotonic glucose and a fall in free water clearance, plasma calcium concentration and plasma solids rose while haematocrit remained unchanged. This apparent paradox was also seen during VIP infusion and could have been due to increased capillary permeability together with splanchnic sequestration of red cells (see Chapter 6.1).

It is difficult to know the physiological significance, if any, of these changes as pentagastrin is not a natural peptide. Levels of circulating gastrin are unknown in the rabbit, but owing to the more-or-less continuous presence of food in the stomach, it is likely that the levels are fairly constant. Vantini et al. (1981) measured serum concentrations of gastrin after food and pentagastrin during infusion in man using the same antibody to G17. Concentrations were expressed as "gastrin" and they recorded peak levels of approximately 80 pg ml^{-1} after a meal and 70 pg ml^{-1} during pentagastrin infusion (0.025 µg kg^{-1} min^{-1}). Unfortunately, due to the differences in antibody specificity for the various circulating forms of gastrin, these levels are not comparable and in molar terms, may differ by several

orders of magnitude. Moreover, differences in potency (on a molar basis) between the various forms of gastrin, including pentagastrin, make interpretation difficult. In one series of pentagastrin infusions in the rabbit (see Appendix), using antiserum to G17 and G4, although changes were erratic, little rise in circulating levels occurred during infusion of 0.1 μg kg^{-1} min^{-1}, but approximately 10 and 100 fold rises in plasma concentration were recorded during infusion of 1 and 10 μg kg^{-1} min^{-1} respectively. These levels were probably at least 10 and 100 times greater than comparable post-prandial concentrations of G17 in man.

The changes observed in this study are likely to reflect pharmacological actions of pentagastrin and do not suggest any significant effect of circulating gastrin (c.f. Chapter 3.2).

CHAPTER 3

PART 2:

PENTAGASTRIN AND RENAL FUNCTION IN MAN

Pentagastrin and renal function in man.

INTRODUCTION

Following the study in conscious rabbits, pentagastrin was infused intravenously in 11 healthy volunteers (4 females and 7 males) aged between 22 and 35 years. Six subjects were given 10 ng kg^{-1} min^{-1} (1 female and 5 males) and 6 were given 50 ng kg^{-1} min^{-1} (3 females and 3 males) of pentagastrin, on separate occasions. One male subject was studied twice, once at each dose. Female subjects were not taking the oral contraceptive pill.

The protocol is described in Chapter 2. In this study, 8 urine collections were completed and pentagastrin was infused from the start of the third until the end of the sixth urine collection. The results are based on comparisons between the first and second (U1 + U2) and fifth and sixth (U5 + U6) urine collections (see Fig. 2.4).

RESULTS

Systemic and renal haemodynamics (Tables 3.4a and 3.4b)

Heart rate and blood pressure were recorded in only two subjects during infusion of 10 and 50 ng kg^{-1} min^{-1} of pentagastrin. No changes occurred and the readings are shown in Table 3.3. Pentagastrin did not affect ERPF and GFR at either dose.

Urine composition (Tables 3.5a and 3.5b)

Both doses of pentagastrin increased renal sodium excretion and decreased potassium excretion, including fractional excretion. These changes were dose-related. There were no significant changes in chloride and calcium excretion. The urinary sodium:potassium (Na/K) and sodium:calcium (Na/Ca) ratios both increased significantly at

Table 3.3.

SUBJECT 1

	Pre-	Pentagastrin (10 ng kg^{-1} min^{-1})			
Time (min)	0	+15	+30	+60	+120
SBP	132	142	143	142	143
DBP	77	80	79	77	83
MBP	95	101	100	99	103
HR	73	54	59	55	70

SUBJECT 2

	Pre-	Pentagastrin (50 ng kg^{-1} min^{-1})			
Time (min)	0	+15	+30	+60	+120
SBP	135	128	139	142	135
DBP	87	89	95	87	95
MBP	103	102	110	105	108
HR	57	55	61	57	60

Blood pressure (mm Hg) and heart rate (beats min^{-1}) in 2 male subjects, each receiving different doses of pentagastrin for up to 120 minutes. Abbreviations:- SBP, systolic blood pressure; DBP, diastolic blood pressure; MBP, mean blood pressure; HR, heart (pulse) rate.

Table 3.4a. HAEMODYNAMIC CHANGES

	Pre-(vehicle only)	Pentagastrin (10 ng kg^{-1} min^{-1})	P	Post-U8 (n = 6)
ERPF (ml min^{-1} kg^{-1})	9.4 ± 2.6	8.6 ± 2.1	NS	-
GFR (ml min^{-1} kg^{-1})	2.1 ± 0.3	2.1 ± 0.3	NS	-
FF (%)	22.4 ± 4.5	24.9 ± 4.2	NS	-
RBF (ml min^{-1} kg^{-1})	18.0 ± 4.4	15.5 ± 3.9	NS	-

Values are given as mean ± S.D., n = 12; P = results of paired t-test, NS = not statistically significant ($P > 0.05$). Abbreviations:- ERPF, effective renal plasma flow; GFR, glomerular filtration rate; FF, filtration fraction; RBF, estimated renal blood flow.

Table 3.4b. HAEMODYNAMIC CHANGES

	Pre- (vehicle only)	Pentagastrin (50 ng kg^{-1} min^{-1})	P	Post- U8 (n = 6)
ERPF (ml min^{-1} kg^{-1})	8.9 ± 2.1	9.5 ± 1.7	NS	-
GFR (ml min^{-1} kg^{-1})	2.0 ± 0.7	2.1 ± 0.3	NS	-
FF (%)	23.6 ± 9.0	22.7 ± 4.8	NS	-
RBF (ml min^{-1} kg^{-1})	16.1 ± 3.8	17.0 ± 2.8	NS	-

Values are given as mean ± S.D., n = 12; P = results of paired t-test, NS = not statistically significant ($P > 0.05$). Abbreviations:- ERPF, effective renal plasma flow; GFR, glomerular filtration rate; FF, filtration fraction; RBF, estimated renal blood flow.

Table 3.5a. URINARY CHANGES

	Pre- (vehicle only)	Pentagastrin (10 ng kg^{-1} min^{-1})	P	Post- U8 (n = 6)
$\dot{V}$ ($\mu l\ min^{-1}kg^{-1}$)	170.6±77.0	184.4±60.5	NS	-
C_{Osm} ($\mu l\ min^{-1}kg^{-1}$)	50.7±10.7	57.2±20.7	NS	-
C_{H_2O} ($\mu l\ min^{-1}kg^{-1}$)	119.9±72.6	127.2±52.0	NS	-
$U_{Na}V$ ($\mu mol\ min^{-1}kg^{-1}$)	3.20±0.92	3.97±0.88	<0.01	3.72±1.00
$U_{K}V$ ($\mu mol\ min^{-1}kg^{-1}$)	1.43±0.51	0.99±0.33	<0.02	0.98±0.22
$U_{Cl}V$ ($\mu mol\ min^{-1}kg^{-1}$)	1.91±1.84	1.80±0.80	NS	-
$U_{Ca}V$ ($\mu mol\ min^{-1}kg^{-1}$)	0.15±0.04	0.16±0.03	NS	-
FEK (%)	14.3±4.7	11.6±5.2	<0.01	11.1±4.4
Na/K ratio	2.41±0.91	4.32±1.35	<0.01	3.99±1.48
Na/Ca ratio	22.0±5.3	25.6±3.7	<0.05	28.0±4.1

Values are given as mean ± S.D., n = 12; P = results of paired t-test, NS = not statistically significant ($P > 0.05$). Abbreviations:- $\dot{V}$, urine flow; C_{Osm}, osmolar clearance; C_{H_2O}, free water clearance; $U_{Na}V$, $U_{K}V$, etc., renal excretion; FE., fractional excretion.

Table 3.5b. URINARY CHANGES

	Pre- (vehicle only)	Pentagastrin (50 ng kg^{-1} min^{-1})	P	Post-U8 (n = 6)
$\dot{V}$ (µl $min^{-1}kg^{-1}$)	166.9±73.9	167.9±66.2	NS	-
C_{Osm} (µl $min^{-1}kg^{-1}$)	55.6±18.2	52.2±9.0	NS	-
C_{H_2O} (µl $min^{-1}kg^{-1}$)	111.3±76.5	115.7±66.7	NS	-
$U_{Na}V$ (µmol $min^{-1}kg^{-1}$)	3.32±0.97	4.77±1.25	<0.01	3.68±1.44
$U_{K}V$ (µmol $min^{-1}kg^{-1}$)	1.69±0.55	1.07±0.33	<0.01	0.94±0.42
$U_{Cl}V$ (µmol $min^{-1}kg^{-1}$)	1.69±0.77	2.12±0.74	NS	-
$U_{Ca}V$ (µmol $min^{-1}kg^{-1}$)	0.15±0.06	0.15±0.05	NS	-
FEK (%)	20.7±9.0	13.0±5.3	<0.04	12.2±5.8
Na/K ratio	2.17±1.12	4.66±1.42	<0.01	4.22±1.78
Na/Ca ratio	24.8±9.3	33.6±7.6	<0.01	31.3±12.7

Values are given as mean ± S.D., n = 12; P = results of paired t-test, NS = not statistically significant ($P > 0.05$). Abbreviations:- $\dot{V}$, urine flow; C_{Osm}, osmolar clearance; C_{H_2O}, free water clearance; $U_{Na}V$, $U_{K}V$, etc., renal excretion; FE., fractional excretion.

each dose. Urine flow, osmolar clearance and free water clearance were unaffected by either dose of pentagastrin.

Plasma composition (Tables 3.6a and 3.6b)

The dose of 10 ng kg^{-1} min^{-1} did not alter plasma electrolytes, plasma solids or haematocrit. The dose of 50 ng kg^{-1} min^{-1} produced a small increase in plasma solids and fall in plasma chloride concentration. A significant decrease in plasma renin activity (PRA) also occurred at this dose.

The control period after pentagastrin (U8)

Sodium excretion after each dose of pentagastrin showed a change towards the basal (pre-infusion) value, but potassium excretion remained low. In both cases, the changes remained significantly different from baseline, as did the changes in Na/K and Na/Ca ratios. PRA also returned towards basal after infusion of 50 ng kg^{-1} min^{-1} pentagastrin.

DISCUSSION

It is difficult to compare the results from this study with those obtained in the rabbit (Chapter 3.1). The dose of 50 ng kg^{-1} min^{-1} was the maximum dose of pentagastrin tolerated without symptoms of nausea and in some cases vomiting, abdominal cramp and diarrhoea. This dose is half the smallest used in the rabbit, a dose which had no effect on renal function and in contrast to the findings in the present study, did not alter the urinary Na/K and Na/Ca ratios (Fig. 3.5). It is therefore not surprising that no change in free water clearance was observed in the present study. This serves to emphasise that in the rabbit, changes in urine flow and free water clearance were unphysiological or even toxicological responses to

Table 3.6a. PLASMA CHANGES

	Pre- (vehicle only)	Pentagastrin (10 ng kg^{-1} min^{-1})	P	Post- U8 (n = 6)
PS (%)	6.81 ± 1.70	7.02 ± 1.39	NS	-
Hct (%)	43 ± 7	43 ± 7	NS	-
P_{Na} (mmol l^{-1})	138.4 ± 1.7	139.7 ± 2.4	NS	-
P_K (mmol l^{-1})	4.26 ± 0.21	4.21 ± 0.31	NS	-
P_{Cl} (mmol l^{-1})	102.3 ± 3.5	101.9 ± 3.1	NS	-
P_{Ca} (mmol l^{-1})	1.52 ± 0.17	1.52 ± 0.24	NS	-
P_{Osm} (mmol l^{-1})	277 ± 7	279 ± 7	NS	-
$^{+}$PRA (pg ml^{-1} hr^{-1})	1050 ± 1003	858 ± 874	NS	-

Values are given as mean ± S.D., n = 12. $^{+}$ Transformed variable expressed as geometric mean ± approximate S.D.; P = results of paired t-test, NS = not statistically significant ($P > 0.05$).

Abbreviations:- PS, plasma solids; Hct, haematocrit; P_{Na}, P_K, etc., plasma concentration; PRA, plasma renin activity.

Table 3.6b. PLASMA CHANGES

	Pre- (vehicle only)	Pentagastrin (50 ng kg^{-1} min^{-1})	P	Post- U8 (n = 6)
PS (%)	6.90 ± 0.35	7.57 ± 0.94	< 0.01	
Hct (%)	42 ± 3	42 ± 3	NS	-
P_{Na} (mmol l^{-1})	140.8 ± 2.8	141.6 ± 3.1	NS	-
P_K (mmol l^{-1})	4.29 ± 0.35	4.05 ± 0.35	NS	-
P_{Cl} (mmol l^{-1})	101.5 ± 3.1	99.5 ± 5.5	< 0.05	100.7 ± 1.7
P_{Ca} (mmol l^{-1})	1.90 ± 0.38	2.03 ± 0.35	NS	-
P_{Osm} (mmol l^{-1})	278 ± 7	278 ± 7	NS	-
$^{+}$PRA (pg ml^{-1} hr^{-1})	695 ± 457	490 ± 218	< .001	506 ± 211
$^{+}$ALDO (pmol l^{-1})	262 ± 110	204 ± 45	NS	-

Values are given as mean ± S.D., n = 12. $^{+}$ Transformed variable expressed as geometric mean ± approximate S.D.; P = results of paired t-test, NS = not statistically significant (P > 0.05).

Abbreviations:- PS, plasma solids; Hct, haematocrit; P_{Na}, P_K, etc., plasma concentration; PRA, plasma renin activity; ALDO, plasma aldosterone concentration.

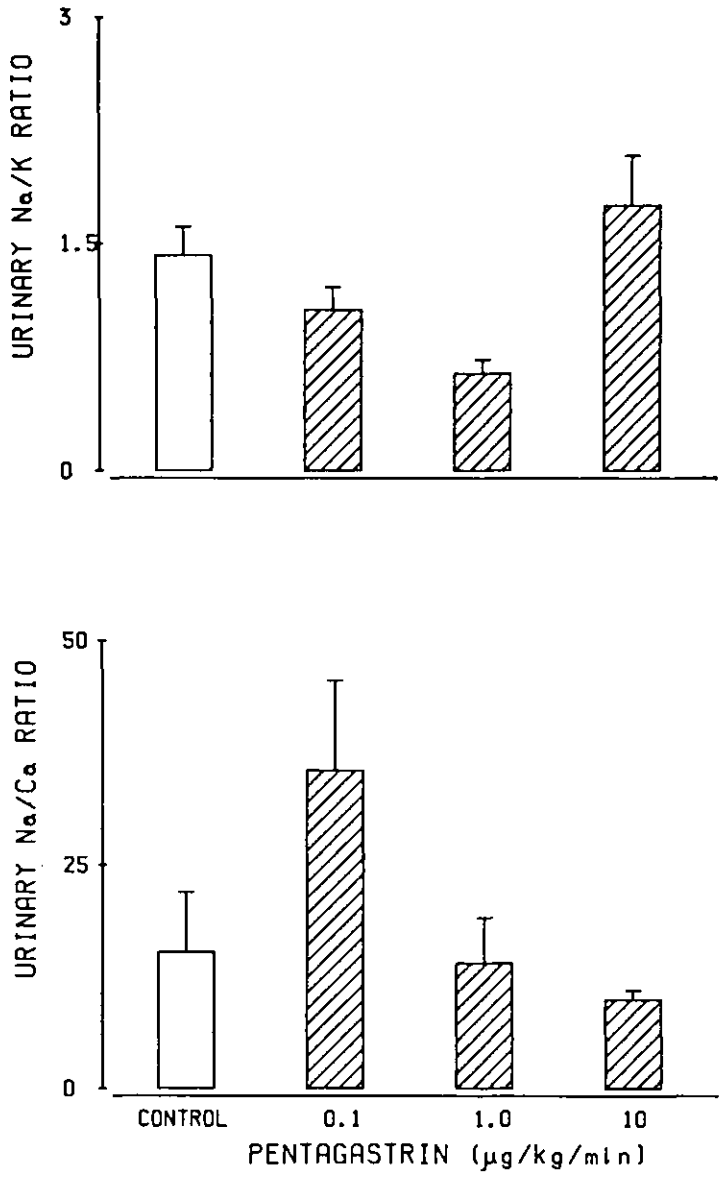


Fig. 3.5.

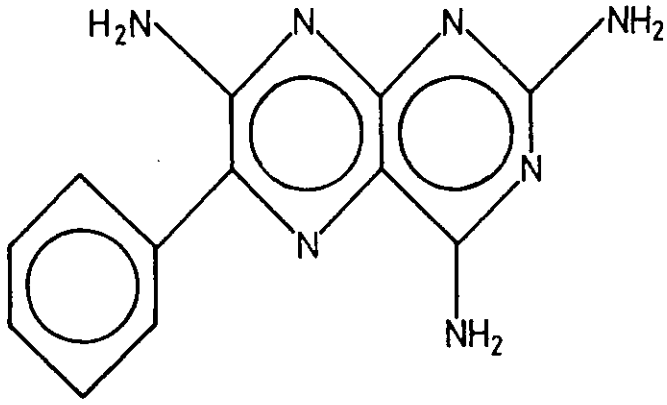
Effect of increasing doses of pentagastrin on urinary sodium : potassium (Na/K) and sodium:calcium (Na/Ca) ratios in the rabbit. Results are expressed as mean ± S.E. of mean, n = 11 for each treatment group.

circulating "gastrin".

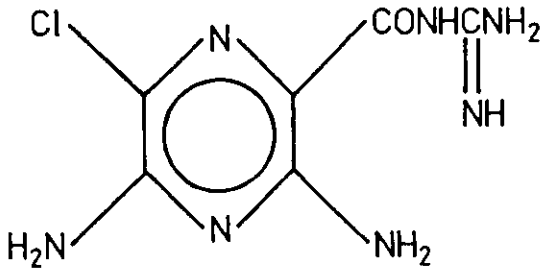
Both doses used in the present study are supra-physiological at least in terms of gastric acid secretion. The low dose of 10 ng kg^{-1} min^{-1} is that recommended for use in testing gastric acid secretion in patients (Peptavlon Data Sheet, ICI). However, the effect of pentagastrin on sodium excretion may have a physiological counterpart in the natriuretic response to feeding observed in dogs (Summer ill, 1974). Unlike the rabbit, man and dog are usually carnivorous and this difference is likely to have important physiological and adaptive consequences. In the present study, the increased sodium excretion was associated with a fall in potassium excretion and was independent of any change in GFR. In the dog, postprandial natriuresis was associated with increased potassium excretion and GFR (as estimated by creatinine clearance; Summer ill, 1974).

The inverse relationship between sodium and potassium excretion and the dissociation between urinary sodium and calcium, may indicate a distal tubular site of action of pentagastrin with reversal of normal Na^+/K^+ exchange i.e., an opposite effect to aldosterone and a thiazide diuretic-like dissociation of sodium and calcium reabsorption (Suki, 1979). Plasma aldosterone levels did not fall significantly during the high dose infusion of pentagastrin. Moreover, inhibition of aldosterone secretion or direct antagonism at its site of action would seem unlikely in view of the slow response to aldosterone, which depends upon protein synthesis (Ross, Reddy, Rivera & Thorn, 1959; Chapter 1).

Pentagastrin may be acting in a similar way to the so-called potassium-sparing diuretes triamterene and amiloride (Fig. 3.6). These substances can produce potassium retention and a mild



TRIAMTERENE



AMILORIDE

Fig. 3.6.

Chemical structures of triamterene and amiloride. Both these potassium-sparing diuretics are pyrazine derivatives. Triamterene is related chemically to folic acid.

natriuresis (Giebisch, Malnic & Berliner, 1981; Reineck & Stein, 1981). Their mechanism of action is unclear, but they are thought to reduce distal tubular permeability to sodium. Like thiazide diuretics, they also dissociate distal reabsorption of sodium and calcium, with a fall in calcium clearance, perhaps by accelerating contraluminal calcium efflux and Ca/Na exchange (Suki, 1979), but the mechanism is thought to be different, as this effect is additive with thiazides (Sutton & Dirks, 1981). So, like pentagastrin in the present study, these diuretics increase both Na/K and Na/Ca ratios in urine and decrease the fractional excretion of potassium (Reineck & Stein, 1981).

A proximal tubular site of action is also possible, with primary inhibition of sodium reabsorption (perhaps through changes in intrarenal blood flow rather than a direct tubular effect) resulting in increased distal tubular reabsorption of Na^+ in exchange for H^+ rather than K^+. Alternatively, pentagastrin may directly stimulate distal tubular H^+ secretion, but this seems unlikely.

The increase in sodium excretion may also involve the action of acid-stimulated gut hormones such as cholecystokinin and secretin, but the time-course of these hormone responses cannot be predicted. Cholecystokinin (C-terminal homology with gastrin) can produce natriuresis in the rabbit and man (Chapter 4) and secretin has also been reported to increase sodium excretion in man (Viteri, Poppell, Lasater & Dyck, 1975). Their mode of action is unknown, but appears to be independent of changes in filtered load, although it must be stated that an undetectable (within 5-10% by clearance methods) increase in GFR could account for the apparent natriuretic response (O'Connor, 1962). However, the inverse relationship with potassium

excretion and the return towards basal values make this unlikely. Gastrin and cholecystokinin also stimulate release of calcitonin and parathormone (Vantini et al., 1981), both of which are natriuretic (Lindheimer & Katz, 1977; Slatopolsky et al., 1979), but again, the latency and time-course of such responses are unknown.

The small rise in plasma solids at the higher dose of pentagastrin may not be significant in the non-statistical sense of the word, although it is similar to the pattern observed in the rabbit and the same explanation may apply (Part 1 of this chapter). This rise would tend to oppose sodium excretion. The reduction in PRA, again only at the higher dose of pentagastrin, may be related to the similar effect produced by CCK8 in the rabbit.

Finally, gastrin and related peptides may be the physiological counterpart of triamterene and amiloride and contribute to the regulation of sodium and potassium balance.

SUMMARY TO CHAPTER 3

1. Pentagastrin is the active C-terminal tetrapeptide sequence of gastrin plus beta-alanine. It is believed to possess all the biological actions of circulating gastrin. Gastrin has been implicated in the postprandial changes in renal sodium excretion and haemodynamics.

2. Three different doses were infused intravenously in 4 conscious rabbits (0.1, 1.0 and 10 $\mu g\ kg^{-1}\ min^{-1}$) and 2 different doses were also infused in 2 separate groups of 6 human volunteers (10 and 50 $ng\ kg^{-1}\ min^{-1}$ respectively).

3. In the rabbit, pentagastrin produced a dose-related fall in urine flow and free water clearance, but no change in systemic and renal haemodynamics, electrolyte excretion and plasma composition, including arginine vasopressin levels. In contrast, in human subjects, pentagastrin increased renal sodium excretion and reduced potassium excretion. Renal haemodynamics did not change, but at the high dose PRA fell.

4. The effects of pentagastrin on renal function in conscious water-loaded rabbits closely resemble those of vasopressin. In man, its renal effects are similar to those of the potassium sparing diuretics triamterene and amiloride.

CHAPTER 4

PART 1:

RENAL FUNCTION DURING CHOLECYSTOKININ OCTAPEPTIDE (CCK8) INFUSION IN CONSCIOUS RABBITS

Renal function during cholecystokinin octapeptide (CCK8) infusion in conscious rabbits.

INTRODUCTION.

In 1928 Ivy & Oldberg gave the name cholecystokinin to a substance released by fat from the small intestine that would stimulate gallbladder contraction. Later, Harper & Raper (1943) discovered a hormone released from duodenal mucosa which stimulated pancreatic secretion and named it pancreozymin. It was not until Mutt & Jorpes (1968) attempted to purify these substances that they were found to be the same peptide with 33 amino acid residues, CCK33. In recent years, the biologically active COOH-terminal octapeptide of CCK (CCK8) has been found in high concentrations in both brain and peripheral neurones (Rehfeld et al., 1979). Apart from the presence of CCK in nerves of the colon and ileum, perhaps indicating regulation of motility, it has been found in nerves to the muscle of the urinary bladder (Rehfeld, 1980), but to date has not been reported in the kidney. CCK8 is chemically similar to pentagastrin (Chapter 3, Fig. 3.1) and is the predominant form of CCK in plasma and gut endocrine cells (Dockray, 1981). It is thought to have a dual role as a neurotransmitter and gut hormone and to be involved in feeding behaviour, control of gallbladder contraction and pancreatic enzyme secretion (Dockray, 1982).

Continuing the search for possible mediators of postprandial changes in renal function (Reinhardt et al., 1975), the renal effects of intravenously infused CCK8 (5, 50 and 450 pmol kg^{-1} min^{-1}) in conscious rabbits were investigated.

RESULTS.

Systemic blood pressure and heart rate (Fig. 4.1)

Mean arterial blood pressure fell by approximately 10 mm Hg during infusion of the middle and high doses (50 and 450 pmol kg^{-1} min^{-1} respectively). Heart rate fell during infusion of the high dose by approximately 30 beats min^{-1}. No changes occurred at the low dose (5 pmol kg^{-1} min^{-1}) of CCK8.

Renal haemodynamics

Figure 4.2 shows the 30-40% rise in ERPF and GFR during infusion of the high dose of CCK8 with no change in filtration fraction. No significant changes occurred during infusion of the low and middle doses.

Urine composition (Table 4.1)

Excretion of sodium more than doubled during infusion of the middle and high doses of CCK8, but renal potassium excretion did not change significantly. The pattern of change in excretion of chloride was similar to sodium. The renal and fractional excretions of calcium fell by more than half during infusion of the middle and high doses, but were unaffected by the low dose of CCK8. Renal and fractional excretions of phosphate did not change significantly at any dose. Urine flow did not alter significantly, but there was a dose-related increase in osmolar clearance and fall in free water clearance.

Plasma composition and haematocrit (Table 4.2)

Table 4.2 shows the changes in plasma composition and haematocrit during CCK8 infusion, including the range of plasma concentrations of

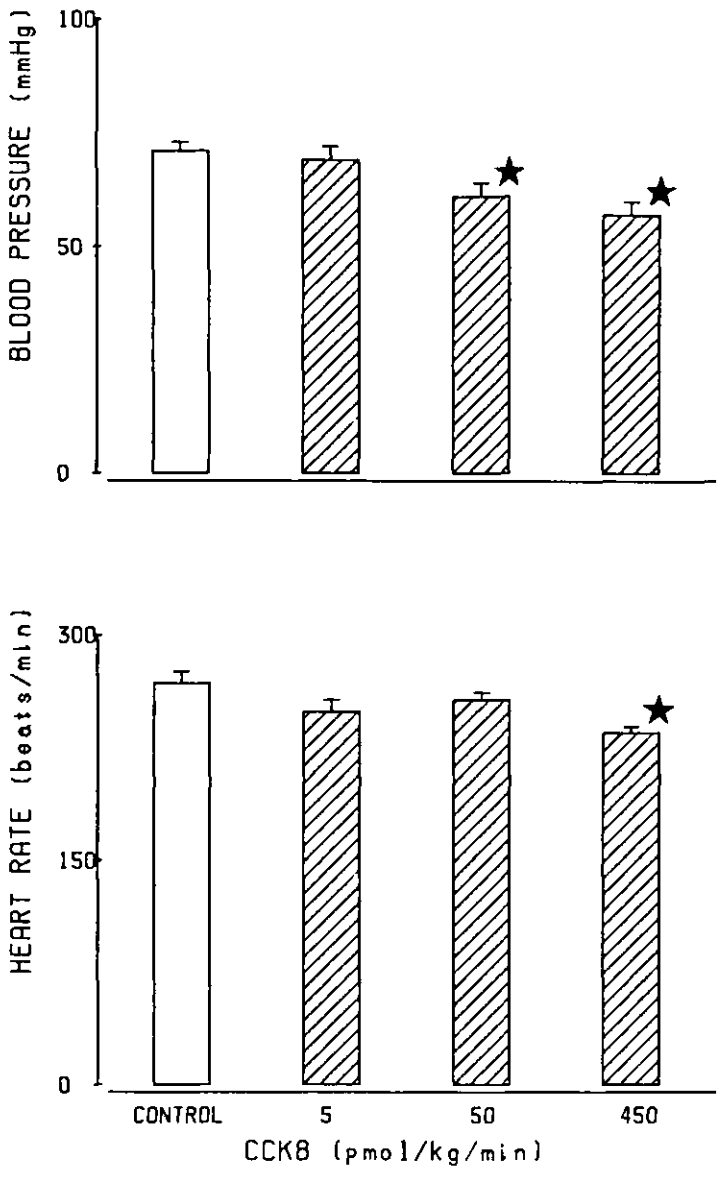


Fig. 4.1.

Effect of increasing doses of CCK8 on heart rate and mean arterial blood pressure. Results are expressed as mean ± S.E. of mean, n = 12 for each treatment group. * indicates significant difference ($p < 0.05$) from control value, as determined by Dunnett's test after analysis of variance.

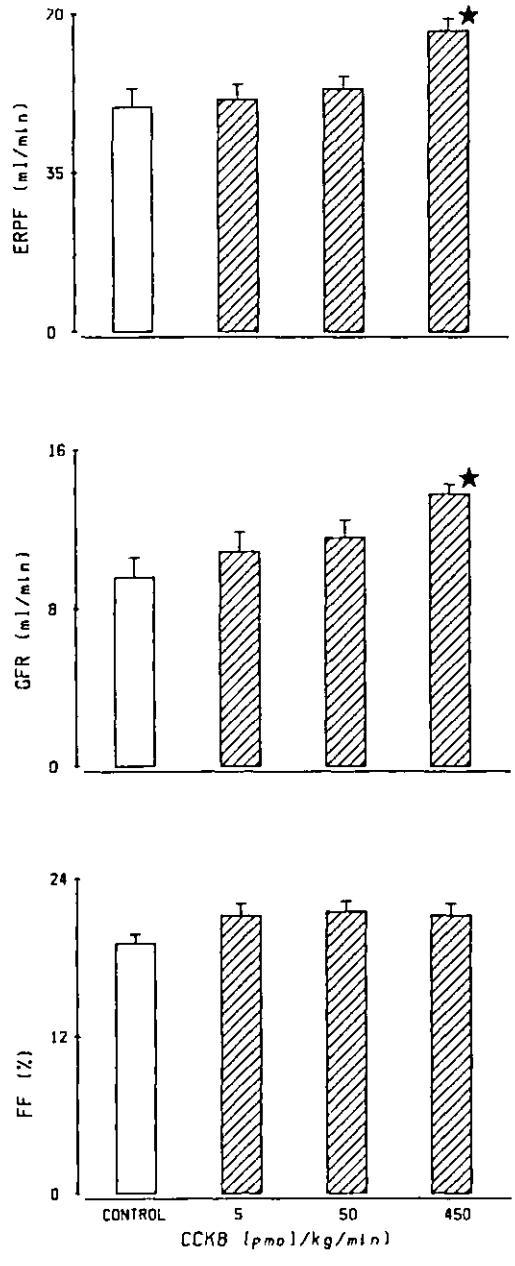


Fig. 4.2.

Effect of increasing doses of CCK8 on effective renal plasma flow (ERPF), glomerular filtration rate (GFR) and filtration fraction (FF). Results are expressed as mean ± S.E. of mean, n = 12 for each treatment group. * indicates significant difference ($p < 0.05$) from control value, as determined by Dunnett's test after analysis of variance.

Table 4.1. Effects of CCK8 on renal electrolyte excretion, urine flow and solute excretion.

		Control (vehicle only)	CCK8 in pmol kg^{-1} min^{-1} 5 (low)	50 (middle)	450 (high)	n
$^{+}U_{Na}V$	(µmol min^{-1})	5.5 ± 1.7	3.1 ± 0.7	*13.9 ± 1.7	**23.0 ± 6.6	12
$^{+}U_{K}V$	(µmol min^{-1})	7.3 ± 1.1	6.5 ± 1.0	5.6 ± 0.6	7.2 ± 0.9	12
$^{+}U_{Cl}V$	(µmol min^{-1})	7.1 ± 1.9	5.0 ± 0.9	9.7 ± 1.2	**17.2 ± 3.5	12
$^{+}U_{Ca}V$	(µmol min^{-1})	1.49 ± 0.62	1.31 ± 0.62	**0.41 ± 0.10	**0.60 ± 0.10	12
$^{+}FE_{Ca}$	(%)	9.5 ± 3.8	7.5 ± 3.1	**2.3 ± 0.6	**3.0 ± 0.4	12
$^{+}U_{Pi}V$	(µmol min^{-1})	0.41 ± 0.15	0.32 ± 0.12	0.46 ± 0.07	0.61 ± 0.14	12
$^{+}FE_{Pi}$	(%)	5.1 ± 1.4	3.7 ± 0.9	5.5 ± 0.9	6.6 ± 1.8	12
$^{+}\dot{V}$	(µl min^{-1})	121.0 ± 36.7	136.6 ± 40	73.3 ± 7.9	123.5 ± 29.1	12
$^{+}C_{Osm}$	(µl min^{-1})	137.7 ± 38.7	180.4 ± 16.1	230.8 ± 20.8	**388.0 ± 69.2	12
C_{H_2O}	(µl min^{-1})	29.1 ± 48.1	23.6 ± 59.2	-168.3 ± 23.2	**-289.8 ± 42.0	12

Values are expressed as mean ± S.E. of mean. $^{+}$Transformed variable, in which case the geometric mean and approximate S.E. of mean are given. n = number of observations. * and ** signify $P < 0.05$ and < 0.01 respectively, when compared with the control value by Dunnett's test after analysis of variance. Abbreviations are as follows:- $U_{Na}V$, $U_{K}V$, etc., renal excretion; F.E., fractional excretion; $\dot{V}$, urine flow; C_{Osm}, osmolar clearance; C_{H_2O}, free water clearance.

Table 4.2. Effects of CCK8 on plasma composition and haematocrit.

	Control (vehicle only)	CCK8 in pmol kg^{-1} min^{-1} 5 (low)	50 (middle)	450 (high)	n
CCK8-Ll (pmol l^{-1})	< 10	10 - 157	677 - 937	4877 - 15877	4
P_{Na} (mmol l^{-1})	135.8 ± 0.5	137.7 ± 1.4	135.8 ± 0.6	134.4 ± 0.6	12
P_K (mmol l^{-1})	3.52 ± 0.08	**4.04 ± 0.09	*3.20 ± 0.09	**3.21 ± 0.07	12
P_{Cl} (mmol l^{-1})	97.8 ± 0.7	98.8 ± 1.2	95.8 ± 0.9	97.3 ± 0.9	12
P_{Glu} (mmol l^{-1})	7.6 ± 0.3	8.5 ± 0.5	9.7 ± 1.0	**12.7 ± 1.1	8
P_{Osm} (m Osm kg^{-1})	265 ± 3	*274 ± 4	**279 ± 3	**274 ± 5	12
PS (%)	5.60 ± 0.12	6.37 ± 0.11	5.18 ± 0.30	5.81 ± 0.21	8
Hct (%)	36 ± 1	**33 ± 2	*34 ± 1	35 ± 1	8
+PGPS (%)	6.92 ± 0.19	8.14 ± 0.20	6.54 ± 0.37	7.21 ± 0.29	8

Values are expressed as mean ± S.E. of mean. +Transformed variable, in which case the geometric mean and approximate S.E. of mean are given. n = number of observations. * and ** signify $P < 0.05$ and < 0.01 respectively, when compared with the control value by Dunnett's test after analysis of variance. Abbreviations are as follows:- P_{Na}, P_K, etc., plasma concentration; PS, plasma solids; Hct, haematocrit; PGPS, post-glomerular plasma solids.

CCK8-like immunoreactivity (CCK8-LI) achieved at each dose. Plasma potassium concentration rose during infusion of the low dose, but fell during infusion of the middle and high doses. There were dose-dependent falls in plasma calcium and phosphate concentrations and plasma renin activity (PRA) (Fig. 4.3). Plasma solids did not change significantly, although there was a small increment during infusion of the low dose. Haematocrit fell slightly during infusion of the low and middle doses of CCK8. Plasma osmolality rose at each dose of CCK8 and there was a dose-related increase in plasma glucose concentration.

DISCUSSION.

Cholecystokinin is known to increase splanchnic blood flow and reduce systemic blood pressure (Said, 1983). Fronek & Stahlgren (1968) have confirmed earlier reports of increased splanchnic flow during digestion in dogs, but did not observe any significant changes in blood pressure, cardiac output or total peripheral vascular resistance. The fall in arterial blood pressure, together with the increases in ERPF and GFR produced by the middle and high doses of CCK8 in the conscious rabbit, are consistent with systemic vasodilatation and a fall in renal vascular resistance. However, these changes occurred at plasma CCK8-LI levels many fold greater than those observed postprandially (Dockray, 1982) and it is therefore unlikely that CCK8, at least alone, is responsible for the haemodynamic changes observed after feeding. Moreover, even the lowest dose of CCK8 infused, when no significant haemodynamic changes occurred, produced plasma CCK8-LI levels 2-5 times greater than reported postprandial levels.

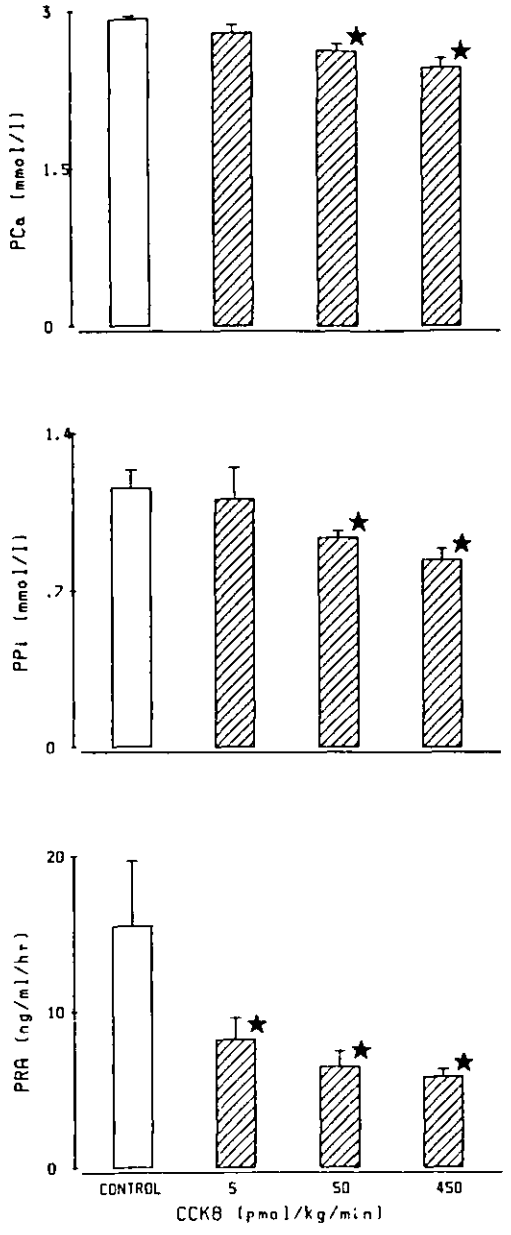


Fig. 4.3.

Effect of increasing doses of CCK8 on plasma calcium and phosphate concentrations (P_{Ca} and P_{Pi}) and plasma renin activity (PRA). PRA was found to be lognormally distributed and therefore results are expressed as geometric mean ± approximate S.E. of mean, n = 8 (PRA) or 12 (P_{Ca} and P_{Pi}) for each treatment group. * indicates significant difference ($p < 0.05$) from control value, as determined by Dunnett's test after analysis of variance.

The natriuresis produced by infusion of the middle and high doses of CCK8 is most likely due to the increase in renal perfusion and filtered load of sodium. Although no significant changes were observed in filtration fraction, plasma solids, and PGPS, the small fall in plasma solids and PGPS together with the haematocrit, may have contributed to the increase in renal sodium excretion at the middle dose. CCK8 may also have a direct tubular action and decrease tubular reabsorption of sodium. It is known to inhibit absorption of water and electrolytes from the jejunum and ileum (Go, 1978), with which the nephron shares some transport characteristics (Guyton, 1976b), but this effect may be due to local increases in mucosal blood flow rather than a direct action on mucosal cells.

Reinhardt et al. (1975) considered that the increase in renal blood flow and natriuresis (which was independent of dietary sodium) observed after feeding in dogs might be due to release of gastrointestinal hormones. They subsequently reported an increase in renal blood flow during intravenous infusion of pentagastrin (Gatzka et al., 1975). In the conscious rabbit, however, no increase in renal perfusion with pentagastrin was observed (Chapter 3.1). Also, the plasma concentration of CCK8-LI at which haemodynamic changes did occur in this study is supra-physiological, making it more likely that this is a pharmacological and not a physiological effect of CCK8.

CCK8 has been shown to affect calcium homeostasis (Stulberg, Norberg & Kaplan, 1976; Vantini et al., 1981). It is known to release calcitonin and parathormone when infused intravenously (Kaplan, North, Norberg, Schulak & Hill, 1977; Vantini et al., 1981), but whether the rise in parathormone is a direct effect, or

secondary to calcitonin-induced hypocalcaemia, is unknown. There is even uncertainty as to whether or not this effect of CCK8 is calcitonin mediated or dependent upon an intact stomach. Kaplan et al. (1977) have reported abolition of CCK-induced hypocalcaemia after gastrectomy in rats, but no such effect was seen in post-gastrectomy human subjects (Vantini et al., 1981). Calcitonin release may be responsible for the fall in plasma calcium concentration and may contribute to the increase in renal perfusion and sodium excretion (Katz & Lindheimer, 1977). Although there have been reports of an anticalciuric action of calcitonin, it is generally believed to produce calciuria (Katz & Lindheimer, 1977). The reduction in calcium excretion observed in this study may reflect the fall in plasma calcium concentration and a net reduction in filtered load in spite of the rise in GFR. However, the fall in fractional excretion of calcium suggests increased tubular reabsorption of calcium. Plasma phosphate concentration also fell, but renal and fractional phosphate excretions did not change significantly. These results could be due to an alteration in tubular handling of both calcium and phosphate and reflect a balance between the actions of calcitonin and parathormone; although it should be stated that changes in renal phosphate handling in the rabbit do not reliably indicate alterations in circulating levels of parathormone (Berndt, Marchand, Sell, Haas, Dousa & Knox, 1978; see also Chapter 10, Fig. 10.5). It is conceivable that many of these effects of CCK8 are due to increased calcitonin release. Berndt & Knox (1980) found that in the rabbit, calcitonin produced a marked natriuresis and a small reduction in calcium excretion, but little change in phosphate excretion.

CCK8 elicits release of insulin and glucagon, both of which may

lower plasma potassium concentration (Natelson, Pincus & Rannazzisi, 1963). The rise in plasma glucose concentration may be a direct effect of CCK8 on peripheral utilisation of glucose and hepatic metabolism, or secondary to glucagon and growth hormone release, although the latter has only been reported after intracerebral injection (Dockray, 1981). Clearly, this effect may not be physiological, but an increase in plasma glucose concentration could be involved in the suppression of appetite produced by intravenous CCK8 (Dockray, 1982).

CCK8, like substance P (Gullner, Campbell & Pettinger, 1979), is unusual as a renal vasodilator, in that it inhibits renin release (Peart, 1978). Parathormone is reported to stimulate renin secretion (Smith, Mouw & Vander, 1979; Peart, Roddis & Unwin, 1981), but calcitonin and hypocalcaemia (in vivo) are without effect (Smith et al., 1979; Llach, Weidmann, Reinhart, Maxwell, Coburn & Massry, 1974). Fray (1980) has argued that although renal (afferent arteriole) vasodilatation may be associated with a rise in renin, the relationship is not causal and provided that renal perfusion pressure remains unaltered, vasodilatation inhibits renin release. Peart (1978) has proposed that renin release depends upon changes in juxta-glomerular cell cytosolic calcium: an increase inhibiting and decrease stimulating renin secretion. Changes in net cellular calcium flux may be produced by alterations in membrane permeability, directly, or through cell stretch: (? vasodilatation rather than vasoconstriction) → increased cell stretch → increased permeability to calcium → elevated intracellular calcium → renin inhibition. CCK8 is believed to stimulate pancreatic enzyme secretion through changes in acinar cell calcium flux and elevation

of intracellular calcium (Christophe & Waelbroeck, 1981; Petersen, 1982). If CCK8 produced similar changes within the juxta-glomerular cell, this would lead to inhibition of renin release.

Finally, of the effects observed, the fall in PRA and the small changes in plasma calcium and glucose concentrations at the low dose of infused CCK8, may occur at physiological concentrations of circulating CCK8. These actions may be important in diseases such as pancreatitis in which plasma CCK levels are reported to be high (Harvey, Dowsett, Hartog & Read, 1973; Dockray, 1981).

CHAPTER 4

PART 2:

CHOLECYSTOKININ OCTAPEPTIDE (CCK8) AND RENAL FUNCTION IN MAN

Cholecystokinin octapeptide (CCK8) and renal function in man.

INTRODUCTION

Following the study in conscious rabbits, CCK8 was infused in 6 healthy male volunteers aged between 22 and 35 years at a dose of 2 pmol kg^{-1} min^{-1} according to the protocol outlined in Chapter 2.

RESULTS

Systemic and renal haemodynamics (Table 4.3)

Small, but statistically significant increases in systolic and mean blood pressures and heart rate occurred during CCK8 infusion. GFR and ERPF did not change.

Urine composition (Table 4.4)

Sodium excretion increased and potassium excretion fell, each by about 15%, approximately half the response to pentagastrin. The urinary sodium:potassium (Na/K) ratio increased, but the sodium : calcium (Na/Ca) ratio remained unchanged. Compared with the values obtained in the pentagastrin and neurotensin studies in man (see Chapters 3.2 and 8.2), the Na/Ca ratios were very much higher in the present study due to lower calcium excretion throughout. Phosphate excretion increased by about 30% during CCK8 infusion. Urine flow and osmolar clearance did not change significantly.

Plasma composition (Table 4.5)

The mean rise in CCK8-LI one hour after the start of infusion was 31 ± 17 pmol l^{-1} (mean ± S.D., n = 6). Plasma solids fell by about 10%, but haematocrit did not change. Plasma phosphate concentration rose during CCK8 infusion.

Table 4.3. HAEMODYNAMIC CHANGES

	Pre-(vehicle only)	CCK8 (2 pmol kg^{-1} min^{-1})	P	Post-U7 (n = 6)
SBP (mm Hg)	130 ± 7	135 ± 10	< 0.02	134 ± 15
DBP (mm Hg)	74 ± 7	76 ± 7	NS	-
MBP (mm Hg)	92 ± 7	96 ± 7	< 0.05	97 ± 10
HR (beats min^{-1})	57 ± 7	61 ± 10	< 0.02	64 ± 10
ERPF (ml min^{-1} kg^{-1})	9.8 ± 2.1	9.4 ± 2.7	NS	-
GFR (ml min^{-1} kg^{-1})	2.1 ± 0.3	2.0 ± 0.6	NS	-
FF (%)	21.9 ± 6.6	22.3 ± 5.5	NS	-
RBF (ml min^{-1} kg^{-1})	17.8 ± 3.8	16.9 ± 4.8	NS	-

Values are given as mean ± S.D., n = 12; P = results of paired t-test, NS = not statistically significant (P > 0.05). Abbreviations:- SBP, systolic blood pressure; DBP, diastolic blood pressure; MBP, mean blood pressure; HR, heart rate; ERPF, effective renal plasma flow; GFR, glomerular filtration rate; FF, filtration fraction; RBF, estimated renal blood flow.

Table 4.4. URINARY CHANGES

	Pre-(vehicle only)	CCK8 (2 pmol kg^{-1} min^{-1})	P	Post-U7 (n = 5)
$\dot{V}$ ($\mu l\ min^{-1}kg^{-1}$)	153.6±58.6	143.2±61.3	NS	-
C_{Osm} ($\mu l\ min^{-1}kg^{-1}$)	60.4±17.4	62.2±14.9	NS	-
C_{H_2O} ($\mu l\ min^{-1}kg^{-1}$)	93.2±45.7	81.0±50.9	NS	-
$U_{Na}V$ ($\mu mol\ min^{-1}kg^{-1}$)	4.76±2.22	5.44±2.29	<0.02	4.67±2.33
$U_{K}V$ ($\mu mol\ min^{-1}kg^{-1}$)	1.69±0.90	1.45±0.83	<0.05	1.01±0.58
$U_{Cl}V$ ($\mu mol\ min^{-1}kg^{-1}$)	3.20±1.70	3.44±1.45	NS	-
$U_{Ca}V$ ($\mu mol\ min^{-1}kg^{-1}$)	0.06±0.03	0.05±0.03	NS	-
$U_{Pi}V$ ($\mu mol\ min^{-1}kg^{-1}$)	0.21±0.10	0.27±0.07	<0.01	0.21±0.11
Na/K ratio	4.01±3.05	5.27±3.74	<0.01	6.12±3.71
Na/Ca ratio	102.8±57.9	110.6±59.2	NS	-

Values are given as mean ± S.D., n = 12; P = results of paired t-test, NS = not statistically significant ($P > 0.05$). Abbreviations:- $\dot{V}$, urine flow; C_{Osm}, osmolar clearance; C_{H_2O}, free water clearance; $U_{Na}V$, $U_{K}V$, etc., renal excretion.

Table 4.5. PLASMA CHANGES

	Pre- (vehicle only)	CCK8 (2 pmol kg^{-1} min^{-1})	P	Post-U7 (n = 6)
PS (%)	6.97 ± 0.80	6.42 ± 0.87	< 0.01	6.31 ± 1.05
Hct (%)	43 ± 3	42 ± 3	NS	-
P_{Na} (mmol l^{-1})	140.3 ± 1.7	140.5 ± 1.0	NS	-
P_K (mmol l^{-1})	4.30 ± 0.24	4.28 ± 0.28	NS	-
P_{Cl} (mmol l^{-1})	102.3 ± 1.7	102.3 ± 1.4	NS	-
P_{Ca} (mmol l^{-1})	2.45 ± 0.07	2.45 ± 0.07	NS	-
P_{Pi} (mmol l^{-1})	1.06 ± 0.07	1.12 ± 0.07	< 0.01	1.17 ± 0.07
P_{Osm} (mmol l^{-1})	274 ± 3	273 ± 3	NS	-
$^{+}$PRA (pg ml^{-1} hr^{-1})	1037 ± 705	1130 ± 730	NS	-

Values are given as mean ± S.D., n = 12. $^{+}$ Transformed variable expressed as geometric mean ± approximate S.D.; P = results of paired t-test, NS = not statistically significant ($P > 0.05$).

Abbreviations:- PS, plasma solids; Hct, haematocrit; P_{Na}, P_K, etc., plasma concentration; PRA, plasma renin activity.

The control period after CCK8 (U7)

Haemodynamic and plasma changes did not return towards basal values, but sodium and phosphate excretions did. Potassium excretion remained low. The persistence of changes after CCK8 infusion makes interpretation of such effects difficult, and if small, doubtful.

DISCUSSION

The effect of CCK8 on sodium and potassium excretion was qualitatively similar to pentagastrin, with an inverse relationship and increase in the urinary Na/K ratio. However in contrast, the urinary Na/Ca ratio was unaffected. The effect on renal sodium and potassium handling may depend upon the C-terminal tetrapeptide of CCK8 which is shared with pentagastrin (Fig. 3.1). The N-terminal extension might alter potency and specificity of action (Grossman, 1976). The changes in urinary Na/K and Na/Ca ratios in the rabbit during CCK8 infusion (Fig. 4.4) were more like the responses observed with pentagastrin in man rather than CCK8.

The actions of CCK8 on electrolyte excretion were not associated with any detectable change in GFR or ERPF. Distributional changes in blood flow within the kidney may occur, but there is considerable uncertainty as to the pattern of such changes and indeed whether they actually take place (Swales, 1975a). As with pentagastrin, these results do not allow any firm conclusions to be drawn with regard to the mechanism of this response, but do indicate the importance of the C-terminal tetrapeptide amino acid sequence and suggest the need for further definition of structure-activity relationships (see Chapter 11).

The increase in phosphate excretion is probably related to the

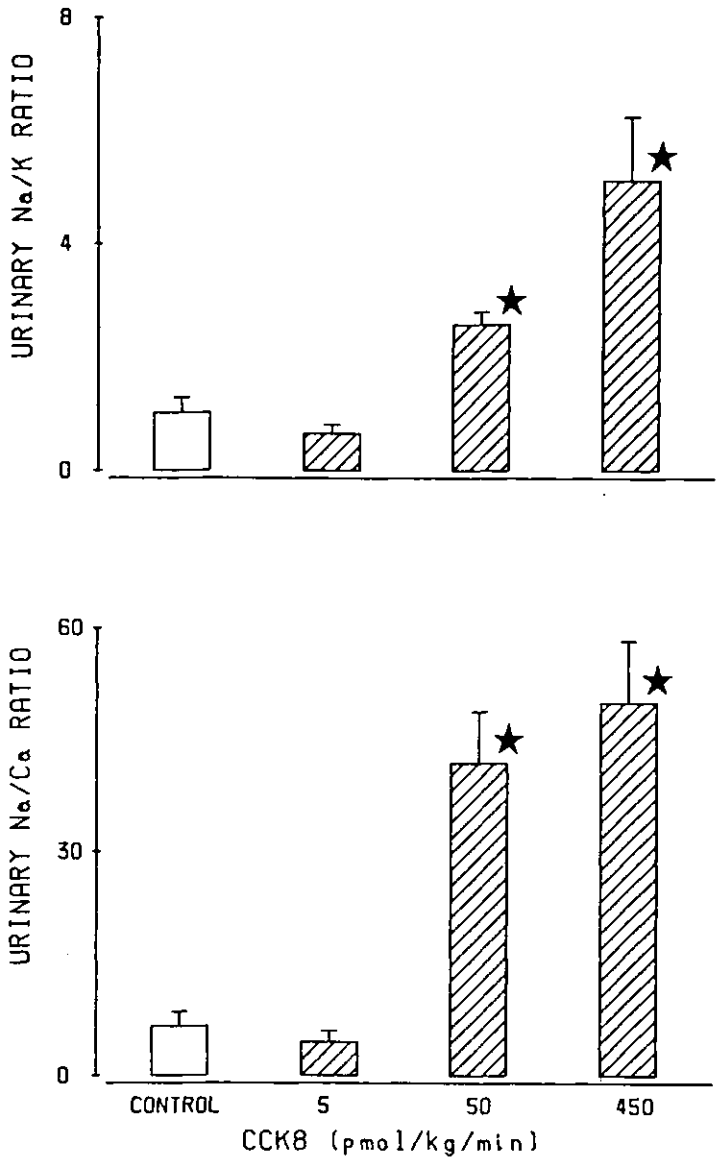


Fig. 4.4.

Effect of increasing doses of CCK8 on urinary sodium:potassium (Na/K) and sodium:calcium (Na/Ca) ratios in the rabbit. Results are expressed as mean ± S.E. of mean, n = 12 for each treatment group. * indicates $p < 0.05$ by Dunnett's test after analysis of variance.

rise in plasma phosphate concentration, but CCK-induced calcitonin and parathormone release may also be factors.

The 10% reduction in plasma solids during CCK8 infusion persisted and may have been dilutional through excessive water intake relative to urine output, although haematocrit did not change. Even so, this fall may have contributed to the increase in sodium and phosphate excretion, but does not explain the decrease in potassium excretion. Moreover, plasma solids continued to fall after CCK8 infusion, but sodium and phosphate excretion returned to basal values.

The dose of CCK8 infused in the present study was less than half the lowest dose given in the rabbit, but still produced a rise in plasma CCK8-LI concentration greater than reported after food (Dockray, 1982). In the rabbit, the lowest dose of 5 pmol kg^{-1} min^{-1}, which produced increases in plasma CCK8-LI levels slightly more than observed in the present study, did not alter renal function except by reducing PRA.

Once again, it is difficult to extrapolate between species, but the effects of CCK8 in the rabbit and of CCK8 and pentagastrin in man do suggest fairly specific actions on renal sodium and potassium handling and perhaps even calcium. The analogous effects of the potassium-sparing diuretics triamterene and amiloride may be relevant and these similarities may enable a clearer understanding of the actions of these peptides.

SUMMARY TO CHAPTER 4

1. Continuing the search for possible mediators of postprandial changes in renal function, the renal effects of infused CCK8 (sulphated) in 4 conscious rabbits and 6 human subjects were studied. CCK8 is chemically similar to pentagastrin and is the predominant form of CCK in plasma and gut endocrine cells, as well as central and peripheral neurones.

2. The doses used were 5, 50 and 450 pmol kg^{-1} min^{-1} in the rabbit and 2 pmol kg^{-1} min^{-1} in man. Plasma CCK8-like immunoreactivity concentrations were supraphysiological at <u>all</u> doses, according to previously reported postprandial levels.

3. In the rabbit, CCK8 produced a dose-related fall in PRA, plasma calcium concentration and mean arterial blood pressure. It also caused dose-dependent increases in ERPF, GFR and renal sodium excretion. In man, changes similar to pentagastrin were observed with an increase in sodium excretion and decrease in potassium excretion. No changes in PRA and plasma calcium concentration occurred, nor in blood pressure, ERPF and GFR.

4. The haemodynamic changes in the rabbit indicate that vasodilatation occurred during infusion of CCK8. As a renal vasodilator CCK8 is unusual in that it inhibits rather than stimulates renin release. Once again, in man, its renal effects resemble those of triamterene and amiloride. In this species at least, together with gastrin, CCK may be involved in renal responses to feeding.

CHAPTER 5

EFFECTS OF PANCREATIC GLUCAGON ON PLASMA ELECTROLYTES AND RENAL FUNCTION IN CONSCIOUS RABBITS

Effects of pancreatic glucagon on plasma electrolytes and renal function in conscious rabbits.

INTRODUCTION

Kimball & Murlin (1923) first detected the hyperglycaemic substance glucagon in aqueous bovine pancreatic extracts. This factor was later isolated and purified by Staub, Sinn & Behrens (1955) and shortly after this, the amino acid sequence of porcine glucagon was determined by Bromer, Sinn, Staub & Behrens (1956).

All mammalian glucagons are similar to porcine glucagon (Sundby, 1976). Glucagon is found within pancreatic A-cells and A-cells of the cat and dog oxyntic mucosa (Larsson, Holst, Håkanson & Sundler, 1975). Few A-cells are found within the gastrointestinal tract of man and rats, but glucagon-like immunoreactivity and immunoreactants (GLI: largely glicentin, a probable biosynthetic precursor of glucagon; Moody, Jacobsen & Sundby, 1978) have been found within endocrine-like cells of the ileum and colon (Larsson et al., 1975; Moody & Thim, 1981). These cells have been designated L-cells and are identified using N-terminal specific antisera. GLI does not react with C-terminal (pancreatic glucagon) specific antisera.

Although pancreatic glucagon has a well established role in glucose homeostasis, the actions of glucagon-like immunoreactants (GLIs) are at present speculative. Smaller GLIs, some of which could be artefacts produced during preparations for assay and may only appear in small amounts in peripheral blood, bind to glucagon receptors (Moody, 1980). These may therefore have glucagon-like effects on the liver (Moody, 1980). The larger GLIs, which do not bind to glucagon receptors, may have no action, or bind to as yet

unidentified GLI receptors with trophic effects or influences on gut motility (Moody, 1980).

While studying the fate of ^{131}I-labelled glucagon in vivo, Staub, Springs, Stoll & Elrick (1957) observed a greater rise in free iodide excretion when compared with excretion after an injection of an equivalent amount of inorganic ^{131}I alone. They concluded that glucagon increased free iodide excretion and proceeded to demonstrate an increase in electrolyte excretion and renal perfusion. This action was shown to be independent of hyperglycaemia and the natriuresis was thought to be due to a direct tubular effect of glucagon rather than increased filtered load (Staub et al., 1957; Elrick, Huffman, Hlad, Whipple, Staub & Staub, 1958; Elrick, Whipple, Arai & Hlad, 1959). Since then, there has been much experimental work to confirm the natriuretic effect of pharmacological doses of glucagon (Katz & Lindheimer, 1977; Uranga, Fuenzalida, Rapoport & del Castillo, 1977; Kirschenbaum & Zawada, 1980), but uncertainty remains as to its exact mode of action. Several workers believe glucagon to have a direct tubular action, while others regard the increase in glomerular filtration rate and filtered load to be sufficient explanation (Katz & Lindheimer, 1977).

Carey (1978) suggested that the differential natriuresis following oral versus intravenous routes of saline administration may involve release of a gut natriuretic hormone such as glucagon (Chapter 1). Glucagon has also been implicated in starvation natriuresis (Spark, Arky, Boulter, Saudek & O'Brian, 1975) and plasma levels observed during fasting in man (expressed in pg ml^{-1} using pancreatic glucagon specific antiserum) have been shown to be associated with increased renal sodium excretion when reproduced by infusion of pancreatic glucagon (Saudek, Boulter & Arky, 1973).

High circulating levels of glucagon are found in patients with renal failure and under normal conditions, renal uptake of glucagon is high (Lefebvre et al., 1974). This may relate to its action on renal electrolyte handling and a physiological contribution to salt and water balance (Lefebvre et al., 1974). Hyperglucagonaemia is also found in patients with liver cirrhosis (Bloom & Polak, 1982; Levy, 1978). In these patients the elevated levels appear to be due to "true" glucagon, whereas in renal failure biologically inactive fragments are also measured (Christofides, 1982a).

Therefore it was decided to examine the renal effects of intravenous pancreatic glucagon in conscious rabbits as a preliminary to studies in man. Pancreatic glucagon was infused at doses of 1, 10 and 1000 ng kg^{-1} min^{-1}.

RESULTS

Systemic blood pressure and heart rate (Fig. 5.1)

No significant change in mean arterial blood pressure occurred. There was a dose-related rise in heart rate with a maximum rise of 30 beats min^{-1} during infusion of the high dose (1000 ng kg^{-1} min^{-1}).

Renal haemodynamics (Fig. 5.2)

Figure 5.2 shows the 30% fall in ERPF of GFR at the low and middle doses of glucagon (1 and 10 ng kg^{-1} min^{-1}, respectively) and the rise in GFR and filtration fraction during infusion of the high dose.

Urine composition (Table 5.1)

The fractional excretions of sodium and chloride fell by half during infusion of the high dose, but were little changed by infusion

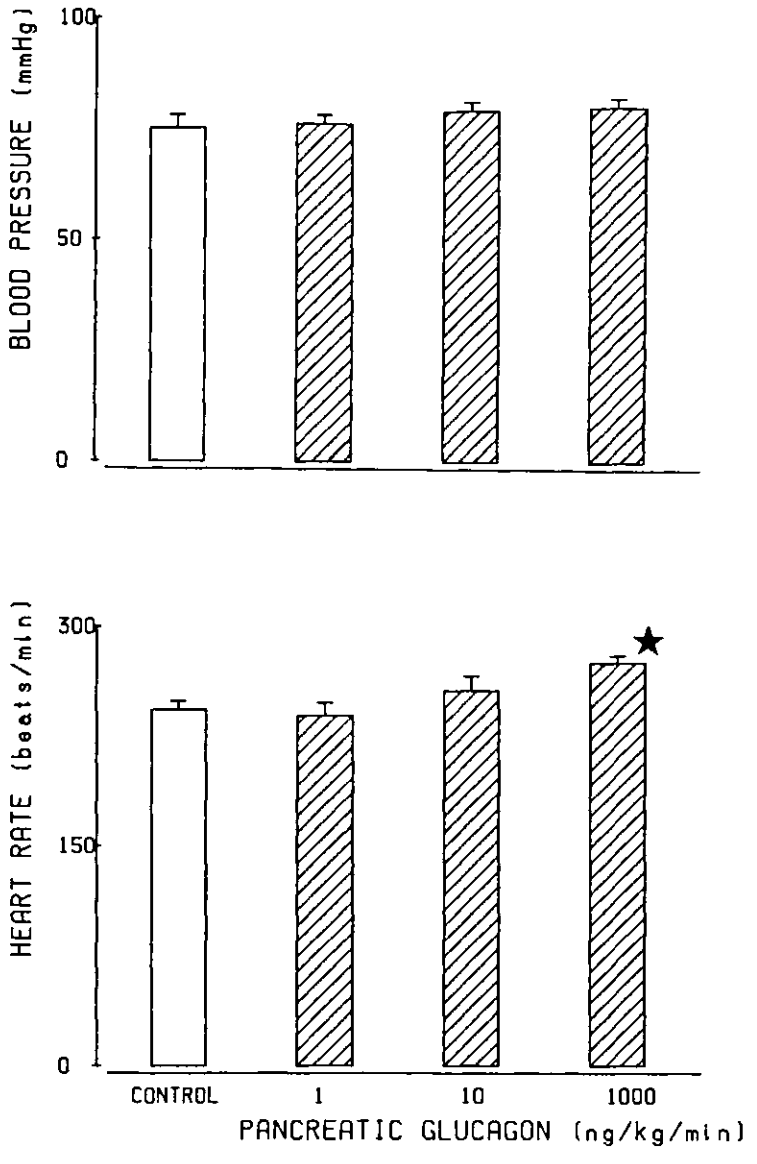


Fig. 5.1.

Effect of increasing doses of pancreatic glucagon on heart rate and mean arterial blood pressure. Results are expressed as mean ± S.E. of mean, n = 27 for control group and n = 15 for each treatment group. * indicates significant difference ($p < 0.05$) from control value as determined by Dunnett's test after analysis of variance.

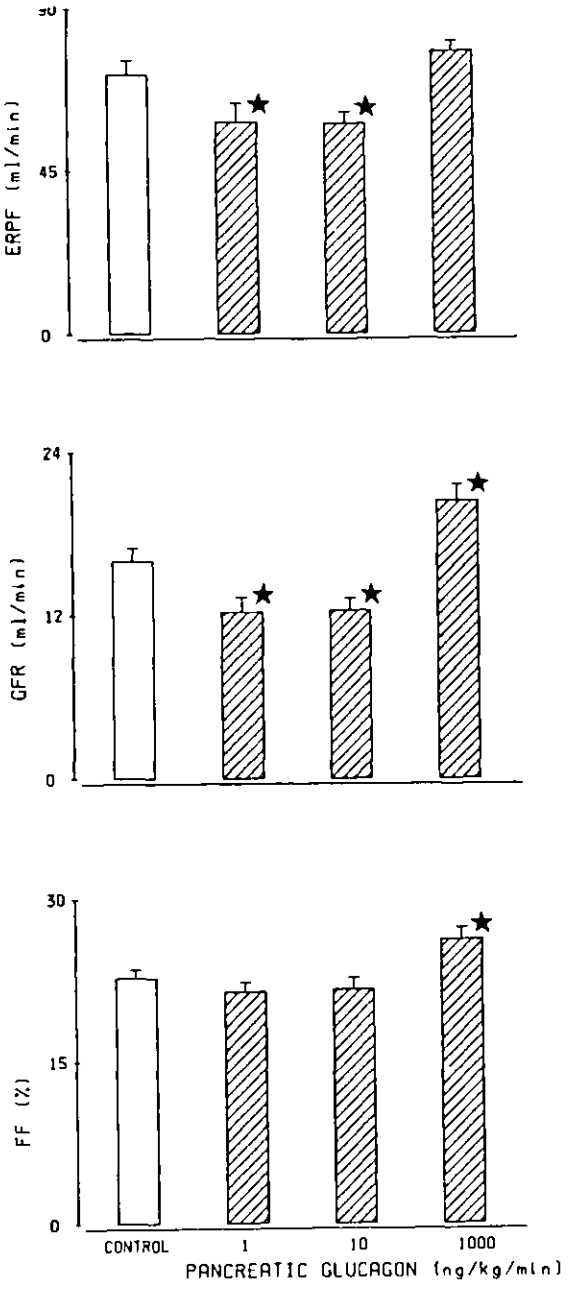


Fig. 5.2.

Effect of increasing doses of pancreatic glucagon on effective renal plasma flow (ERPF), glomerular filtration rate (GFR) and filtration fraction (FF). Results are expressed as mean ± S.E. of mean, n = 27 for control group and n = 15 for each treatment group. * indicates significant difference ($p < 0.05$) from control value as determined by Dunnett's test after analysis of variance.

Table 5.1.

Effects of pancreatic glucagon on fractional electrolyte excretion, urine flow and solute excretion.

	Control (vehicle only)	Glucagon in ng kg^{-1} min^{-1} 1 (low)	10 (middle)	1000 (high)	n
$^{+}FE_{Na}$ (%)	0.65 ± 0.07	0.76 ± 0.14	0.49 ± 0.15	*0.32 ± 0.06	(27) 15
$^{+}FE_{K}$ (%)	18.9 ± 1.8	17.8 ± 2.7	13.7 ± 1.9	13.9 ± 1.9	(27) 15
$^{+}FE_{Cl}$ (%)	0.64 ± 0.08	0.94 ± 0.15	0.88 ± 0.14	**0.30 ± 0.06	(27) 15
$^{+}FE_{Ca}$ (%)	1.41 ± 0.21	2.18 ± 0.43	2.18 ± 0.60	1.35 ± 0.26	(26) 15
$^{+}FE_{Pi}$ (%)	8.48 ± 1.06	3.36 ± 1.54	5.26 ± 1.64	6.31 ± 1.74	(20) 8
$^{+}\dot{V}$ (µl min^{-1})	354.8 ± 60.1	336.1 ± 95.2	242.0 ± 71.6	167.5 ± 30.2	(27) 15
$^{+}C_{Osm}$ (µl min^{-1})	310.0 ± 17.6	261.2 ± 30.3	232.3 ± 23.0	296.1 ± 42.5	(27) 15
C_{H_2O} (µl min^{-1})	163.5 ± 60.6	216.0 ± 73.5	199.6 ± 76.8	*-126.6 ± 55.4	(27) 15

Values are expressed as mean ± S.E. of mean. $^{+}$Transformed variable, in which case the geometric mean and approximate S.E. of mean are given. n = number of observations, n in brackets is number in control group. * and ** signify $P < 0.05$ and < 0.01 respectively, when compared with the control value by Dunnett's test after analysis of variance. Abbreviations are as follows:- FE, fractional excretion; $\dot{V}$, urine flow; C_{Osm}, osmolar clearance; C_{H_2O}, free water clearance.

of the low and middle doses. The fractional excretions of potassium, calcium and phosphate did not alter significantly at any dose. There was an apparent dose-dependent fall in urine flow, but this did not reach statistical significance. Osmolar clearance did not change, but free water clearance fell during the high dose infusion.

Plasma composition and haematocrit (Table 5.2)

Table 5.2 shows the changes in plasma composition and haematocrit during glucagon infusion, including the range of plasma glucagon (pancreatic) concentrations recorded at each dose. During high dose infusion, there were falls in plasma sodium, potassium, chloride, calcium and phosphate concentrations. Plasma osmolality rose in proportion to the rise in plasma glucose concentration. These changes did not appear to be dose-related, although plasma sodium concentration also fell during infusion of the middle dose of glucagon. There were no significant changes in plasma solids or haematocrit. Plasma renin activity (PRA) almost doubled during infusion of the high dose (Fig. 5.3).

DISCUSSION

Glucagon is known to increase cardiac contractility and rate in man and anaesthetised dogs (Parmley, Glick & Sonnenblick, 1968; Lucchesi, 1968), although earlier studies could only demonstrate these actions in isolated heart preparations and demonstrated inhibition by beta-receptor blockade with dichloroisoproterenol (Farah & Tuttle, 1960). However, prior treatment with propranolol has no effect and the cardiac actions of glucagon do not seem to require endogenous catecholamine release (Lucchesi, 1968; Murad & Vaughan, 1969). This is in contrast to the related peptide

Table 5.2. Effects of pancreatic glucagon on plasma composition and haematocrit (legend overleaf).

	Control (vehicle only)	Glucagon in ng kg^{-1} min^{-1} 1 (low)	10 (middle)	1000 (high)	n
Pancreatic glucagon (pmol l^{-1})	10 - 16	12 - 23	22 - 42	3190 - 4127	5
P_{Na} (mmol l^{-1})	135.0 ± 0.4	135.9 ± 0.8	**132.4 ± 0.5	**132.5 ± 0.7	(27) 15
P_{K} (mmol l^{-1})	3.54 ± 0.04	3.67 ± 0.11	3.59 ± 0.10	*3.34 ± 0.05	(27) 15
P_{Cl} (mmol l^{-1})	98.2 ± 0.7	95.5 ± 1.4	96.5 ± 0.84	**93.5 ± 1.1	(27) 15
P_{Ca} (mmol l^{-1})	2.84 ± 0.06	3.08 ± 0.06	2.94 ± 0.06	*2.63 ± 0.06	(26) 15
P_{Pi} (mmol l^{-1})	0.95 ± 0.05	*0.77 ± 0.05	0.80 ± 0.05	**0.77 ± 0.05	(21) 8
P_{Glu} (mmol l^{-1})	10.8 ± 0.6	9.9 ± 0.6	12.8 ± 0.6	**26.9 ± 2.0	(9) 5
P_{Osm} (m Osm kg^{-1})	280 ± 2	284 ± 2	286 ± 3	**290 ± 2	(27) 15
PS (%)	5.19 ± 0.23	5.04 ± 0.30	4.31 ± 0.34	4.82 ± 0.43	(18) 9
Hct (%)	33 ± 2	32 ± 1	30 ± 2	29 ± 1	(8) 5
$^{+}$PGPS (%)	6.53 ± 0.32	6.23 ± 0.37	5.31 ± 0.38	6.31 ± 0.72	(18) 9

Table 5.2.

Values are expressed as mean ± S.E. of mean. $^{+}$Transformed variable, in which case the geometric mean and approximate S.E. of mean are given. n = number of observations, n in brackets is number in control group. * and ** signify $P < 0.05$ and < 0.01 respectively, when compared with the control value by Dunnett's test after analysis of variance. Abbreviations are as follows:- P_{Na}, P_{K}, etc. plasma concentration; PS, plasma solids; Hct, haematocrit; PGPS, post-glomerular plasma solids.

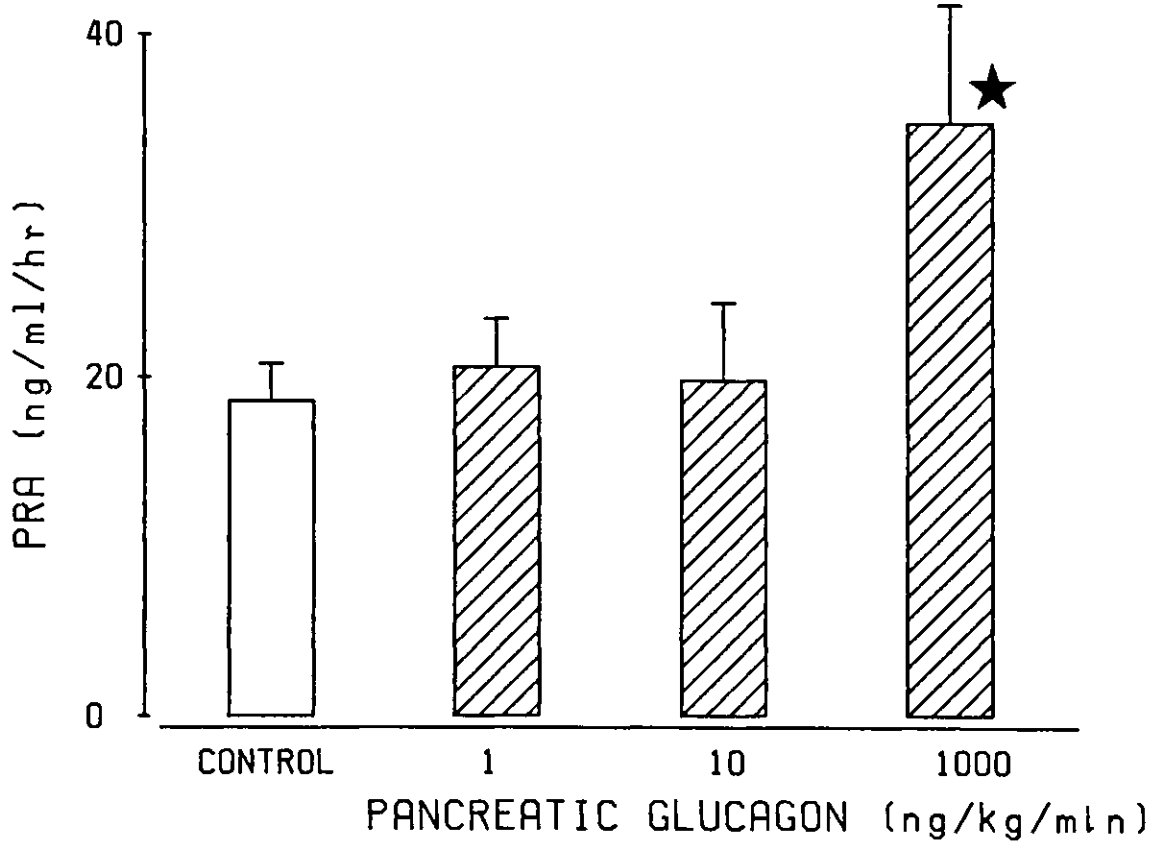


Fig. 5.3.

Effect of increasing doses of pancreatic glucagon on plasma renin activity (PRA). This variable was found to be lognormally distributed and therefore results are expressed as geometric mean ± approximate S.E. of mean, n = 18 for control group and n = 10 for each treatment group. * indicates significant difference ($p < 0.01$) from control value as determined by Dunnett's test after analysis of variance.

VIP (vasoactive intestinal polypeptide; Chapter 6.3).

Blood pressure did not change, but ERPF and GFR fell during infusion of the low and middle doses of glucagon. This indicates a rise in renal vascular resistance and as filtration fraction did not change significantly, this was probably due to afferent arteriole vasoconstriction. Although inconsistent effects of glucagon on GFR have been reported (Katz & Lindheimer, 1977), it is well known to produce renal vasodilatation when given both intravenously and intrarenally, (Katz & Lindheimer, 1977; Ueda, Nakanishi, Miyazaki & Abe, 1977) and its site of action is believed to be largely pre-glomerular. However in this study, even during infusion of the high dose of glucagon, achieving plasma levels between 50 and 100 times those found after a meal in man (Holst, 1978), ERPF did not increase significantly, although GFR did; this suggests pre-glomerular vasodilatation. The fall in renal perfusion at the higher doses may reflect selective vasodilatation of some vascular beds resulting in reduced perfusion of others, perhaps by reflex sympathetic stimulation to maintain blood pressure. Heart rate did rise at the middle dose, but not during infusion of the low dose of glucagon. These disimilarities with previous work may be due to species differences, absence of anaesthetic or the different doses used.

The middle dose produced circulating levels of pancreatic glucagon comparable to those of pancreatic-type glucagon found after a mixed meal in normal man (Holst, 1978). Similar changes (lower basal values) have also been reported during insulin-induced hypoglycaemia (Christofides, 1982a). This dose produced a small rise in heart rate and a 20% fall in ERPF, but no change in fractional electrolyte

excretion. A similar dose in man has been shown to inhibit jejunal salt and water absorption (Hicks & Turnberg, 1974).

The high dose of glucagon did produce a 30% increase in GFR, but a decrease in fractional excretion of sodium and chloride. This latter effect was probably related to afferent arteriole vasodilatation and the relative rise in post-glomerular renal resistance (increased filtration fraction, although PGPS did not rise significantly), rather than any direct antinatriuretic action of glucagon. Moreover, this effect occurred in the presence of marked hyperglycaemia which, with glycosuria, would be expected to promote a natriuresis. Also, urine flow and free water clearance fell while osmolar clearance remained essentially unchanged. This may indicate increased arginine vasopressin secretion, but is again more likely to reflect the relative changes in the pre- and post-glomerular circulations, with associated changes in peritubular capillary physical factors (Chapter 1). The insulin response to glucagon-induced hyperglycaemia may be important here, as these hormones have antagonistic actions and there have been several reports of insulin producing an antidiuresis and antinatriuresis (Miller & Bogdonoff, 1954; Murdaugh, Robinson & Doyle, 1959; Nizet, Lefebvre & Crabbe, 1971).

The associated rise in PRA and by inference, increased aldosterone secretion, is unlikely to have influenced renal sodium handling as the renal action of aldosterone is probably too slow (> 8 hr; Lennane, Stockigt & Peart, 1976). This dose also reduced plasma potassium, phosphate and calcium concentrations. Glucagon has been previously reported to lower serum potassium and phosphate concentrations in the rabbit, presumably through increased cellular influx (Natelson et al., 1963). This may also occur with calcium,

as glucagon has been reported to produce hypocalcaemia, probably by stimulation of calcitonin release (Avioli, Birge, Scott & Shieber, 1969; Care, Bates, Phillippo, Lequin, Hackeny, Barlet & Larvor, 1970).

PRA rose during the high dose infusion only. Glucagon-induced renin release is believed to occur via a direct renal action related to its vasodilator effect (Vandongen, Peart & Boyd, 1973; Peart, 1978). Although no significant increase in ERPF occurred, the marked rise in GFR during high dose infusion suggests afferent arteriole vasodilatation. Both hypokalaemia and hypocalcaemia may have contributed to the increased renin secretion (Himathongkam, Dluhy & Williams, 1975; Fray, 1977).

It is not possible from this study to infer that glucagon has any physiological role in the regulation of renal function. Most changes were produced by a pharmacological dose of glucagon, but even this did not alter renal sodium handling in the conscious rabbit. Finally, the effects of glucagon are qualitatively similar to those of the related peptide, VIP (Chapter 6.1).

SUMMARY TO CHAPTER 5

1. It has been suggested that the differential natriuresis following oral and intravenous routes of saline administration may involve release of a gut natriuretic hormone such as glucagon. Glucagon has also been implicated in starvation natriuresis. High circulating levels of glucagon-like immunoreactivity have been found in patients with renal insufficiency and liver disease.

2. The renal actions of pancreatic glucagon were studied in 5 conscious rabbits by infusing intravenously doses of 1, 10 and 1000 ng kg^{-1} min^{-1}.

3. Pancreatic glucagon produced a dose-dependent rise in heart rate, but no change in blood pressure. ERPF fell during infusion of the low and middle doses and GFR increased at the high dose. Infusion of the high dose also led to a fall in plasma sodium, potassium, calcium and phosphate concentrations, but increases in PRA and plasma glucose concentration. At this dose, fractional excretion of sodium and chloride decreased. The plasma glucagon (pancreatic) concentrations achieved during infusion of the middle dose, approximated previously reported postprandial levels.

4. These results do not indicate a natriuretic effect of pancreatic glucagon, but together with the fall in plasma electrolyte concentration, are qualitatively similar to the actions of the related peptide VIP.

CHAPTER 6

PART 1:

EFFECTS OF VASOACTIVE INTESTINAL POLYPEPTIDE (VIP) ON RENAL FUNCTION AND PLASMA RENIN ACTIVITY IN THE CONSCIOUS RABBIT.

Effects of vasoactive intestinal polypeptide (VIP) on renal function and plasma renin activity in the conscious rabbit.

INTRODUCTION

Vasoactive intestinal polypeptide (VIP) was originally isolated from porcine duodenum by Said & Mutt (1970a,b). Since then it has been found to be distributed widely throughout the gut, but was initially thought to be present only in endocrine cells (Polak, Pearse, Garaud & Bloom, 1974). VIP is now considered to be primarily located in neurones throughout the central and peripheral nervous system (Larsson, Fahrenkrug, Schaffalitzky de Muckadell, Sundler, Håkanson & Rehfeld, 1976; Giachetti, Said, Reynolds & Koniges, 1977; Bryant, Bloom, Polak, Albuquerque, Modlin & Pearse, 1976), chiefly associated with small blood vessels, smooth muscle cells and ganglia of the gastrointestinal and genitourinary tracts (Uddman, Alumets, Edvinsson, Håkanson & Sundler, 1981; Jessen, Polak, van Noorden, Bloom & Burnstock, 1980; Alm, Alumets, Håkanson, Owman, Sjöberg, Sundler & Walles, 1980; Larsson, Fahrenkrug & Schaffalitzky de Muckadell, 1977a,b).

VIP is a basic 28-amino acid peptide, structurally related to secretin, glucagon, and gastric inhibitory peptide (GIP) (Fig. 6.1). It is known to affect electrolyte and water transport across intestinal and gallbladder epithelium (Barbezat & Grossman, 1971; Mailman, 1978; Camilleri, Cooper, Adrian, Bloom & Chadwick, 1981; Wood, Brennan & Hormbrey, 1982) and to produce local vasodilatation and smooth muscle relaxation (Said, 1974, 1981). Nerve fibres containing VIP-like material were recently found within the guinea pig renal cortex adjacent to small blood vessels (Hökfelt et al.,

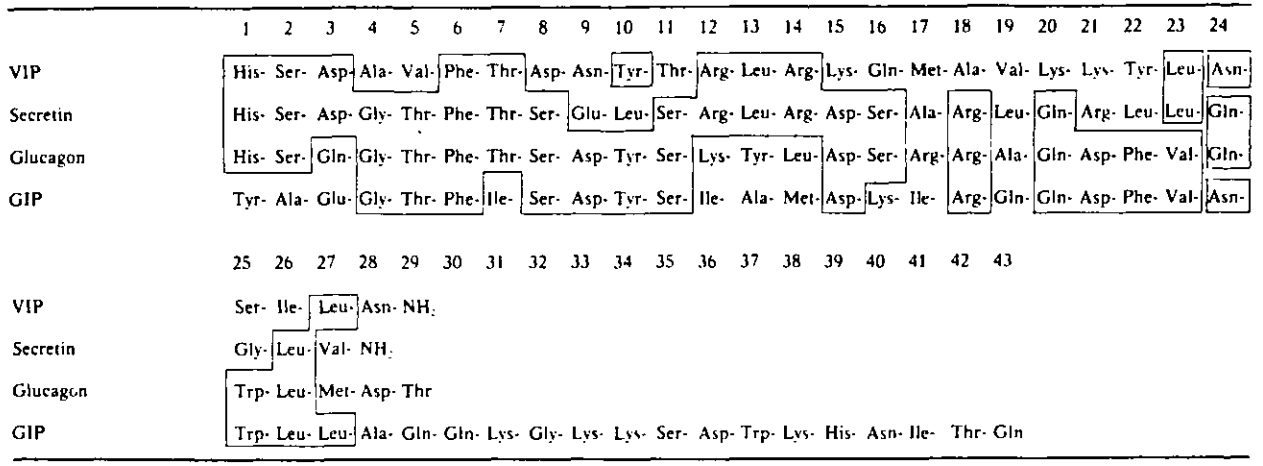


Fig. 6.1.

The amino acid sequence of VIP, secretin, glucagon and GIP (all from pig). Identical residues are grouped into boxes (taken from Fahrenkrug, 1980).

1978) and it has since been shown that VIP is a potent stimulus to release of renin (Porter, Reid, Said & Ganong, 1980).

The effects of VIP have largely been studied in vitro or in anaesthetised animals. Therefore it was decided to examine the renal effects of VIP in the conscious rabbit. VIP was infused at doses of 1, 10 and 25 pmol kg^{-1} min^{-1}.

RESULTS

Systemic blood pressure and heart rate (Fig. 6.2)

A mean rise of 10 mm Hg occurred during infusion of the middle dose (10 pmol kg^{-1} min^{-1}). There was a dose-related rise in heart rate and a maximum rise of 100 beats min^{-1} during infusion of the high dose (25 pmol kg^{-1} min^{-1}).

Renal Haemodynamics

Figure 6.3 shows the appreciable fall in ERPF and GFR at the middle and high doses of VIP and the small, but significant, rise in filtration fraction at the middle dose. No changes occurred during infusion of the low dose (1.0 pmol kg^{-1} min^{-1}).

Urine composition (Table 6.1)

The fractional excretions of sodium, potassium and chloride approximately doubled during infusion of the high dose, but were little changed by infusion of the low and middle doses of VIP. The fractional excretion of calcium did not alter at any dose, but the fractional excretion of phosphate fell during infusion of the high dose. There were dose-dependent falls in urine flow rate, osmolar clearance and free water clearance. Urine pH fell at each dose of VIP, but this was not significantly different from the control value

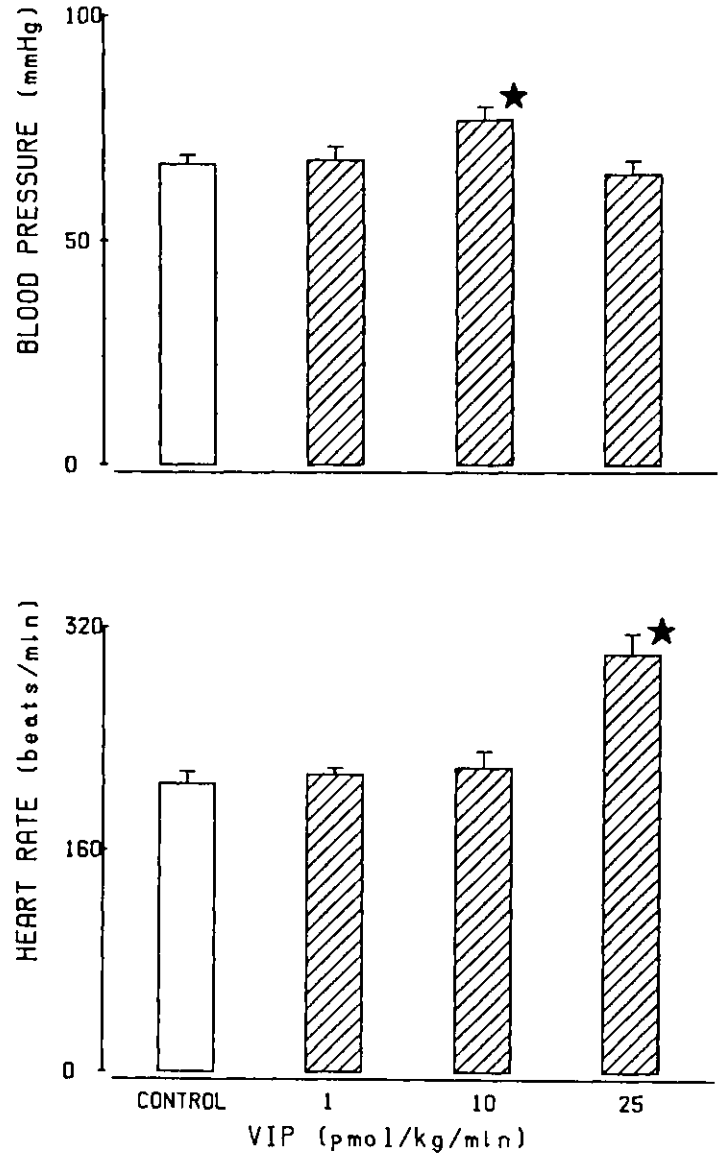


Fig. 6.2.

Effect of increasing doses of VIP on heart rate and mean arterial blood pressure. Results are expressed as mean ± S.E. of mean, n = 12 for each treatment group. * indicates significant difference ($p < 0.05$) from control value as determined by Dunnett's test after analysis of variance.

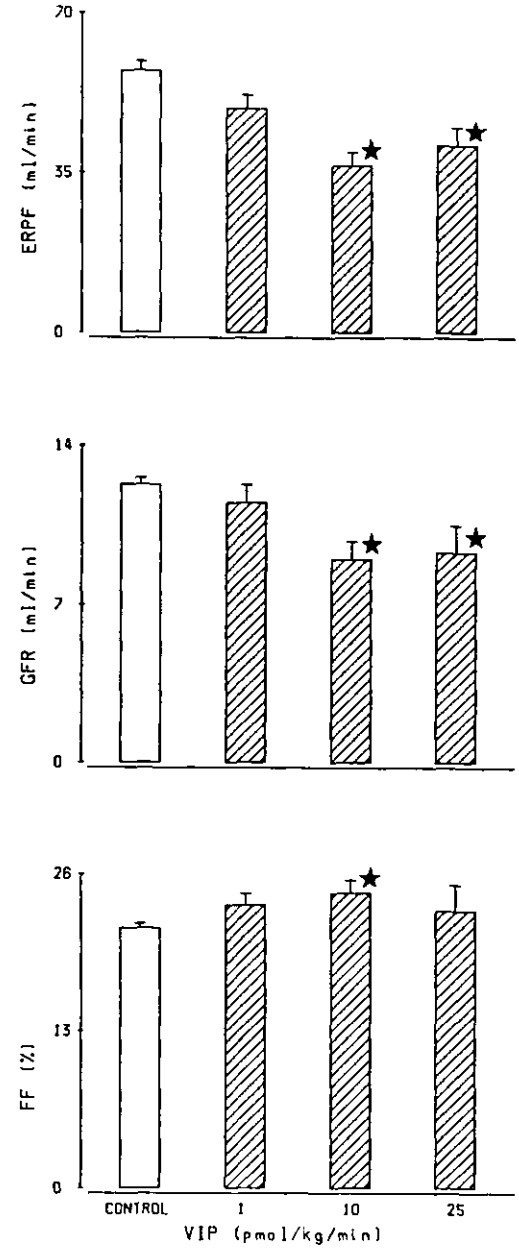


Fig. 6.3.

Effect of increasing doses of VIP on effective renal plasma flow (ERPF), glomerular filtration rate (GFR) and filtration fraction (FF). Results are expressed as mean ± S.E. of mean, n = 12 for each treatment group. * indicates significant difference ($p < 0.05$) from control value as determined by Dunnett's test after analysis of variance.

Table 6.1.

Effects of VIP on fractional electrolyte excretion, urine flow, solute excretion and urine pH.

	Control (vehicle only)	VIP in pmol kg^{-1} min^{-1} 1 (low)	10 (middle)	25 (high)	n
$^{+}FE_{Na}$ (%)	0.32 ± 0.08	0.52 ± 0.06	0.41 ± 0.06	*0.61 ± 0.13	12
$^{+}FE_{K}$ (%)	12.7 ± 1.5	12.3 ± 0.8	11.8 ± 0.6	**23.7 ± 2.8	12
$^{+}FE_{Cl}$ (%)	0.69 ± 0.09	0.87 ± 0.05	0.51 ± 0.12	*1.25 ± 0.20	12
$^{+}FE_{Ca}$ (%)	2.10 ± 0.60	1.92 ± 0.46	1.26 ± 0.31	1.36 ± 0.36	12
$^{+}FE_{Pi}$ (%)	10.3 ± 0.8	11.0 ± 0.9	9.64 ± 0.39	**4.5 ± 0.86	12
$^{+}\dot{V}$ ($\mu l\ min^{-1}$)	410.4 ± 66.0	324.3 ± 43.8	**62.7 ± 7.1	**80.1 ± 17.9	12
$^{+}C_{Osm}$ ($\mu l\ min^{-1}$)	210.8 ± 18.7	161.1 ± 16.9	**107.2 ± 15.9	*146.8 ± 23.5	12
C_{H_2O} ($\mu l\ min^{-1}$)	243.7 ± 56.7	185.7 ± 53.4	**-50.2 ± 13.8	*-48.8 ± 44.7	12
pH	6.61 ± 0.20	**5.71 ± 0.15	**5.38 ± 0.25	5.91 ± 0.23	12

Values are expressed as mean ± S.E. of mean. $^{+}$ Transformed variable, in which case the geometric mean and approximate S.E. of mean is given. n = number of observations. * and ** signify $P < 0.05$ and < 0.01 respectively, when compared with the control value by Dunnett's test after analysis of variance. Abbreviations are as follows:- F.E., fractional excretion; $\dot{V}$, urine flow; C_{Osm}, osmolar clearance; C_{H_2O}, free water clearance.

during infusion of the high dose. Although the reduction in urine flow during infusion of the middle and high doses led to fluid retention, recovery began after stopping the infusion of VIP (Fig. 6.4) with the result that no weight gain had occurred by the end of each experiment.

Plasma composition and haematocrit (Table 6.2)

Table 6.2 shows the changes in plasma composition and haematocrit during VIP infusion, including the range of plasma VIP concentrations achieved at each dose. During infusion of the high dose, there were falls in plasma sodium, potassium and phosphate concentrations and plasma osmolality. At this dose plasma solids rose and this was mirrored by a rise in PGPS. These changes appeared to be dose-related, although at the low dose of VIP, plasma potassium concentration increased slightly. There were no significant changes in plasma chloride and glucose concentrations. In spite of the rise in plasma solids, there was no change in plasma calcium concentration. An increase would be expected if all the changes were entirely due to loss of plasma water and increased concentration of albumin and other plasma proteins. Figure 6.5 shows the large dose-dependent increase in plasma renin activity (PRA).

DISCUSSION

VIP-induced tachycardia appears to be due to reflex sympathetic activation or direct beta-adrenoceptor stimulation, as it is abolished by pre-treatment with propranolol (see Chapter 6.3).

The decrease in ERPF in the absence of a fall in blood pressure indicates a rise in renal vascular resistance. As there was little change in filtration fraction, this rise was largely due to afferent

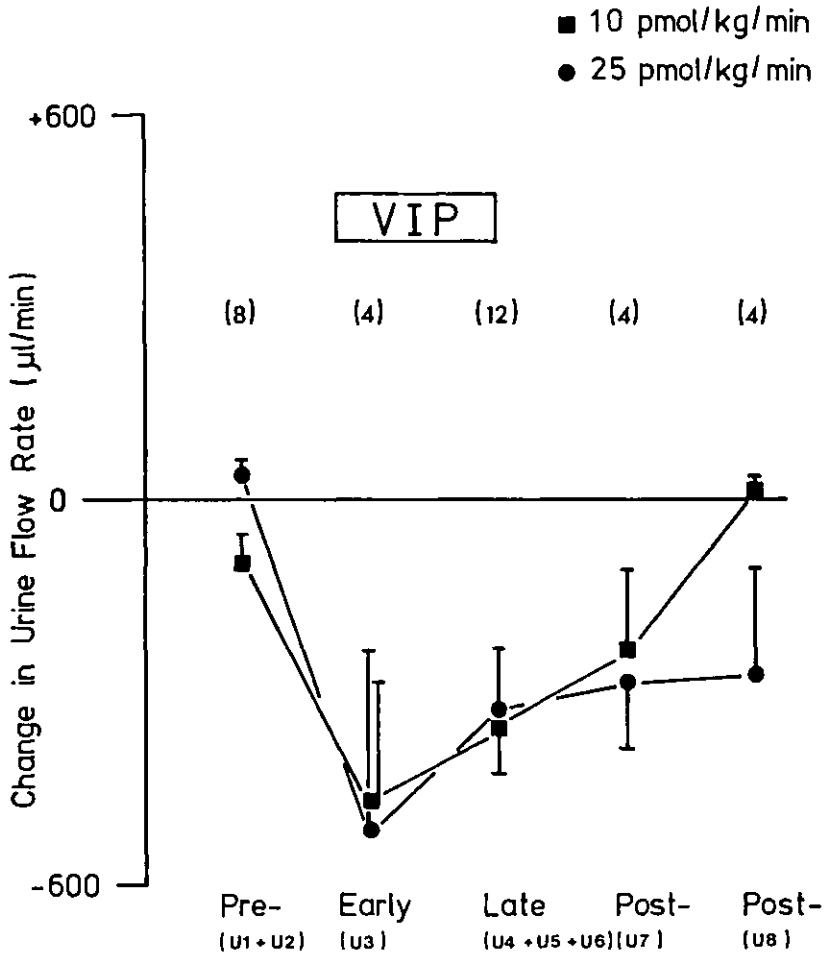


Fig. 6.4.

Effect of the middle and high doses of VIP on urine flow, expressed as the difference from the corresponding control value. Flow fell during VIP infusion, but began to recover after the infusion ended. Urine flow was found to be lognormally distributed and therefore values are given as geometric mean ± approximate S.E. of mean. For ease of presentation, infusions are divided into 5 periods and the observations made during each collection pooled as shown in brackets. Numbers in parentheses indicate n of observations for each period.

Table 6.2. Effects of VIP on plasma composition and haematocrit.

	Control (vehicle only)	VIP in pmol kg^{-1} min^{-1} 1 (low)	10 (middle)	25 (high)	n
VIP (pmol l^{-1})	<10	40 - 70	300 - 460	800 - 2000	4
P_{Na} (mmol l^{-1})	135.7 ± 0.7	135.8 ± 1.1	134.6 ± 1.2	**132.6 ± 0.5	12
P_K (mmol l^{-1})	3.73 ± 0.04	*3.98 ± 0.08	3.79 ± 0.04	**3.39 ± 0.11	12
P_{Cl} (mmol l^{-1})	102.3 ± 0.8	100.4 ± 0.6	100.3 ± 1.4	100.2 ± 1.0	12
P_{Ca} (mmol l^{-1})	2.79 ± 0.03	2.89 ± 0.05	2.77 ± 0.09	2.85 ± 0.06	12
P_{Pi} (mmol l^{-1})	1.56 ± 0.03	1.48 ± 0.04	1.50 ± 0.02	**1.25 ± 0.06	12
P_{Glu} (mmol l^{-1})	8.2 ± 0.3	9.7 ± 1.4	7.9 ± 0.3	10.0 ± 0.8	8
P_{Osm} (m Osm kg^{-1})	284 ± 2	278 ± 2	*271 ± 4	**264 ± 1	12
PS (%)	6.72 ± 0.23	6.50 ± 0.15	6.59 ± 0.23	**7.93 ± 0.24	8
Hct (%)	36 ± 2	*32 ± 1	36 ± 1	36 ± 2	8
+PGPS (%)	8.50 ± 0.31	8.39 ± 0.19	8.73 ± 0.39	**10.59 ± 0.49	8

Values are expressed as mean ± S.E. of mean. + Transformed variable, in which case the geometric mean and approximate S.E. of mean is given. n = number of observations. * and ** signify $P < 0.05$ and < 0.01 respectively, when compared with the control value by Dunnett's test after analysis of variance. Abbreviations are as follows:- P_{Na}, P_K etc., plasma concentration; PS, plasma solids; Hct, haematocrit; PGPS, post-glomerular plasma solids.

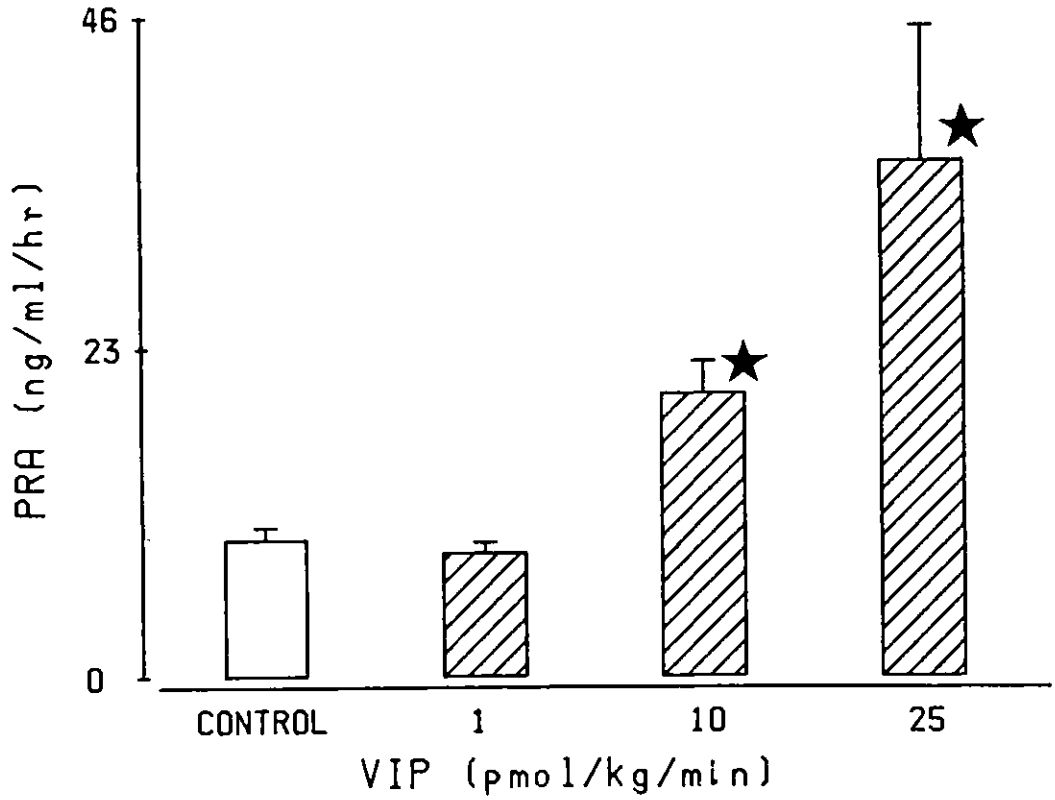


Fig. 6.5.

Effect of increasing doses of VIP on plasma renin activity (PRA). This variable was found to be lognormally distributed and therefore results are expressed as geometric mean ± approximate S.E.of mean, n = 8 for each treatment group. * indicates significant difference ($p < 0.01$) from control value as determined by Dunnett's test after analysis of variance.

arteriole vasoconstriction. A direct vasoconstrictor effect of VIP seems unlikely in view of its well-established vasodilating action (Said, 1981). However, this may be selective (Said, 1981), leading to reduced perfusion of other vascular beds. Reflex sympathetic stimulation secondary to peripheral vasodilatation may produce renal vasoconstriction, but this seems unlikely since VIP-induced renin release is not blocked by propranolol (Chapter 6.3). Systemic VIP is known to raise plasma prolactin levels (Fahrenkrug & Emson, 1982) and in physiological concentrations, prolactin has been shown to reduce renal perfusion and increase renal vascular resistance (Bliss & Lote, 1982). In constrast, Porter et al. (1980) have reported a rise in renal blood flow during intrarenal VIP infusion in anaesthetised dogs, but increased sympathetic tone during anaesthesia (Price, 1966) is likely to alter vascular reactivity to vasoactive substances such as VIP (Uddman et al., 1981).

Reduced renal perfusion would explain the corresponding fall in urine flow but the increased fractional excretion of sodium, potassium and chloride, suggests a direct renal tubular action of VIP. Rosa, Stoff, Silva & Epstein (1977) have reported tubular diuresis during VIP infusion into the isolated rat kidney and the data of Porter & Ganong (1982) showed an increase in renal sodium and potassium excretion during intrarenal VIP infusion (up to 10 pmol kg^{-1} min^{-1}) in the anaesthetised dog. In the latter study, there was no significant change in GFR, but a modest rise in renal blood flow. The present changes in renal electrolyte excretion are the more impressive as they are opposed by the renal haemodynamic changes and the rise in PGPS.

The fall in urine pH at each dose of VIP may be due to a direct

effect on distal tubular H^+ secretion. A similar effect has been observed in man (Chapter 6.2).

The changes in plasma composition are complex and not easily explained. The fall in plasma osmolality suggests plasma dilution, but the rise in plasma solids indicates loss of plasma water and VIP has been shown to produce oedema in rabbit skin (Williams, 1982). Although haematocrit did not alter, this may reflect red cell sequestration perhaps due to splanchnic pooling rather than a change in red cell size (Pessina, 1971; Kaplan & Timmons, 1979). The fall in plasma osmolality is greater than predicted from the change in measured electrolytes; plasma glucose and chloride concentrations did not change. An unmeasured plasma constituent such as bicarbonate might have decreased in concentration. Plasma water may have been lost, hence the rise in plasma solids, but the loss of plasma electrolytes, perhaps into cells, must have been greater. The homologous peptide glucagon is thought to reduce plasma potassium and phosphate in the rabbit by cellular influx (Natelson et al., 1963; Chapter 5).

The renin response to VIP is probably due to the fall in renal perfusion and activation of the intrarenal baroreceptor (Davis & Freeman, 1976). Reflex sympathetic stimulation is unlikely to be involved as propranolol does not alter the renin response to VIP (Chapter 6.3). Changes in the fractional excretion of sodium and chloride seem unimportant, as PRA rose at the middle dose of VIP when no changes occurred. Release of renin is unlikely to be secondary to hypokalaemia, as a marked rise occurred during infusion of the middle dose of VIP with no change in plasma potassium concentration. Finally, a direct effect of VIP cannot be excluded (Porter, Said & Ganong, 1982).

Although VIP is not thought to be physiologically a circulating hormone (Mitchell & Bloom, 1978), the high plasma levels of VIP achieved might mimic the changes produced if VIP was released locally from nerves. Situations do exist in which circulating levels of VIP are high, such as in patients with VIPomas, liver disease and after extensive small bowel resection (Welbourn, Wood, Polak & Bloom, 1981; Hunt, Carlos, Vaamonde, Rattassi, Berian, Said & Papper, 1979; Lezoche, 1978). VIP may be involved in the metabolic and physiological abnormalities found in these patients.

CHAPTER 6

PART 2:

EFFECTS OF VASOACTIVE INTESTINAL POLYPEPTIDE (VIP) ON RENAL FUNCTION IN MAN

Effects of vasoactive intestinal polypeptide (VIP) on renal function in man.

INTRODUCTION

As a follow-up to the infusion study in conscious rabbits, VIP was infused intravenously at a dose of 6 pmol kg^{-1} min^{-1}, in 6 healthy male volunteers aged between 22 and 35 years. Details of the infusion protocol are given in Chapter 2.

RESULTS

Plasma VIP levels were 45 ± 17 pmol l^{-1} (mean ± S.D., n = 12) before VIP infusion and did not approach a steady state until the last hour of infusion, when levels were about 100 pmol l^{-1} above basal (Fig. 6.6): above and below the range of plasma VIP concentrations achieved during the low and middle doses respectively, of VIP infused in the rabbit. Plasma concentrations of this order are only found in pathological states such as in patients with VIP secreting tumours (Welbourn et al., 1981). Plasma VIP levels remained elevated during the hour after VIP infusion (Fig. 6.6).

Systemic and renal haemodynamics (Table 6.3)

During VIP infusion, heart rate increased, mean arterial blood pressure fell and pulse pressure rose due to a fall in diastolic blood pressure. Facial flushing occurred in all subjects, and facial skin temperature increased by $1^{o}C$ during VIP infusion.

Urine composition (Table 6.4)

During VIP infusion the urine flow fell to about a third, and the osmolar clearance rate fell to about a half of control values. The renal excretion of sodium, potassium, chloride and calcium fell to

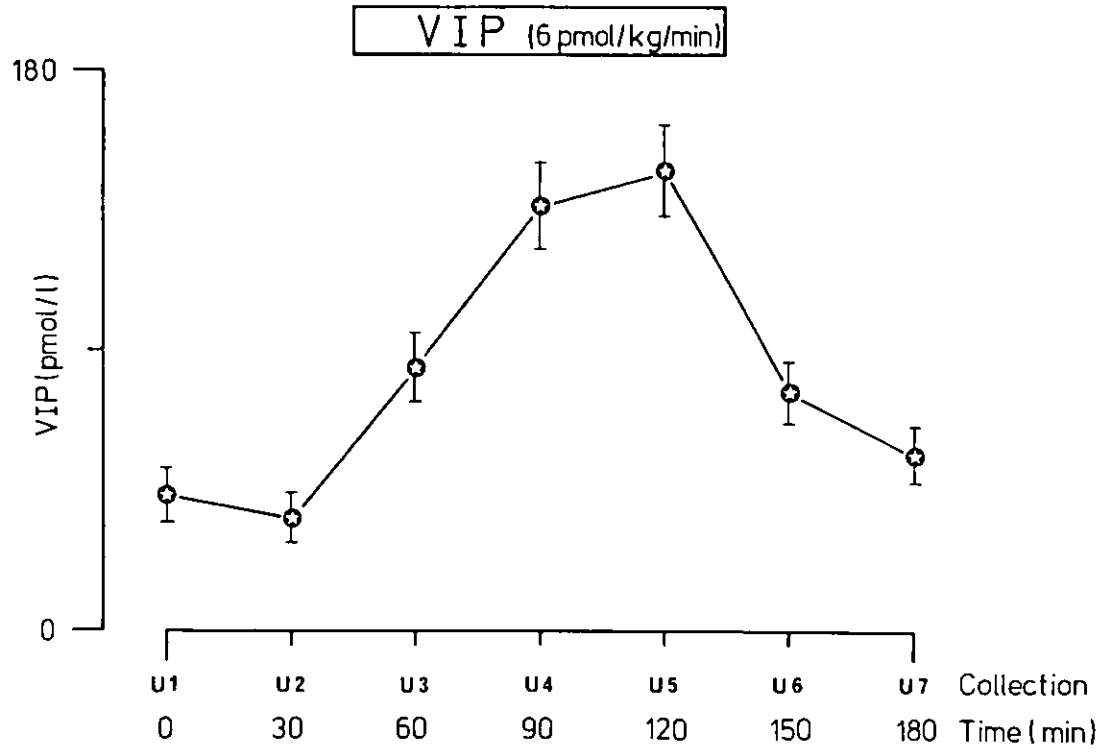


Fig. 6.6.

Mean (± S.E. of mean, n = 6) plasma VIP concentrations, before, during and after intravenous VIP infusion.

Table 6.3. HAEMODYNAMIC CHANGES

	Pre-(vehicle only)	VIP (6 pmol kg^{-1} min^{-1})	P	Post-U7 (n = 6)
SBP (mm Hg)	132 ± 12	133 ± 17	NS	-
DBP (mm Hg)	79 ± 10	70 ± 10	< 0.01	78 ± 10
MBP (mm Hg)	97 ± 10	91 ± 14	< 0.01	96 ± 10
HR (beats min^{-1})	65 ± 7	86 ± 14	< 0.01	74 ± 12
*Facial skin temperature (^{o}C)	35.1 ± 0.4	36.1 ± 0.2	< 0.01	35.2 ± 0.1
ERPF (ml min^{-1} kg^{-1})	8.6 ± 1.7	9.6 ± 4.6	NS	-
GFR (ml min^{-1} kg^{-1})	1.7 ± 0.3	1.7 ± 0.7	NS	-
FF (%)	19.9 ± 2.8	19.2 ± 6.3	NS	-
RBF (ml min^{-1} kg^{-1})	16.2 ± 3.5	*19.2 ± 8.6	NS	-

Values are given as mean ± S.D., n = 12 (*n = 8); P = results of paired t-test, NS = not statistically significant (P > 0.05).

Abbreviations:- SBP, systolic blood pressure; DBP, diastolic blood pressure, MBP, mean blood pressure; HR, heart rate; ERPF, effective renal plasma flow; GFR; glomerular filtration rate; FF, filtration fraction, RBF, estimated renal blood flow.

Table 6.4. URINARY CHANGES

	Pre- (vehicle only)	VIP (6 pmol kg^{-1} min^{-1})	P	Post- U7 (n = 6)
$\dot{V}$ (µl $min^{-1}kg^{-1}$)	213.4 ± 74.8	71.7 ± 54.7	< 0.01	108.7 ± 42.5
C_{Osm} (µl $min^{-1}kg^{-1}$)	100.7 ± 72.0	45.1 ± 22.7	< 0.01	60.0 ± 31.6
C_{H_2O} (µl $min^{-1}kg^{-1}$)	112.8 ± 75.9	26.5 ± 35.5	< 0.01	48.8 ± 50.7
$U_{Na}V$ (µmol $min^{-1}kg^{-1}$)	4.53 ± 1.73	2.87 ± 1.61	< 0.01	2.88 ± 0.92
U_KV (µmol $min^{-1}kg^{-1}$)	1.40 ± 0.35	0.70 ± 0.40	< 0.01	0.67 ± 0.15
$U_{Cl}V$ (µmol $min^{-1}kg^{-1}$)	2.91 ± 1.07	1.70 ± 1.13	< 0.01	1.45 ± 0.78
$U_{Ca}V$ (µmol $min^{-1}kg^{-1}$)	0.22 ± 0.10	0.12 ± 0.06	< 0.01	0.15 ± 0.04
$U_{Pi}V$ (µmol $min^{-1}kg^{-1}$)	0.22 ± 0.07	0.20 ± 0.11	NS	-
pH	6.30 ± 0.35	5.31 ± 0.24	< 0.01	5.41 ± 0.15

Values are given as mean ± S.D., n = 12; P = results of paired t-test, NS = not statistically significant (P > 0.05). Abbreviations:- $\dot{V}$, urine flow; C_{Osm}, osmolar clearance; C_{H_2O}, free water clearance; $U_{Na}V$, U_KV, etc., renal excretion.

between a half and two thirds of initial values. However, the excretion of phosphate fell to a lesser degree and the change was not significant. Urinary pH fell significantly during VIP infusion.

Plasma composition (Table 6.5)

During VIP infusion plasma solids, plasma calcium concentration and haematocrit all rose significantly by about 5%. Plasma phosphate concentration rose by about 10%, but this rise was not significantly greater than the rise in plasma solids. Plasma renin activity rose from 796 ± 402 to 2629 ± 1864 pg ml^{-1} hr^{-1} and levels had fallen to 1229 ± 649 pg ml^{-1} hr^{-1} 45 minutes after discontinuing VIP infusion (U7, Table 6.5). Plasma aldosterone concentration doubled during VIP infusion. There were no changes in the concentrations of sodium, potassium or glucose, but a small fall in plasma chloride concentration did reach statistical significance. Plasma arginine vasopressin (AVP) concentration did not change significantly, nor did plasma osmolality.

The control period after VIP infusion (U7)

Facial flushing disappeared within 15 minutes of stopping VIP infusion. All the haemodynamic changes, including facial skin temperature, showed an obvious change towards basal values within 15 minutes of stopping the infusion. Forty five minutes after the end of the VIP infusion all the changes observed in blood were returning towards basal levels, except for the rise in plasma phosphate concentration. All urinary changes induced by VIP, except for the fall in the fractional excretion of chloride showed some change towards basal levels by the second half of this control period. However, such changes were small and values remained significantly below basal.

Table 6.5. PLASMA CHANGES

	Pre- (vehicle only)	VIP (6 pmol kg^{-1} min^{-1})	P	Post- U7 (n = 6)
PS (%)	7.66 ± 0.35	8.04 ± 0.31	< 0.01	7.63 ± 0.24
Hct (%)	45 ± 3	48 ± 7	< 0.01	46 ± 5
P_{Na} (mmol l^{-1})	143.7 ± 2.4	142.5 ± 3.1	NS	-
P_K (mmol l^{-1})	4.14 ± 0.14	4.07 ± 0.24	NS	-
P_{Cl} (mmol l^{-1})	102.9 ± 3.1	100.0 ± 3.5	< 0.01	101.0 ± 3.2
P_{Ca} (mmol l^{-1})	2.41 ± 0.10	2.56 ± 0.10	< 0.01	2.51 ± 0.10
P_{Pi} (mmol l^{-1})	0.98 ± 0.07	1.09 ± 0.10	< 0.01	1.21 ± 0.12
P_{Osm} (m Osm kg^{-1})	279 ± 7	276 ± 7	NS	-
P_{Glu} (mmol l^{-1})	4.4 ± 0.3	4.6 ± 0.7	NS	-
$^{+}$PRA (pg $ml^{-1}hr^{-1}$)	796 ± 402	2629 ± 1864	< 0.01	1229 ± 649
$^{+}$ALDO (pmol l^{-1})	253 ± 59	490 ± 225	< 0.01	316 ± 56
$^{+}$AVP (pmol l^{-1})	5.1 ± 2.8	5.6 ± 3.5	NS	-

Values are given as mean ± S.D., n = 12. $^{+}$ Transformed variable expressed as geometric mean and approximate S.D.; P = results of paired t-test, NS = not statistically significant (P > 0.05). Abbreviations:- PS, plasma solids; Hct, haematocrit, P_{Na}, P_K, etc., plasma concentration; PRA, plasma renin activity; ALDO, plasma aldosterone concentration; AVP, plasma arginine vasopressin concentration.

DISCUSSION

The increased heart rate, widened pulse pressure, fall in mean arterial blood pressure and facial flushing produced by VIP are consistent with its vasodilator effect and have been previously reported (Domschke, Domschke, Bloom, Mitznegg, Mitchell, Lux & Strunz, 1978). There was no fall in ERPF or GFR as measured by PAH and creatinine clearance respectively, suggesting that renal vascular resistance fell during VIP infusion, and that the urinary changes may be due to altered tubular function. A similar effect on renal vascular resistance has been reported in the anaesthetised dog (Porter et al., 1980), but this contrasts with the reduction in GFR and ERPF and the increase in renal vascular resistance during VIP infusion in the rabbit (Chapter 6.1).

The persistently elevated plasma VIP-like immunoreactivity levels after infusion are surprising in view of VIP's reported plasma half-life of < 1 minute (Modlin, Mitchell & Bloom, 1978). This may be spurious and due to assay interference from non-specific effects of plasma, but could also be interpreted to mean tissue (including neuronal) uptake of VIP with subsequent release, although this seems unlikely. A more plausible explanation is measurement of partially degraded, biologically inactive, circulating fragments of VIP (c.f. neurotensin, Chapter 8).

Plasma solids and haematocrit both rose by about 5% during VIP infusion. A rise in plasma albumin concentration has already been reported during infusion of VIP (Domschke et al., 1978). These changes are most readily explained by a loss of plasma water. Sufficient water might have been secreted into the gut (Fahrenkrug, 1980), although none of our subjects experienced diarrhoea. Alternatively, water could have moved into the interstitial space,

perhaps due to an increase in capillary pressure associated with arteriolar vasodilatation (Taylor, 1981). A 5% rise in the concentration of binding proteins would account for all of the observed rise in plasma calcium, and some of the observed rise in plasma phosphate concentration.

The mechanism of increased tubular reabsorption of water and electrolytes during VIP infusion is not clear. The changes were unlike the effects of vasopressin in that osmolar clearance fell almost as much as urine flow. Plasma AVP concentrations remained low throughout the study in all subjects, presumably because they were water-loaded. Plasma aldosterone levels rose during VIP infusion, but a fall in sodium excretion due to this hormone would be accompanied by a rise, rather than a fall in potassium excretion. Furthermore, onset of the tubular effects of aldosterone is probably too slow to account for the changes seen during a 90 minute VIP infusion (Ross et al., 1959). The rates of excretion of water, sodium, potassium, chloride, calcium and, to a lesser extent, phosphate all decreased, suggesting a non-specific effect. Increased reabsorption of water and electrolytes could have been due to altered physical factors in the peritubular capillaries (Chapter 1). A fall in blood pressure of 6 mm Hg and a 5% rise in plasma protein concentration would both tend to produce this effect on salt and water reabsorption. When VIP was given into the renal artery of anaesthetised dogs, diuresis and natriuresis were seen in association with increased renal blood flow, probably because renal vasodilatation was the predominant effect (Porter & Ganong, 1982). In the present study, salt and water retention occurred during intravenous administration of VIP. Renal function was presumably

influenced by the effects of generalised vasodilatation, such as a fall in blood pressure and reflexly increased sympathetic activity, as well as any direct effect.

VIP induced renin release has been observed in the anaesthetised dog (Porter et al., 1980) and the conscious rabbit (Chapter 6.1), but the mechanism remains unclear. VIP could produce renin release through one of several stimuli (Freeman & Davis, 1976). For example, in the present study, and in the anaesthetised dog, vasodilatation of the afferent arteriole may well have occurred and led to renin release; the fall in blood pressure could have been an added stimulus. In all three studies (Porter et al., 1980; Chapter 6.1), there have been changes in the urinary electrolytes, so that renin release could have been stimulated by changes in ionic fluxes at the macula densa. Beta-adrenergic stimulation produces renin release, and is involved in the renin response to vasodilators such as hydrallazine and minoxidil (Pettinger, Campbell and Keeton, 1973). However, beta-blockade does not alter the renin response to VIP in the conscious rabbit although the tachycardia is abolished (Chapter 6.3). Indomethacin inhibits VIP-induced renin release suggesting mediation by prostaglandins (Chapter 6.3). Once again, a direct effect of VIP on the justaglomerular cells is still possible (Porter et al., 1982).

In the rabbit, VIP appeared to have a direct renal tubular action, altering electrolyte handling. In contrast, the renal electrolyte changes in man can probably be explained by the VIP-induced changes in systemic haemodynamics and plasma composition. This difference may be due to dose and, of course, species. The plasma levels of VIP at which effects occurred in the rabbit were more than twice the levels recorded in man.

The possible mechanism of the renin response in the two species is particularly interesting. In the rabbit, there was no change in blood pressure, but renal perfusion fell, indicating a rise in renal vascular resistance. In man, the opposite occurred, a fall in blood pressure and no change in renal perfusion, indicating a decrease in renal vascular resistance. The common underlying mechanism is probably the so-called renal baroreceptor (Freeman & Davis, 1976), although it could be argued that VIP is acting directly in both instances. According to the baroreceptor theory, renin release depends upon afferent arteriole (and therefore juxtaglomerular cell) stretch, increased stretch inhibiting renin release and vice versa. However, stretch does not only depend upon afferent arteriole tone, but also systemic blood pressure and renal interstitial pressure i.e., a function of transmural pressure (Fray, 1980). A rise in blood pressure would increase stretch and inhibit renin release, whereas a fall in blood pressure would reduce stretch and increase renin release. Paradoxical stimulation of the baroreceptor would occur in the face of a normal blood pressure if constriction of the afferent arteriole proximal to the juxtaglomerular cell took place (see Discussion, Chapter 4.1). Parts 3 and 4 of this chapter describe further studies on the mechanism of VIP-induced renin release, including the role of reflex sympathetic stimulation.

CHAPTER 6

PART 3:

STUDIES ON THE RENIN RESPONSE TO VIP IN THE CONSCIOUS RABBIT

Studies on the renin response to vasoactive intestinal polypeptide (VIP) in the conscious rabbit.

INTRODUCTION

The mechanism of the renin response to VIP is unknown, but vasodilating substances like VIP (Said, 1981) can produce renin release through reflex sympathetic stimulation (Pettinger et al., 1973), as well as activation of the intrarenal baroreceptor (Peart, 1978). The baroreceptor mechanism (afferent arteriole stretch) is thought to be prostaglandin-dependent, but whether or not the adrenergic pathway involves local prostaglandin release is controversial (Oates, Whorton, Gerkens, Branch, Hollifield & Frölich, 1979; Weber & Siess, 1982).

This study examined the relative importance of prostaglandins and reflex sympathetic activation in the haemodynamic and renin responses to VIP. The effects of beta-adrenoreceptor blockade and cyclo-oxygenase inhibition on VIP-induced changes in pulse, blood pressure and renin release in conscious rabbits were studied.

METHODS

Studies were performed in 9 Sandy Half-lop rabbits. Each rabbit underwent 3 separate infusions in random order and at intervals of at least 7 days. The animals were prepared for study in identical fashion. They were fed a standard laboratory diet (RHM R14, Labsure Animal Diets) until 3 hrs before each infusion. An ear vein and artery were cannulated and heart rate and arterial blood pressure were recorded continuously, as described in Chapter 2. Each rabbit rested quietly for at least 1 hr before infusion. An intravenous dose of 25 pmol kg^{-1} min^{-1} VIP was used in all experiments (Fig. 6.7).

INFUSION PROTOCOLS :-

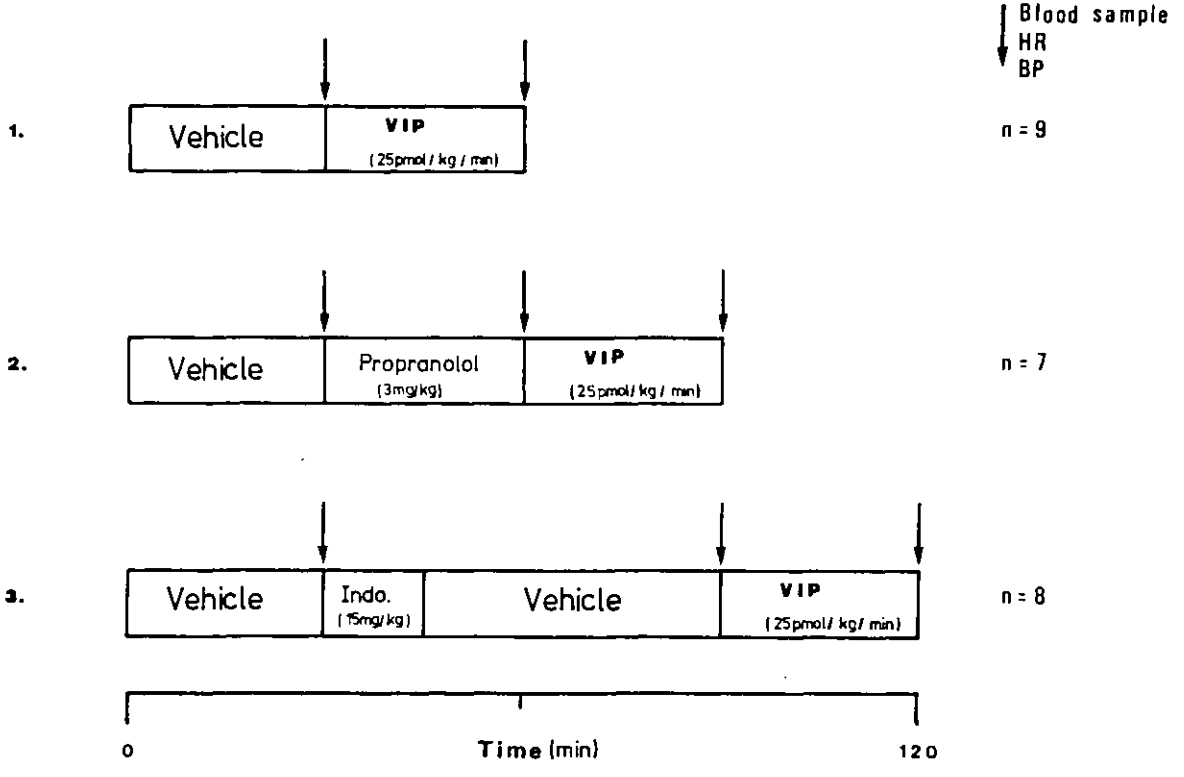


Fig. 6.7. Design of rabbit infusion studies (see text for details).

VIP alone (n = 9)

Each rabbit received an intravenous infusion of the vehicle alone for 30 minutes. The vehicle consisted of 1 g/l rabbit albumin in 0.28 M glucose solution (isotonic). The volume rate in all experiments was 13.33 ml hr^{-1}. VIP was then infused for 30 minutes. Arterial blood samples were taken 5 minutes before the end of the vehicle and VIP infusions respectively.

VIP after propranolol (n = 7)

Vehicle was infused together with DL-propranolol (Inderal, ICI), 3.2 mg kg^{-1} over 30 minutes. This dose has been previously shown to produce complete beta-adrenoreceptor blockade in the rabbit (Gordon, Roddis & Sever, 1980). VIP was then infused for 30 minutes. Blood samples were taken 5 minutes before the start and 5 minutes before the end of the VIP infusion.

VIP after indomethacin (n = 8)

Indomethacin (Merck, Sharp and Dohme Ltd.) was infused at a constant rate over 15 minutes in a dose of 10-15 mg kg^{-1}. The indomethacin was first dissolved in dehydrated ethanol 20 mg ml^{-1}, which was then diluted with NaCl solution (0.154 M: saline) containing 1 mg sodium bicarbonate per 4.5 ml saline as described by Scott (1980). The final concentration of indomethacin was 3.5 mg ml^{-1}. This dose has been previously shown to inhibit prostaglandin synthesis and depress renin levels (Larsson, Weber & Änggård, 1974). After 15 minutes, the vehicle was infused for 30 minutes. This was followed by a 30 minute infusion of VIP. Blood samples were taken for renin estimation (PRA) before and 45 minutes after indomethacin, and 5 minutes before the VIP infusion was stopped. Blood samples were taken for VIP estimation before the start of the VIP infusion and 5 minutes before it ended.

Significance of changes within each set of infusions was assessed by paired t-test and between each set of infusions by two-way analysis of variance. PRA was found to approximate the lognormal distribution and logarithmic transformations were used in the analysis. The mean value given for this variable is the geometric mean and approximate S.E. of mean. Other results are expressed as arithmetic mean ± S.E. of mean.

RESULTS (Table 6.6)

VIP alone produced a 2-3 fold rise in PRA ($P < 0.001$) and a 50% increase in heart rate ($P < 0.01$), but little change in mean blood pressure.

Prior treatment with propranolol did not significantly affect the renin response to VIP, but did inhibit the increase in heart rate. This inhibition was significant when compared with the response during VIP alone and after indomethacin ($P < 0.001$). Propranolol did not alter the resting blood pressure and heart rate. Adequate beta-adrenoreceptor blockade was confirmed by the absence of blood pressure and heart rate responses to bolus injections of 10-20 µg isoprenaline and lack of renin rise after 6 µg isoprenaline.

Indomethacin did not change the resting mean blood pressure, heart rate or PRA, nor did it influence the VIP-induced tachycardia. It did inhibit the renin response to VIP infusion. VIP after indomethacin produced a small but significant fall in mean blood pressure ($P < 0.01$).

DISCUSSION

The VIP-induced rise in renin was unaffected by propranolol, but inhibited by indomethacin. This suggests involvement of

Table 6.6.

Range of plasma VIP concentrations before and during each set of infusions together with changes in plasma renin activity, heart rate and mean arterial blood pressure.

		Plasma VIP (pmol l^{-1})		Plasma renin activity^{+} (ng ml^{-1} hr^{-1})		Heart Rate (beats min^{-1})		Mean Blood Pressure (mm Hg)	
	n	Before	During	Before	During	Before	During	Before	During
VIP alone	9	< 10	460-1100	4.9 ± 0.8	13.3 ± 4.1[a]	201 ± 6	271 ± 9[a]	80 ± 3	76 ± 5
VIP + propranolol	7	< 10	550-850	5.2 ± 1.1	11.6 ± 3.0[a]	187 ± 11	210 ± 7[b]	81 ± 6	78 ± 6
VIP + indomethacin	8	< 10	400-720	3.8 ± 0.6	5.3 ± 0.8[ab]	198 ± 10	283 ± 11[a]	76 ± 2	63 ± 2[ab]

Values are given as mean ± S.E. of mean, n is number of observations. + Geometric mean and S.E. for log normally distributed data. 'a' signifies $P < 0.01$ comparing Before with During and 'b' signifies $P < 0.05$ for comparisons between each set of infusions during VIP infusion.

prostaglandins in the renin response, perhaps through intrarenal baroreceptor stimulation, rather than reflex sympathetic activation and beta-adrenoreceptor stimulation. Although indomethacin may have actions which are independent of cyclo-oxygenase inhibition, such as phosphodiesterase inhibition (Newcombe, Thanassi & Coisek, 1974), or intrinsic vasoconstrictor activity, at the dose described it has not been found to alter renal function (Dunn & Zambraski, 1980).

Propranolol abolished the VIP-induced tachycardia suggesting that this effect is due to either direct or reflex beta-adrenoreceptor stimulation. Chatelain et al. have demonstrated the presence of specific VIP receptors (Chatelain, Robberecht, de Neef, Deschodt-Lanckman, Konig & Christophe, 1980). From our results it seems unlikely that these receptors are involved in the chronotropic action of VIP.

Propranolol and indomethacin did not alter resting blood pressure, but after indomethacin, VIP infusion produced a fall in mean blood pressure. If the adrenergic pathway to renin release is independent of prostaglandin synthesis (Oates et al., 1979), the fall in blood pressure during VIP infusion after indomethacin would be expected to produce renin release through enhanced reflex sympathetic activity. As this did not occur, it seems likely that prostaglandins are involved in the adrenergic pathway. The fall in blood pressure may reflect the reduced renin response, and by inference, diminished circulating levels of angiotensin-II. It is also possible that loss of "pressor prostaglandins" e.g., thromboxanes which are also dependent on the cyclo-oxygenase pathway, might be a factor. Non-steroidal anti-inflammatory agents such as indomethacin are known to inhibit the vasoconstriction produced by thromboxanes and may alter the balance between these and "depressor prostaglandins", such as

prostacyclin (Smith, Schmunk & Lefer, 1981).

The secretory and cerebral vasodilating effects of VIP are also inhibited by indomethacin (Albuquerque, Owens & Bloom, 1979; Wei, Kontos & Said, 1980) and this drug may be of value in patients with disorders associated with excess circulating VIP (Friesen, 1982).

CHAPTER 6

PART 4:

INDOMETHACIN AND VIP-INDUCED RENIN RELEASE IN MAN

Indomethacin and VIP-induced renin release in man.

METHOD

A double-blind crossover study was conducted in 5 healthy male volunteers aged between 26 and 35 years. There was no dietary or other restriction during the study.

On two separate occasions, at least one week apart, each subject was given either indomethacin or an equal number of placebo (containing lactose) capsules orally for 3 days. The dosage of indomethacin was 25 mg t.d.s. on the first day and 50 mg. t.d.s. thereafter. This regimen of indomethacin has been shown to suppress the renin response to a variety of stimuli (Frankel, Mathias, Peart & Unwin, 1982). A comparable total dose of indomethacin has been shown to suppress plasma renin activity and to reduce urinary prostaglandin E excretion by approximately 50% (Frölich, Hollifield, Dormois, Frölich, Seyberth, Michelakis & Oates, 1976). VIP (6 pmol kg^{-1} min^{-1}) was then infused intravenously on the afternoon of the third day, according to the protocol shown in Figure 6.8. The subjects were supine throughout the infusion and cannulated as described in Chapter 2. The VIP and vehicle infusates were prepared as for the study described in Part 2 of this chapter. Pulse rate and systemic blood pressure were recorded by an automatic sphygmomanometer (Dinamapp; Chapter 2). Venous blood samples were taken for estimation of plasma renin activity (PRA) and plasma noradrenaline concentration (PNA). Samples for PNA were collected in chilled commercial tubes containing lithium heparin, centrifuged and the plasma stored at -70^{o}C until thawed for assay by a radioenzymatic method (Henry, Starman, Johnson & Williams, 1975; Sever, Birch, Osikowska & Tunbridge, 1977).

Results presented are based on the measurements made as indicated

Day 1 Indomethacin (25mg t.d.s.) or Placebo
Day 2 " (50mg t.d.s.) "
Day 3 " (50mg t.d.s.) "

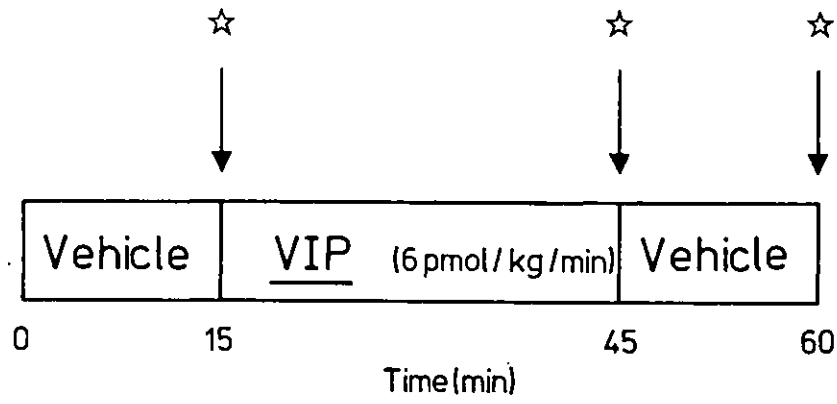


Fig. 6.8.

Diagram of infusion protocol. Starred arrows indicate points at which recordings of pulse and blood pressure were made and venous blood withdrawn. These observations are compared in Table 6.7.

in Figure 6.8. Significance of changes within infusions (vehicle versus VIP) and between infusion (VIP + placebo versus VIP + indomethacin) were assessed by paired t-test. PRA and PNA were found to be lognormally distributed so logarithmic transformations were used in the analysis. For these variables, results are expressed as geometric mean and approximate S.E. of mean, otherwise, arithmetic mean + S.E. of mean are given.

RESULTS (Table 6.7)

Indomethacin significantly lowered resting PRA ($P < 0.01$), but did not affect basal values of the other parameters. Pulse rate and diastolic blood pressure fell during VIP infusion, as observed in the previous study in man (Chapter 6.2). VIP almost doubled PNA concentration and all subjects flushed visibly. These responses to VIP were unaffected by pre-treatment with indomethacin.

PRA more than doubled during VIP infusion in the presence and absence of indomethacin. However, the absolute rise in PRA after indomethacin was significantly less than after the placebo (452 ± 278 versus 1412 ± 582 pg ml^{-1} hr^{-1}; $P < 0.01$).

DISCUSSION

Indomethacin has been previously reported to lower basal PRA in man and other species, and to reduce, although not abolish, the renin response to many different stimuli such as orthostasis, haemorrhage, administration of frusemide and isoprenaline (Weber & Siess, 1982). Prostaglandins may stimulate renin release by direct stimulation of the juxtaglomerular cells, or indirectly through activation of the intrarenal baroreceptor (afferent arteriole stretch or tension). They also interfere with adrenergic transmission and the vascular

Table 6.7 (legend overleaf).

PLACEBO	Basal	VIP	P	15 min after VIP
Systolic blood pressure (mm Hg)	124 ± 5	128 ± 4	NS	124 ± 5
Diastolic blood pressure (mm Hg)	84 ± 1	75 ± 4	< 0.05[a]	82 ± 3
Mean blood pressure (mm Hg)	98 ± 2	92 ± 3	< 0.05[a]	96 ± 3
Pulse rate ($beats\ min^{-1}$)	62 ± 6	77 ± 8	< 0.005[b]	56 ± 4
$^{+}$PNA ($pmol\ l^{-1}$)	*236 ± 33	425 ± 78	< 0.05[b]	309 ± 47
$^{+}$PRA ($pg\ ml^{-1}\ hr^{-1}$)	975 ± 202	2450 ± 775	< 0.005[b]	1545 ± 425

INDOMETHACIN	Basal	VIP	P	15 min after VIP
Systolic blood pressure (mm Hg)	131 ± 6	127 ± 7	NS	128 ± 5
Diastolic blood pressure (mm Hg)	84 ± 4	75 ± 4	< 0.01[b]	87 ± 2
Mean blood pressure (mm Hg)	100 ± 5	91 ± 3	< 0.02[b]	101 ± 2
Pulse rate ($beats\ min^{-1}$)	60 ± 5	78 ± 8	< 0.005[b]	59 ± 5
$^{+}$PNA ($pmol\ l^{-1}$)	210 ± 53	375 ± 101	< 0.005[b]	411 ± 69
$^{+}$PRA ($pg\ ml^{-1}\ hr^{-1}$)	504 ± 149	1019 ± 408	< 0.01[b]	722 ± 189

Table 6.7.

Effects of VIP with and without indomethacin. Abbreviations as given in the text. Results are expressed as mean ± S.E., n = 5 (*n = 4). $^{+}$Transformed variable; P = results of paired t-test, NS = not statistically significant (P > 0.05); 'a' and 'b' indicate P-values for one and two-tailed tests of significance, respectively.

response to catecholamines, but quite how this affects renin release is unclear (McGiff, Malik & Terragno, 1976).

The renin response to VIP in the present study was only attenuated by indomethacin. VIP, as a vasodilator, is likely to produce reflex sympathetic stimulation, which in turn would stimulate renin secretion. The VIP-related rise in PNA concentration probably reflects this increased sympathetic nervous activity (Sever et al., 1977). Elevated PNA levels have been reported in patients taking the antihypertensive vasodilator hydrallazine (Davies, Sever & Rosenthal, 1979) and the renin response to this drug has been shown to be largely due to increased reflex sympathetic discharge to the kidney (Ueda, Kaneko, Takeda, Ikeda & Yagi, 1970). However, the previous study (Chapter 6.3) suggests that in the rabbit at least, beta-adrenergic stimulation is not important in VIP-induced renin release.

Some workers have reported reduced PNA levels in man after treatment with indomethacin (Gullner, Lake, Bartter & Kafka, 1979). In this study, indomethacin (2 mg kg^{-1} day^{-1}) was given for 7 days, but as the authors themselves point out, some prostaglandins in vitro inhibit noradrenaline release (Junstad & Wennmalm, 1973). The mechanism of this inhibitory effect is not understood, but may involve interference with calcium flux across membranes and changes in intracellular free calcium concentration (McGiff et al., 1976). Therefore, indomethacin might be expected to produce a rise rather than a fall in circulating noradrenaline levels. Although just over half the above dose of indomethacin was given in the present study, basal and VIP-stimulated PNA levels were unaffected by indomethacin.

Blood pressure in man has been reported to rise after indomethacin

(Carr & Lee, 1977; Frankel et al., 1982). This is to be expected if prostaglandins are involved in blood pressure control, presumably through loss of vasodilator prostaglandins. However, other workers have found no effect of indomethacin on blood pressure (Carr & Lee, 1977), as in the present study. VIP-induced facial flushing is unaffected by indomethacin, making it unlikely to be mediated by prostaglandins. The vascular effects of VIP are believed to be independent of adrenergic or cholinergic receptors (Fahrenkrug & Emson, 1982) and prostaglandin release (Said, 1983), but the vasodilatation of cerebral arterioles does appear to be prostaglandin dependent (Wei et al., 1980).

The effect of indomethacin on renin release in man is less clear-cut than in the rabbit (Chapter 6.3), but may simply reflect the much larger dose per kg of indomethacin given to the rabbit. Moreover, the dose of indomethacin may have been insufficient to adequately block renal prostaglandin synthesis. It is always so difficult to compare changes when basal values have been altered as in the present study, for it cannot be predicted how this will affect a given response (reduce or magnify), and use of percentage change may not be legitimate. However, bearing these points in mind, it does seem that the renin response to VIP in man is reduced by indomethacin which suggests that, as in the rabbit, it depends to some extent upon local prostaglandin release. The facial flush produced by VIP appears to be independent of prostaglandins and may be a direct effect on cutaneous blood vessels. Although VIP has been reported to release 5-hydroxytryptamine (Fahrenkrug & Emson, 1982), this does not cause facial flushing in man (Robertson, Peart & Andrews, 1962).

SUMMARY TO CHAPTER 6

1. Vasoactive intestinal polypeptide (VIP) was originally isolated from the small intestine and has been found within the renal cortex, and is believed to be a neurotransmitter rather than a circulating hormone.

2. Studies were conducted in conscious rabbits and man to investigate the renal effects of VIP and the mechanism of VIP-induced renin release.

3. Four rabbits each received 3 different doses of VIP (1, 10 and 25 pmol kg^{-1} min^{-1}) intravenously on separate occasions. Subsequently, 9 rabbits (including the original 4) were given further infusions of VIP at 25 pmol kg^{-1} min^{-1} alone and again after pre-treatment with the beta-adrenoreceptor blocker propranolol, and then the cyclo-oxygenase inhibitor indomethacin.

4. Six healthy males received 6 pmol kg^{-1} min^{-1} of VIP. Subsequently, another 5 males were given the same dose of VIP on 2 separate occasions after 3 days of placebo or oral indomethacin, as a double-blind crossover study.

5. In the rabbit, VIP led to decreases in ERPF and GFR at the middle and high doses. Mean blood pressure rose slightly and filtration fraction increased at the middle dose. The high dose produced an increase in heart rate, a fall in plasma sodium, potassium and phosphate concentrations and a rise in plasma solids. In spite of the renal haemodynamic effects and changes in plasma composition during infusion of the high dose, fractional

excretion of sodium, potassium and chloride doubled, suggesting a direct renal tubular action of VIP.

6. In man, VIP produced facial flushing, a fall in blood pressure and an increase in heart rate; all consistent with generalised vasodilatation. However, ERPF and GFR did not change significantly. Urine flow fell to about 1/3 and fractional excretion of sodium, potassium and chloride and calcium to between 1/2 and 2/3 of control values. Phosphate excretion did not alter. Plasma solids and haematocrit increased, indicating haemoconcentration.

7. In both rabbit and man, PRA rose by between 2 and 3-fold. In the rabbit, the rise in PRA was unaltered by pre-treatment with propranolol, but attenuated by indomethacin. However, the tachycardia was inhibited by propranolol, but unaffected by indomethacin. In man, VIP also produced a rise in plasma noradrenaline concentration. Indomethacin reduced basal PRA and altered the renin response to VIP, but did not influence the haemodynamic (including facial flushing) and noradrenaline responses.

8. These results, together with the reported presence of VIP-like material in the renal cortex, may indicate a role for VIP in the regulation of renal function, including renin release. With regard to renin, the response appears to be in part prostaglandin dependent and the heart rate response seems due to direct or reflex beta-adrenoreceptor stimulation.

CHAPTER 7

EFFECTS OF INTRAVENOUS PORCINE SECRETIN INFUSION ON PLASMA COMPOSITION AND RENAL FUNCTION IN CONSCIOUS RABBITS

Effects of intravenous porcine secretin infusion on plasma composition and renal function in conscious rabbits.

INTRODUCTION

In 1902, Bayliss and Starling gave the name secretin to a hypothetical messenger which produced secretion from a denervated pancreas after duodenal acid stimulation and after injection of small bowel extracts. The term "hormone" (from the Greek hormao, "I arouse") was later used to describe such circulating messengers. Almost 60 years elapsed before secretin was isolated by Jorpes and Mutt (1961) from large quantities of hog small intestine. Five years later, these same workers identified its amino acid sequence (Jorpes & Mutt, 1966).

Secretin is a basic polypeptide and a member of the glucagon, VIP (vasoactive intestinal polypeptide) and GIP (gastric inhibitory peptide) family. These peptides have many amino acid sequences in common (Chapter 6, Fig. 6.1) and it might be expected that their actions should be qualitatively similar, or that competitive antagonism could exist between them.

Secretin is found within endocrine cells (S-cells) of the small intestine, with highest concentrations in the duodenum (Häcki, 1980). Recently, a secretin-like substance has been isolated from brain (Mutt, Carlquist & Tatemoto, 1979). The precise structure of human secretin is unknown, but evidence suggests a single molecular form almost identical with porcine secretin (Greenberg, 1981). The pharmacological actions of secretin are listed in Table 7.1. Many are controversial and result from administration of high doses, in excess of 1 clinical unit (CU) per kilogram.

CARDIOVASCULAR		↑	cardiac output
		↓	splanchnic blood flow
GUT	Oesophagus	↓	lower oesophageal sphincter
	Stomach	↓	acid secretion
		↓	gastrin release
		↑	pepsin secretion
		↑	mucus secretion
		↑	mucosal blood flow
		↓	motility
	Pancreas	↑	water and electrolyte secretion
		↑	protein washout
		↑	blood flow
		↑	insulin release
	Liver	↑	biliary secretion of water and electrolytes
	Small bowel	↑	Brunner's gland secretion
		↓	motility
	Large bowel	↓	motility (but also reported to ↑ food stimulated persistalsis)
RENAL		↑	salt and water excretion

Table 7.1. Some pharmacological actions of secretin: ↑ increase; ↓ decrease; (based on Häcki, 1980; Chey, Chang, Lee, Raninger, Rhodes & You, 1981).

The main difficulty in identifying the physiological role of secretin has been in the measurement of fasting and postprandial circulating levels. Originally, basal levels in man of ca. 100 pmol l^{-1} (< 0.4 ng ml^{-1}) were reported (Young, Lazarus & Chisolm, 1968). However, in recent years with improvements in assay systems and antibody specificity, fasting plasma levels in healthy human subjects have been in the range of 1.5 - 5 pmol l^{-1} (Greenberg, 1981). Higher levels have been found in patients with gastrinomas (Zollinger-Ellison Syndrome) of > 10 pmol l^{-1} (Greenberg, 1981). Postprandial levels, after a solid meal, range between 2.5 - 7 pmol l^{-1} i.e., a very small increment compared with other circulating gut hormones (Greenberg, 1981, 1982). Infusions of secretin (porcine) at doses which produce a rise in plasma concentration well within the physiological ranges as given above, have been reported to significantly increase pancreatic bicarbonate output (Greenberg, 1981, 1982). Effects on gastric acid secretion, gastrin release, oesophageal sphincter tone and insulin release are probably pharmacological.

It has been suggested that secretin is responsible for the post-prandial rise in urine pH (Viteri et al., 1975), but its renal effects are controversial. Table 7.2 summarises some of the reported renal actions of secretin. Many studies have been in anaesthetised dogs, using large doses of secretin and without measurement of plasma concentrations. The early study in man of Baron, Newman & Warwick (1958) also involved patients with pancreatic disease in whom responses were less marked and even the more recent study of Viteri et al. (1975) in healthy subjects, used only bolus injections of secretin. Therefore studies were performed in conscious rabbits

Table 7.2. Results from some studies of the renal effects of secretin (see text).

Reference	Species	Dose	Route	BP	RBF	GFR	$\dot{V}$	$U_{Na}V$
Baron et al., 1958	Man (c)	1 CU kg^{-1}	i.v. bolus	-	-	-	↑	↑
Marchand et al., 1972	Dog (a)	22.5-90 mCU $kg^{-1}min^{-1}$	i.a	→	↑	↑	→	→
Barbazet et al., 1972	Dog (a)	0.5 µg kg^{-1} min^{-1}	i.v.	-	-	-	↑	↑
Edwards & Brehme, 1973	Dog (-)	-	-	-	-	-	-	↓
Levy, 1975	Dog (a)	5-10 µg kg^{-1} min^{-1}	i.v.	→	→	→	→	→
"	"	"	i.a.	→	→	→	→	→
Viteri et al., 1975	Man (c)	1 CU kg^{-1}	i.v. bolus	-	-	-	↑	↑
Nakanishi et al., 1977	Dog (a)	0.4-2 CU kg^{-1} min^{-1}	i.v.	-	↑	→	→	→
"	"	0.2-1 CU kg^{-1} min^{-1}	i.a.	-	↑	→	→	→
Marchand et al., 1977	Dog (a)	50-100 mCU $kg^{-1}min^{-1}$	i.a.	→	↑	→	-	↑

Abbreviations:- a, anaesthetised; c, conscious; i.a., intrarenal; i.v., intravenous; BP, mean blood pressure; RBF, renal blood flow or estimate; GFR, glomerular filtration rate; $\dot{V}$, urine flow; $U_{Na}V$, renal sodium excretion; ↑, increase; ↓, decrease; →, no change; -, no record.

using 3 different doses of porcine secretin (5, 50 and 100 mCU kg^{-1} min^{-1}) and with measurement of plasma levels.

RESULTS

Tables 7.3, 7.4 and 7.5 show the effects of increasing doses of secretin on mean arterial blood pressure, heart rate, renal function and plasma composition, compared with control (vehicle only) infusions. The plasma levels attained were a little lower than expected from the doses infused, but those observed during infusion of the lowest dose (5 mCU kg^{-1} min^{-1}) are close to postprandial values in man. Control levels are comparable with those previously reported in fasting human subjects (see above).

Systemic and renal haemodynamics (Table 7.3)

Blood pressure rose significantly during infusion of the low dose of secretin and heart rate increased at the middle dose (50 mCU kg^{-1} min^{-1}). There were no significant changes in ERPF, GFR or filtration fraction at any dose of infused secretin.

Urine composition (Table 7.4)

Urine flow and free water clearance fell significantly at the middle dose and potassium excretion decreased at the high dose of secretin (100 mCU kg^{-1} min^{-1}). Renal phosphate excretion increased significantly during infusion of the low dose. The changes in phosphate and potassium excretion were approximately 100 and 30% of their control values respectively. Although there was an apparent increase in phosphate excretion during infusion of the high dose, this did not reach statistical significance.

Plasma composition and haematocrit (Table 7.5)

Alterations in plasma composition were complex. Plasma renin

Table 7.3. Effects of secretin on mean arterial blood pressure, heart rate and renal haemodynamics.

	Control (vehicle only)	Secretin in mCU kg^{-1} min^{-1} 5 (low)	50 (middle)	100 (high)	n
BP (mm Hg)	79 ± 3	**95 ± 4	78 ± 3	78 ± 2	15
HR (beats min^{-1})	241 ± 9	249 ± 5	**263 ± 8	245 ± 4	15
ERPF (ml min^{-1})	57.9 ± 3.2	60.3 ± 3.3	65.1 ± 3.9	66.3 ± 4.7	15
GFR (ml min^{-1})	13.0 ± 0.9	14.1 ± 1.1	14.9 ± 0.4	14.4 ± 1.2	15
FF (%)	22.3 ± 0.6	23.1 ± 0.9	23.4 ± 1.5	21.7 ± 1.0	15

Values are expressed as mean ± S.E. of mean. +Transformed variable, in which case the geometric mean and approximate S.E. of mean are given. n = number of observations. * and ** signify $P < 0.05$ and < 0.01 respectively, when compared with the control value by Dunnett's test after analysis of variance. Abbreviations are as follows:- BP, mean arterial blood pressure; HR, heart rate; ERPF, effective renal plasma flow; GFR, glomerular filtration rate; FF, filtration fraction.

Table 7.4. Effects of secretin on renal electrolyte excretion, urine flow and solute excretion.

	Control (vehicle only)	Secretin in mCU kg^{-1} min^{-1} 5 (low)	50 (middle)	100 (high)	n
$^{+}U_{Na}V$ (μmol min^{-1})	15.1 ± 1.9	18.6 ± 1.4	13.5 ± 2.0	16.4 ± 1.9	15
$^{+}U_{K}V$ (μmol min^{-1})	8.7 ± 1.0	9.3 ± 0.7	7.9 ± 0.7	*6.3 ± 0.4	15
$^{+}U_{Cl}V$ (μmol min^{-1})	15.4 ± 1.9	16.1 ± 1.8	17.1 ± 1.8	12.9 ± 2.0	15
$^{+}U_{Ca}V$ (μmol min^{-1})	0.46 ± 0.08	0.83 ± 0.16	0.41 ± 0.08	0.40 ± 0.10	15
$^{+}U_{Pi}V$ (μmol min^{-1})	0.76 ± 0.23	*1.56 ± 0.17	0.86 ± 0.24	1.77 ± 0.21	15
$^{+}\dot{V}$ (μl min^{-1})	405.7 ± 102.5	566.5 ± 94.3	**112.5 ± 23.2	235.2 ± 72.4	15
$^{+}C_{Osm}$ (μl min^{-1})	303.9 ± 32.2	353.9 ± 16.1	259.8 ± 22.6	285.6 ± 27.0	15
C_{H_2O} (μl min^{-1})	221.0 ± 63.1	304.0 ± 83.3	**-124.3 ± 21.1	106.2 ± 73.8	15

Values are expressed as mean ± S.E. of mean. $^{+}$Transformed variable, in which case the geometric mean and approximate S.E. of mean are given. n = number of observations. * and ** signify $P < 0.05$ and < 0.01 respectively, when compared with the control value by Dunnett's test after analysis of variance. Abbreviations are as follows:- $U_{Na}V$, $U_{K}V$, etc., renal excretion; $\dot{V}$, urine flow, C_{Osm}, osmolar clearance; C_{H_2O}, free water clearance.

Table 7.5. Effects of porcine secretin on plasma composition and haematocrit.

	Control (vehicle only)	Secretin in mCU kg^{-1} min^{-1} 5 (low)	50 (middle)	100 (high)	n
Secretin (pmol l^{-1})	1.4 - 5.0	8.0 - 12.8	72 - 92	142 - 248	5
P_{Na} (mmol l^{-1})	133.9 ± 0.5	135.0 ± 0.6	** 130.5 ± 0.7	132.5 ± 0.8	15
P_{K} (mmol l^{-1})	3.52 ± 0.09	3.68 ± 0.09	3.52 ± 0.10	3.57 ± 0.10	15
P_{Cl} (mmol l^{-1})	99.0 ± 1.3	99.2 ± 1.5	97.7 ± 0.9	98.9 ± 1.5	15
P_{Ca} (mmol l^{-1})	2.99 ± 0.06	3.12 ± 0.06	3.11 ± 0.05	** 3.24 ± 0.09	15
P_{Pi} (mmol l^{-1})	1.24 ± 0.04	1.30 ± 0.06	1.21 ± 0.03	1.32 ± 0.07	15
P_{Glu} (mmol l^{-1})	10.7 ± 1.1	10.5 ± 0.9	9.2 ± 0.5	11.0 ± 1.5	5
P_{Osm} (m Osm kg^{-1})	281 ± 1	282 ± 2	** 271 ± 2	* 276 ± 2	15
PS (%)	5.44 ± 0.29	5.61 ± 0.23	5.12 ± 0.15	** 6.12 ± 0.19	10
Hct (%)	31 ± 1	33 ± 1	* 35 ± 1	32 ± 1	5
+PGPS (%)	6.88 ± 0.30	7.19 ± 0.29	6.60 ± 0.21	** 7.81 ± 0.30	10
+PRA (ng ml^{-1} hr^{-1})	18.9 ± 3.7	** 11.9 ± 1.6	19.7 ± 3.8	** 11.4 ± 2.4	10

Values are expressed as mean ± S.E. of mean. +Transformed variable, in which case the geometric mean and approximate S.E. of mean are given. n = number of observations. * and ** signify $P < 0.05$ and < 0.01 respectively, when compared with the control value by Dunnett's test after analysis of variance. Abbreviations are as follows:- P_{Na}, P_{K}, etc., plasma concentrations; PS, plasma solids; Hct, haematocrit; PGPS, post-glomerular plasma solids; PRA, plasma renin activity.

activity levels fell significantly during infusion of the low and high doses of secretin. Plasma sodium concentration decreased and haematocrit rose at the middle dose. Plasma osmolality fell during infusion of the middle and high doses of secretin. At the high dose, plasma solids, PGPS and plasma calcium concentration increased. There was no change in plasma glucose concentration at any dose of secretin.

DISCUSSION

The increments in plasma secretin concentration at each dose were smaller than expected. In man, a dose of 5 mCU kg^{-1} min^{-1} (equivalent to the lowest dose infused in the present study) given intravenously, produced plasma secretin levels of around 20 pmol l^{-1} (Greenberg, 1981).

Ross (1970) observed that bolus injections of secretin (1-10 CU) produced initial transient hypotension followed by systemic hypertension. The former he attributed to mesenteric vasodilatation and the latter was associated with small increases in cardiac stroke volume, heart rate and total peripheral vascular resistance. However, an infusion of approximately 50 mCU kg^{-1} min^{-1} produced no change in blood pressure, but increased mesenteric blood flow. In the rabbit, the changes in blood pressure and heart rate were not consistent or dose-related.

The changes in phosphate excretion and urine flow were not dose-dependent and therefore difficult to interpret. Unlike glucagon, secretin is not known to stimulate calcitonin release or affect parathormone levels (Care, Bruce, Boelkins, Kenny, Conaway & Anast, 1971) and there are no previous reports of the effect of secretin on

renal phosphate handling. Several workers have reported secretin-induced diuresis, but others have observed no change in urine flow (Table 7.2). The fall in potassium excretion may be related to the rise in plasma solids and PGPS promoting increased tubular reabsorption. However, this is unlikely in the absence of any change in the excretion of other electrolytes.

The fall in plasma osmolality, together with the rise in plasma solids and lack of change in haematocrit, resemble the effects of the closely related peptide VIP (see Chapter 6.1). The decrease in plasma osmolality indicates plasma dilution, whereas the increase in plasma solids suggests loss of plasma water. The absence of any change in haematocrit may again reflect red cell sequestration due to splanchnic pooling (mesenteric vasodilatation) (Pessina, 1971).

Secretin increased plasma calcium concentration, but only at the highest dose infused and in association with elevated plasma solids, and therefore probably of albumin. The percentage rise in plasma solids may be enough to explain the increase in plasma calcium concentration, although secretin has been reported to increase ionised calcium in man at a dose of 50 mCU kg^{-1} min^{-1} (Isenberg, et al., 1973).

The apparent bimodal response in terms of plasma renin activity suppression may be explained by the 3% fall in plasma sodium concentration at the middle dose. This could mask an inhibitory effect of secretin on plasma renin activity, as decreases in plasma sodium concentration are known to increase renin release (Davis & Freeman, 1976). In the conscious rabbit, the only other gut peptide found to inhibit renin release is CCK8 (Chapter 3.1). Although these peptides share certain effects on gastrointestinal function (pancreas

and gallbladder) and may potentiate one another (Greenberg, 1981), they are structurally unrelated. Other peptides reported to produce renin suppression include substance P (Gullner et al., 1978) and somatostatin (Patel, Zingg, Fitz-Patrick & Srikant, 1981), but this effect was not observed in the present series of experiments in the conscious rabbit (Chapters 9 and 10).

In conclusion, secretin at plasma levels approximating post-prandial levels in man, produced no change in renal perfusion, but increased systemic blood pressure, renal phosphate excretion and decreased plasma renin activity. Further experiments in man are necessary.

SUMMARY TO CHAPTER 7

1. Secretin was the first so-called hormone to be discovered and is structurally related to glucagon and VIP. It was originally isolated from hog small intestine and is believed to be the physiological stimulus to pancreatic bicarbonate secretion. Secretin has been reported to be diuretic, but its renal effects are controversial.

2. The renal actions of porcine secretin were investigated in 5 conscious rabbits by infusing intravenously doses of 5, 50 and 100 mCU kg^{-1} min^{-1}.

3. Secretin at plasma levels simulating postprandial concentrations recorded in man (5 mCU kg^{-1} min^{-1}) produced no change in ERPF and GFR, but increased mean arterial blood pressure and renal phosphate excretion, and decreased PRA.

4. Further studies in man are necessary to determine the significance of these observations in terms of human physiology.

CHAPTER 8

PART 1:

NEUROTENSIN AND ANTINATRIURESIS IN THE CONSCIOUS RABBIT

Neurotensin and antinatriuresis in the conscious rabbit.

INTRODUCTION

Neurotensin is a tridecapeptide with the amino acid sequence shown in Figure 8.1 (Carraway & Leeman, 1975). The biologically active part of the molecule is the C-terminal end (Hammer & Leeman, 1981). It was originally isolated as another vasoactive constituent of bovine hypothalamus while screening for substance P (Carraway & Leeman, 1973). Shortly after, an identical peptide was discovered in the gastrointestinal tract (Kitabgi, Carraway & Leeman, 1976) and neurotensin has now been found throughout the nervous system and gut, with high concentrations in the hypothalamus, brain stem and ileum. Most of the body's neurotensin is present in the gastrointestinal tract, especially the ileum, and here it is located within discrete endocrine cells known as N-cells (Rosell & Rökaeus, 1981). Some of the pharmacological effects of neurotensin, together with possible physiological actions, are listed in Table 8.1. Although some of these effects are similar to bradykinin, there is no structural resemblance (Fig. 8.1).

Neurotensin is an interesting peptide because it may act as both a circulating hormone and neurotransmitter. Fasting plasma levels in man have been reported between < 15 to 70 pmol l^{-1}, with post-prandial rises of between 2 and 4-fold (Blackburn, 1982). Fat is a good stimulus to gut neurotensin release and this, together with inhibition of gastric acid secretion and gastrointestinal motility by neurotensin, have led some authors to claim it as the long postulated "enterogastrone" (Rosell & Rökaeus, 1981). However, in trying to establish a physiological role for neurotensin, studies have relied

PHARMACOLOGICAL EFFECTS:

Central administration:

- Hypothermia
- Hypotension
- Stimulation of somatostatin release
- Inhibition of GH, LH and prolactin release (opposite occurs when given peripherally)
- Inhibition of gastric acid secretion
- Antinociceptive
- Modulation of dopaminergic, noradrenergic and cholinergic neurones

Peripheral administration:

- Vasodilatation and hypotension
- Vasoconstriction of portal, coronary and adipose vasculature
- Increases vascular permeability
- Inhibits gastric acid secretion
- Inhibits lower oesophageal sphincter, gastric emptying and intestinal peristalsis
- Hyperglycaemia (stimulates glucagon and inhibits insulin release)
- Increases intestinal secretion
- Releases histamine

POSSIBLE PHYSIOLOGICAL ACTIONS:-

? enterogastrone (fat stimulated release and inhibition of gastric acid secretion and motility)

? mediation of gastrocolic reflex

? role in postprandial distribution of fat

? role in glucose homeostasis

? role in temperature control

Table 8.1. Pharmacological effects and possible physiological actions of neurotensin (based on Rosell & Rökaeus, 1981; Brown & Miller, 1982; Calam, Peart & Unwin, 1983).

Neurotensin

Glp - Leu - Tyr - Glu - Asn - Lys - Pro - Arg - Arg - Pro - Tyr - Ile - Leu

Mol. wt. 1673

Bradykinin

Arg - Pro - Pro - Gly - Phe - Ser - Pro - Phe - Arg

Mol. wt. 1060

Fig. 8.1.

Amino acid sequences of bovine neurotensin and bradykinin.

upon the effects observed during neurotensin infusion at doses which simulated postprandial plasma concentrations of neurotensin-like immunoreactivity (NTLI) (Blackburn, Fletcher, Adrian & Bloom, 1980). Such data must be interpreted with caution as measured NTLI may not reflect biologically active neurotensin, but inactive N-terminal fragments, particularly as most antisera used to date have been N-terminally directed (Hammer & Leeman, 1981).

Recently, neurotensin and neurotensin receptors have been found within the kidney (Forsmann et al., 1982; Quirion, Evarist, Rioux, St.-Pierre, Pert & Pert, 1982). Neurotensin may affect the kidney as both a circulating hormone and locally released neurotransmitter. Thus, the renal effects of intravenously infused neurotensin (2, 20 and 200 pmol kg^{-1} min^{-1}) were studied in conscious rabbits. A C-terminal specific antibody was used to measure changes in plasma NTLI (Chapter 2).

RESULTS (Tables 8.2 and 8.3)

There was no effect of neurotensin on mean blood pressure, heart rate , ERPF, filtration fraction, plasma electrolytes, plasma glucose, plasma osmolality and plasma solids at the doses infused (Table 8.1). Although changes in plasma renin activity (PRA), GFR and urine flow occurred at the highest dose infused, the fall in renal sodium excretion was significant even at the low and middle doses, with plasma neurotensin levels within the human postprandial range (Blackburn, 1982). The fractional excretion of sodium also fell during neurotensin infusion, but the fall was only significant during infusion of the low and high doses. There was no significant change in the fractional excretion of potassium at any dose (Table 8.2).

Table 8.2. Effects of bovine neurotensin on mean arterial blood pressure, heart rate and plasma composition.

		Control (vehicle only)	Neurotensin in pmol kg^{-1} min^{-1} 2	20	200	n
P_{NTLI}	(pmol l^{-1})	< 3	15 ± 2	117 ± 22	1165 ± 91	4
MBP	(mm Hg)	60 ± 2	62 ± 4	58 ± 4	64 ± 3	12
HR	(beats min^{-1})	271 ± 9	243 ± 7	262 ± 8	242 ± 5	12
P_{Na}	(mmol l^{-1})	137 ± 1	136 ± 1	134 ± 1	134 ± 1	12
P_K	(mmol $^{1-1}$)	3.6 ± 0.1	3.5 ± 0.1	3.5 ± 0.1	3.4 ± 0.1	12
P_{Osm}	(m Osm kg^{-1})	275 ± 1	278 ± 2	276 ± 2	275 ± 2	12
P_{Glu}	(mmol l^{-1})	8.5 ± 0.4	8.5 ± 0.2	10.5 ± 1.3	9.1 ± 0.5	8
PS	(%)	7.68 ± 0.20	7.22 ± 0.16	7.35 ± 0.14	7.59 ± 0.26	8
$^{+}$PRA	(pg ml^{-1} hr^{-1})	12.9 ± 1.9	13.6 ± 2.4	17.9 ± 3.2	*22.8 ± 3.7	8

Values are expressed as mean ± S.E. of mean. $^{+}$Transformed variable, in which case the geometric mean and approximate S.E. of mean are given. n = number of observations. * signifies $P < 0.05$, when compared with the control value by Dunnett's test after analysis of variance. Abbreviations are as follows:- P_{NTLI}, plasma neurotensin-like immunoreactivity; MBP, mean arterial blood pressure; HR, heart rate; P_{Na}, P_K, plasma electrolytes; P_{Glu}, plasma glucose; P_{Osm}, plasma osmolality; PS, plasma solids; PRA, plasma renin activity.

Table 8.3. Effects of bovine neurotensin on renal haemodynamics and electrolyte excretion.

		Control (vehicle only)	Neurotensin in pmol kg^{-1} min^{-1} 2	20	200	n
ERPF	(ml min^{-1})	54.6 ± 4.9	54.7 ± 4.8	48.7 ± 2.8	42.6 ± 3	12
GFR	(ml min^{-1})	11.0 ± 0.9	10.9 ± 1.0	9.6 ± 0.9	*7.9 ± 0.8	12
FF	(%)	20.2 ± 0.4	20.0 ± 0.6	19.4 ± 1.1	18.5 ± 1.2	12
$^{+}\dot{V}$	(µl min^{-1})	362.6 ± 96.6	171.7 ± 49	167.5 ± 47.6	*78.9 ± 12.3	12
$^{+}U_{Na}V$	(µmol min^{-1})	9.6 ± 1.4	*4.0 ± 1.3	*4.9 ± 1.2	*1.9 ± 1.2	12
$^{+}U_{K}V$	(µmol min^{-1})	9.7 ± 1.1	8.7 ± 1.0	7.2 ± 0.9	*5.3 ± 0.6	12
$^{+}$FENa	(%)	0.67 ± 0.17	*0.29 ± 0.06	0.40 ± 0.10	*0.19 ± 0.04	12
$^{+}$FEK	(%)	25.7 ± 3.2	24.0 ± 2.1	23.0 ± 3.3	21.1 ± 2.2	12

Values are expressed as mean ± S.E. of mean. $^{+}$Transformed variable, in which case the geometric mean and approximate S.E. of mean are given. n = number of observations. * and ** signify $P < 0.05$ and < 0.01 respectively, when compared with the control value by Dunnett's test after analysis of variance. Abbreviations are as follows:- FF, filtration fraction $\dot{V}$, urine flow; $U_{Na}V$, $U_{K}V$, electrolyte excretion; FENa, FEK, fractional excretion.

DISCUSSION

Originally, neurotensin was found to produce vasodilatation and hypotension in the rat (Carraway & Leeman, 1973), but has since been shown to have no effect on blood pressure or heart rate in doses of 10-20 pmol kg^{-1} min^{-1} (Rosell, Burcher, Chang & Folkers, 1976). Thus it seems unlikely that the changes observed were due to haemodynamic effects of neurotensin at the low and middle doses, although distributional changes within the kidney cannot be excluded. Neurotensin is thought to interact with dopaminergic systems and to behave as a dopaminergic antagonist (Brown & Miller, 1982). Dopamine binding sites are present in renal tissue (Roberts, Woodruff & Poat, 1977) and dopamine is known to be natriuretic (Lee, 1982). Therefore, it is conceivable that neurotensin alters dopaminergic activity peripherally and perhaps even centrally. It may influence sympathetic nervous function and release of other hormones such as prolactin (Brown & Vale, 1977), thus affecting sodium excretion, GFR and PRA (Bliss & Lote, 1982). Furthermore, neurotensin-like immunoreactivity has been demonstrated within renal peptidergic nerves (Forssmann et al., 1982) and neurotensin may act directly to alter intrarenal blood flow or tubular function.

The derived variable of fractional excretion minimizes the changes in sodium excretion due to alterations in GFR (Chapter 2). Thus, the fall in sodium excretion during infusion of the middle dose seems largely due to the small reduction in GFR. However, the decrease in fractional sodium excretion during infusion of the low and high doses seems to suggest a direct tubular action of neurotensin, for even at the high dose, the reduction in GFR does not fully explain the fall in sodium excretion. The fact that the relationship is not strictly

dose-dependent may simply indicate a changing balance between haemodynamic and tubular effects of neurotensin.

The rise in PRA seen at the high dose infused could be the result of macula densa stimulation due to reduced sodium delivery, although the exact nature of the stimulus perceived, if any, by the macula densa is uncertain (Davis & Freeman, 1976). The finding that renal potassium excretion did not change until the fall in filtered load (GFR) may reflect increased distal tubular Na^+/K^+ exchange. The small fall in GFR at pharmacological concentrations of plasma neurotensin may be a direct effect or secondary to some other factor such as increased local production of angiotensin-II.

Haematocrit and plasma solids did not rise as anticipated had vascular permeability increased during neurotensin infusion (Table 8.1). Neurotensin did not elevate plasma glucose concentration. There is evidence that neurotensin-induced changes in circulating levels of insulin and glucagon depend upon the degree of glycaemia and in the presence of hyperglycaemia (ca. 9 mmol l^{-1}), insulin release is enhanced by neurotensin and glucagon release diminished (Blackburn, Bloom & Edwards, 1981). In the present study, this effect could explain the absence of neurotensin-induced hyperglycaemia, as basal plasma glucose levels were already elevated by continuous glucose infusion.

Finally, neurotensin may influence yet another aspect of sodium homeostasis, that of sodium appetite. Like some other neuropeptides, it seems to be involved in the regulation of appetite (Koopmans, 1981), and this might also include sodium appetite. Therefore, it seems possible that neurotensin could play a role in the control of sodium balance. As implied in the introduction to this chapter, it

will be necessary to isolate the circulating fragments of neurotensin and determine their actions, before firm conclusions can be reached concerning the effects of postprandial changes in plasma neurotensin concentration.

CHAPTER 8

PART 2:

NEUROTENSIN AND RENAL FUNCTION IN MAN

Neurotensin and renal function in man.

INTRODUCTION

As shown in the previous section of this chapter, neurotensin is antinatriuretic in the rabbit. Unlike man, the sodium intake of this animal is relatively low (14 - 28 mmol sodium and 18 - 37 mmol potassium per 24 hrs; estimate based on standard laboratory diet, Chapter 2). Therefore, the renal effects of bovine neurotensin in man were studied on 2 separate occasions under conditions of differing dietary sodium. In the first study, the 3 day equilibrium ("run-in") period consisted of the standard diet described in Chapter 2 containing 150 mmol sodium per day. In the second study, the calorie content was identical, but only 100 mmol sodium per day were given. It is known from previous work that by the third day of a low sodium diet, a new steady state has been achieved (Gordon & Peart, 1979).

Neurotensin (2.3 pmol kg^{-1} min^{-1}) was infused intravenously in 6 healthy male volunteers aged between 22 and 35 years according to the protocol described in Chapter 2. This dose of neurotensin has been shown to produce plasma neurotensin-like immunoreactivity (NTLI) levels comparable with those reported by Mashford et al. (Mashford, Nilsson, Rökaeus & Rosell, 1978) after a mixed meal (Blackburn et al., 1980). However, the reservations discussed in Part 1 of this chapter still apply i.e., antibody specificity and activity of neurotensin fragments.

RESULTS

Systemic and renal haemodynamics (Tables 8.4a and 8.4b)

Small, but statistically significant increases in diastolic and

Table 8.4a. HAEMODYNAMIC CHANGES (150 MMOL SODIUM)

	Pre- (vehicle only)	Neurotensin (2.3_1 pmol kg^{-1} min^{-1})	P	Post- U7 (n = 6)
SBP (mm Hg)	137 ± 10	139 ± 10	NS	-
DBP (mm Hg)	81 ± 7	83 ± 7	NS	-
MBP (mm Hg)	100 ± 7	101 ± 7	NS	-
HR (beats min^{-1})	64 ± 7	64 ± 7	NS	-
ERPF (ml min^{-1} kg^{-1})	9.3 ± 1.7	9.0 ± 0.7	NS	-
GFR (ml min^{-1} kg^{-1})	1.9 ± 0.3	1.9 ± 0.3	NS	-
FF (%)	21.5 ± 0.7	21.1 ± 0.3	NS	-
RBF (ml min^{-1} kg^{-1})	16.5 ± 3.5	16.1 ± 1.4	NS	-

Values are given as mean ± S.D., n = 12; P = results of paired t-test, NS = not statistically significant ($P > 0.05$). Abbreviations:- SBP, systolic blood pressure; DBP, diastolic blood pressure; MBP, mean blood pressure; HR, heart rate; ERPF, effective renal plasma flow; GFR, glomerular filtration rate; FF, filtration fraction; RBF, estimated renal blood flow.

Table 8.4b. HAEMODYNAMIC CHANGES (100 MMOL SODIUM)

	Pre- (vehicle only)	Neurotensin (2.3_1 pmol kg^{-1} min^{-1})	P	Post- U7 (n = 6)
SBP (mm Hg)	130 ± 7	132 ± 7	NS	-
DBP (mm Hg)	74 ± 10	81 ± 10	< 0.05	79 ± 7
MBP (mm Hg)	93 ± 10	98 ± 7	< 0.05	97 ± 7
HR (beats min^{-1})	59 ± 7	59 ± 7	NS	-
ERPF (ml min^{-1} kg^{-1})	8.9 ± 1.0	7.2 ± 2.4	< 0.01	8.0 ± 0.9
GFR (ml min^{-1} kg^{-1})	1.4 ± 0.7	1.2 ± 0.7	NS	-
FF (%)	16.1 ± 9.7	23.9 ± 26.5	NS	-
RBF (ml min^{-1} kg^{-1})	16.0 ± 2.1	12.9 ± 4.5	< 0.01	14.6 ± 1.6

Values are given as mean ± S.D., n = 12; P = results of paired t-test, NS = not statistically significant (P > 0.05). Abbreviations:- SBP, systolic blood pressure; DBP, diastolic blood pressure; MBP, mean blood pressure; HR, heart rate; ERPF, effective renal plasma flow; GFR, glomerular filtration rate; FF, filtration fraction; RBF, estimated renal blood flow.

mean blood pressures occurred during neurotensin infusion and ERPF fell slightly. These changes only occurred while on the low (100 mmol) sodium diet.

Urine composition (Tables 8.5a and 8.5b)

Sodium excretion did not change significantly during neurotensin infusion, but chloride and potassium excretions did fall in both studies. Not surprisingly, basal excretion rates of sodium and chloride were much lower on the low sodium diet, but the percentage decrease in chloride excretion was similar at approximately 30% in each study. There was a small increment in phosphate excretion and the urinary sodium:potassium (Na/K) ratio also increased during neurotensin infusion. Calcium excretion was unaffected.

Plasma composition (Tables 8.6a and 8.6b)

On the "normal" (150 mmol) sodium diet, plasma calcium and phosphate concentrations rose slightly during neurotensin infusion. On the low sodium diet, plasma potassium and chloride concentrations fell a little during neurotensin infusion. There were no changes in plasma solids, haematocrit and plasma renin activity (PRA). Figures 8.2a and 8.2b show the changes in plasma NTLI concentrations during neurotensin infusion in the "normal" and "low" sodium studies respectively.

The control period after neurotensin (U7)

In the second study (low sodium), the changes in blood pressure persisted, making it less likely that these were real effects of neurotensin even under these conditions. However, the change in ERPF did return towards the basal (pre-infusion) value.

The changes in potassium and phosphate excretion persisted after neurotensin infusion in both studies, but the comparable falls in chloride excretion returned towards basal values.

Table 8.5a. URINARY CHANGES (150 MMOL SODIUM)

	Pre- (vehicle only)	Neurotensin (2.3_1 pmol kg^{-1} min^{-1})	P	Post-U7 (n = 6)
$\dot{V}$ (µl $min^{-1}kg^{-1}$)	204.9±46.1	180.4±39.7	NS	-
C_{Osm} (µl $min^{-1}kg^{-1}$)	58.7±8.7	48.7±9.4	<0.01	51.5±7.1
C_{H_2O} (µl $min^{-1}kg^{-1}$)	146.2±43.6	131.8±31.5	NS	-
$U_{Na}V$ (µmol $min^{-1}kg^{-1}$)	3.06±0.97	2.75±1.11	NS	-
$U_{K}V$ (µmol $min^{-1}kg^{-1}$)	2.12±0.52	1.27±0.55	<0.01	1.01±0.54
$U_{Cl}V$ (µmol $min^{-1}kg^{-1}$)	2.39±0.80	1.64±0.80	<0.01	1.98±0.56
$U_{Ca}V$ (µmol $min^{-1}kg^{-1}$)	0.13±0.03	0.13±0.03	NS	-
$U_{Pi}V$ (µmol $min^{-1}kg^{-1}$)	0.19±0.07	0.21±0.07	<0.02	0.28±0.10
Na/K ratio	1.52±0.55	2.61±1.59	<0.01	4.31±3.09
Na/Ca ratio	24.0±10.4	21.1±7.3	NS	-

Values are given as mean ± S.D., n = 12; P = results of paired t-test, NS = not statistically significant ($P > 0.05$). Abbreviations:- $\dot{V}$, urine flow; C_{Osm}, osmolar clearance; C_{H_2O}, free water clearance; $U_{Na}V$, $U_{K}V$, etc., renal excretion.

Table 8.5b. URINARY CHANGES (100 MMOL SODIUM)

	Pre-(vehicle only)	Neurotensin (2.3_{-1} pmol kg^{-1} min^{-1})	P	Post-U7 (n = 5)
$\dot{V}$ ($\mu l\ min^{-1}kg^{-1}$)	184.0±46.1	145.6±42.5	<0.01	168.8±26.0
C_{Osm} ($\mu l\ min^{-1}kg^{-1}$)	49.7±6.5	43.3±7.6	<0.01	44.3±10.9
C_{H_2O} ($\mu l\ min^{-1}kg^{-1}$)	134.3±44.1	102.4±41.2	<0.01	124.5±17.3
$U_{Na}V$ ($\mu mol\ min^{-1}kg^{-1}$)	2.63±0.58	2.56±0.79	NS	-
$U_{K}V$ ($\mu mol\ min^{-1}kg^{-1}$)	1.73±0.78	1.23±0.81	<0.01	1.17±0.94
$U_{Cl}V$ ($\mu mol\ min^{-1}kg^{-1}$)	1.57±1.06	1.23±1.03	<0.05	1.49±1.25
$U_{Ca}V$ ($\mu mol\ min^{-1}kg^{-1}$)	0.13±0.04	0.12±0.03	NS	-
$U_{P_i}V$ ($\mu mol\ min^{-1}kg^{-1}$)	0.23±0.10	0.28±0.07	<0.01	0.36±0.08
Na/K ratio	1.76±0.76	2.67±1.47	<0.01	3.70±1.58
Na/Ca ratio	22.8±9.2	23.4±9.8	NS	-

Values are given as mean ± S.D., n = 12; P = results of paired t-test, NS = not statistically significant ($P > 0.05$). Abbreviations:- $\dot{V}$, urine flow; C_{Osm}, osmolar clearance; C_{H_2O}, free water clearance; $U_{Na}V$, $U_{K}V$, etc., renal excretion.

Table 8.6a. PLASMA CHANGES (150 MMOL SODIUM)

	Pre- (vehicle only)	Neurotensin (2.3_1 pmol kg^{-1} min^{-1})	P	Post-U7 (n = 6)
PS (%)	8.11 ± 0.21	8.23 ± 0.24	NS	-
Hct (%)	41 ± 3	41 ± 3	NS	-
P_{Na} (mmol l^{-1})	140.1 ± 1.7	140.8 ± 2.8	NS	-
P_K (mmol l^{-1})	4.18 ± 0.17	4.10 ± 0.17	NS	-
P_{Cl} (mmol l^{-1})	99.9 ± 1.7	100.5 ± 1.4	NS	-
P_{Ca} (mmol l^{-1})	2.02 ± 0.07	2.10 ± 0.10	< 0.01	2.09 ± 0.07
P_{Pi} (mmol l^{-1})	0.94 ± 0.14	1.01 ± 0.10	< 0.02	1.10 ± 0.15
P_{Osm} (mmol l^{-1})	278 ± 3	278 ± 3	NS	-
P_{Glu} (mmol l^{-1})	4.6 ± 0.3	4.7 ± 0.3	NS	-
$^{+}$PRA (pg ml^{-1} hr^{-1})	676 ± 301	796 ± 461	NS	-

Values are given as mean ± S.D., n = 12. $^{+}$ Transformed variable expressed as geometric mean ± approximate S.D.; P = results of paired t-test, NS = not statistically significant ($P > 0.05$).

Abbreviations:- PS, plasma solids; Hct, haematocrit; P_{Na}, P_K, etc., plasma concentration; PRA, plasma renin activity.

Table 8.6b. PLASMA CHANGES (100 MMOL SODIUM)

	Pre- (vehicle only)	Neurotensin (2.3_1 pmol kg^{-1} min^{-1})	P	Post- U7 (n = 5)
PS (%)	7.35 ± 0.55	7.42 ± 0.48	NS	-
Hct (%)	42 ± 3	42 ± 3	NS	-
P_{Na} (mmol l^{-1})	139.7 ± 0.7	139.3 ± 1.4	NS	-
P_K (mmol l^{-1})	4.33 ± 0.45	4.21 ± 0.31	< 0.05	4.07 ± 0.32
P_{Cl} (mmol l^{-1})	106.3 ± 3.1	104.6 ± 3.5	< 0.05	103.5 ± 3.0
P_{Ca} (mmol l^{-1})	2.53 ± 0.07	2.57 ± 0.10	NS	-
P_{Pi} (mmol l^{-1})	1.02 ± 0.21	1.10 ± 0.17	NS	-
P_{Osm} (mmol l^{-1})	278 ± 3	278 ± 3	NS	-
$^{+}$PRA (pg ml^{-1} hr^{-1})	963 ± 249	921 ± 211	NS	-

Values are given as mean ± S.D., n = 12. $^{+}$ Transformed variable expressed as geometric mean ± approximate S.D.; P = results of paired t-test, NS = not statistically significant ($P > 0.05$).

Abbreviations:- PS, plasma solids; Hct, haematocrit; P_{Na}, P_K, etc., plasma concentration; PRA, plasma renin activity.

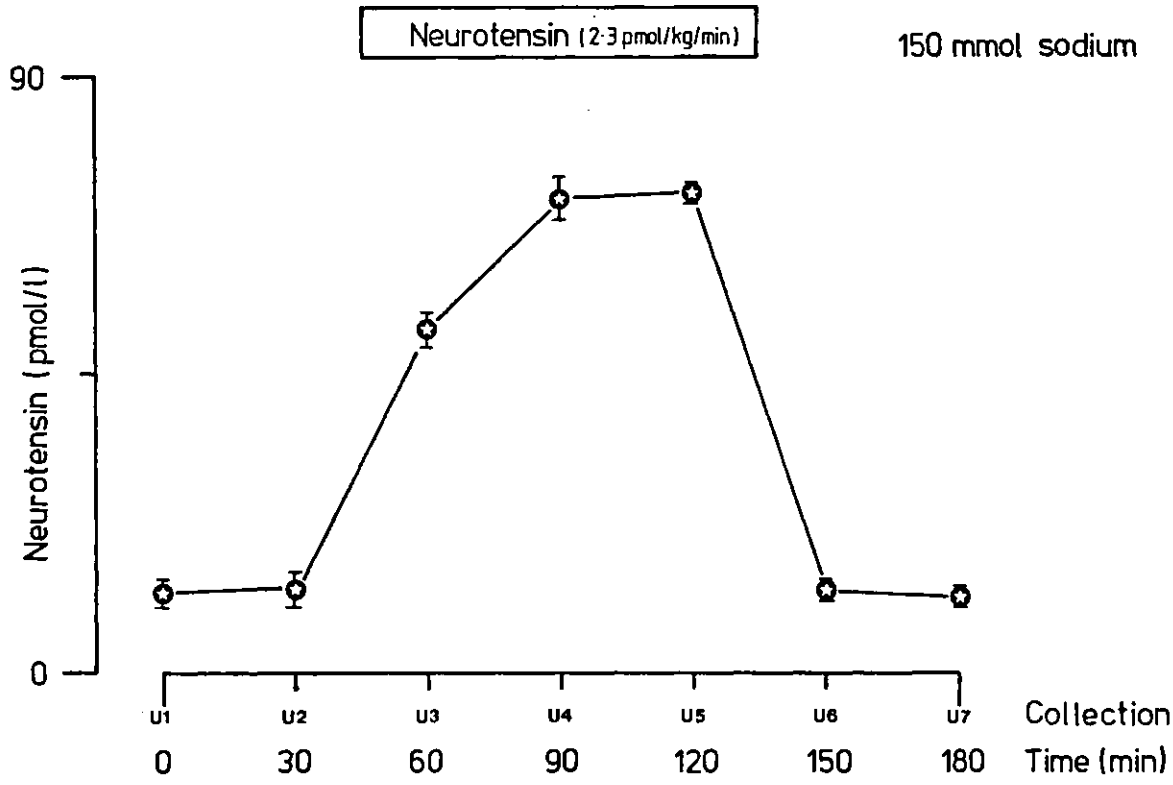


Fig. 8.2a (normal sodium diet).

Mean (± S.E. of mean, n = 6) plasma NTLI levels, before, during and after intravenous neurotensin infusion.

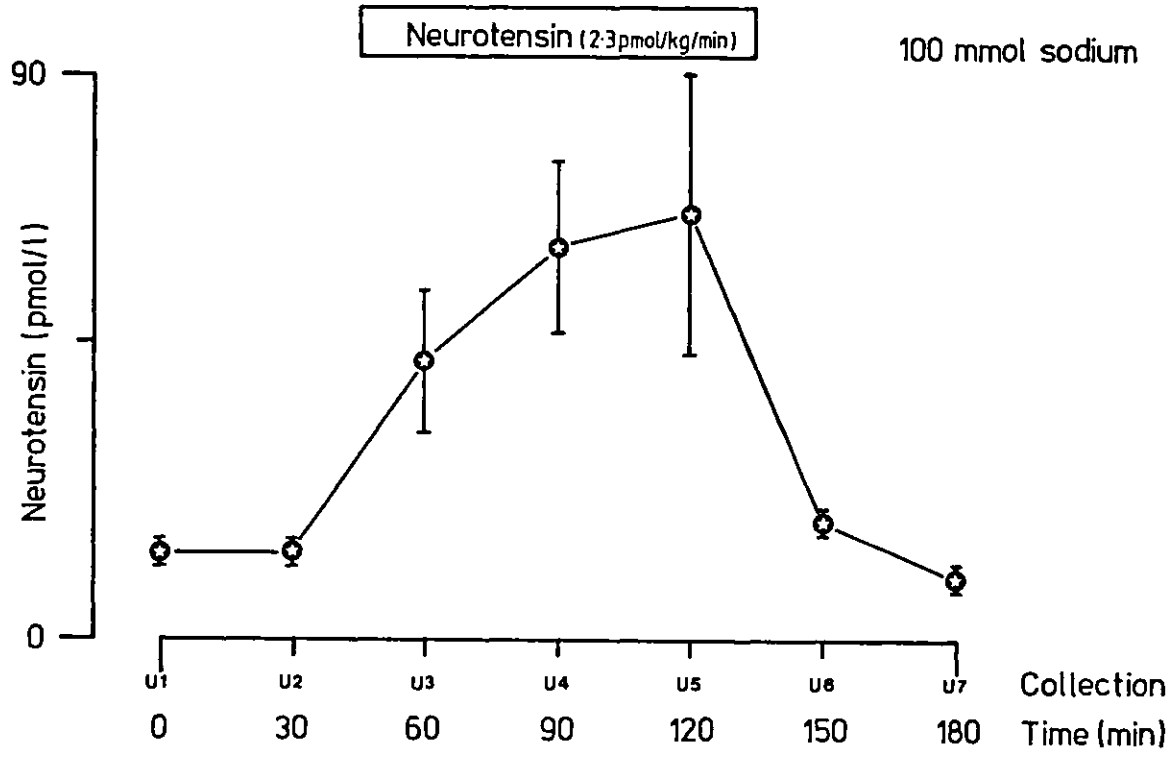


Fig. 8.2b (low sodium diet).

Mean (± S.E. of mean, n = 6) plasma NTLI levels, before, during and after intravenous neurotensin infusion.

DISCUSSION

One problem of interpretation of changes in potassium excretion in all these human studies has been a fall which has persisted after ceasing the peptide infusion. The antikaliuretic action of these peptides (pentagastrin, CCK8 and neurotensin) could be considered as a specific renal effect. However, absence of recovery, or at least a move towards pre-infusion values in such longitudinal "off-on-off" studies, inevitably makes recognition of peptide-related effects difficult and interpretation hazardous (see Chapter 2).

In contrast to the study in the rabbit, neurotensin in man did not alter renal sodium excretion, even under conditions of differing sodium balance. However, chloride excretion did fall and recover after infusion, in both studies. Could this indicate a primary effect of neurotensin on renal handling of chloride rather than sodium? The increase in phosphate excretion would maintain the electrochemical balance, but this does not imply any necessary or causal relationship. It seems fair to broadly conclude that neurotensin may have effects on tubular handling of sodium and chloride under physiological conditions i.e., during feeding.

The small rise in plasma phosphate concentration may have contributed to the increase in phosphate excretion. Consistently in these studies (CCK8 included), phosphate excretion has risen while potassium excretion has fallen. This inverse association or relationship may depend upon renal acid-base balance and increased distal tubular H^+ secretion (as potassium secretion falls), buffered by urinary phosphate.

In conclusion, changes in dietary sodium intake do not appear to alter the response to neurotensin infusion. Of all the peptides

examined, neurotensin is potentially one of the most exciting and perhaps fruitful in terms of future work. It is both a neurotransmitter and circulating hormone with possible renal actions at physiological plasma concentrations found in man. The plasm NTLI concentrations achieved in this study may be regarded as post-prandial, although in terms of the response to the "Hammersmith Test Meal" (see Appendix), they are high.

SUMMARY TO CHAPTER 8

1. In the search for a gut peptide which may influence renal sodium excretion and be involved in the renal response to sodium deprivation, neurotensin is of particular interest because of its dual role as a circulating hormone and putative neurotransmitter. Moreover, neurotensin receptors and neurotensin-like immunoreactivity (NTLI; peptidergic nerves) have been found in the rat renal cortex.

2. The renal actions of neurotensin were examined by infusion intravenously in 4 conscious rabbits in doses of 2, 20 and 200 pmol kg^{-1} min^{-1}, and in 6 human subjects at 2.3 pmol kg^{-1} min^{-1}. Two human studies were carried out in 2 separate groups each on 2 different sodium diets, containing 150 ("normal") and 100 ("low") mmol sodium respectively. Plasma NTLI concentrations at the two lower doses in the rabbit and at the dose used in the human study were comparable with previously reported post-prandial levels in man.

3. In the rabbit, neurotensin produced a significant reduction in renal sodium excretion at each dose, with similar responses at 2 and 20 pmol kg^{-1} min^{-1}. At these doses, there were no significant changes in plasma composition and renal haemodynamics. Only at the highest dose was there a significant fall in GFR and rise in PRA. In man, although sodium excretion did not fall significantly during neurotensin infusion on either diet, chloride excretion did. Potassium excretion also fell, but no other consistent changes were observed.

4. Neurotensin is thought to inhibit dopaminergic neurones, but the role of dopamine in the kidney is unclear. The action of neurotensin in the rabbit may involve inhibition of peripheral or central dopaminergic neurones. In man at least, it is possible that neurotensin is predominantly affecting renal tubular chloride transport rather than sodium. These changes occurred at plasma NTLI levels similar to those found after feeding in man and raise the possibility that neurotensin contributes to the regulation of postprandial renal salt excretion.

CHAPTER 9

RENAL FUNCTION DURING SUBSTANCE P (SP) INFUSION IN CONSCIOUS RABBITS

Renal function during substance P (SP) infusion in conscious rabbits.

INTRODUCTION

SP was the first "gut-brain" peptide to be discovered. It was originally isolated from equine intestine and brain by von Euler and Gaddum (1931), during work on the distribution of acetylcholine, and named after the "powdery" appearance of the crude preparation. Forty years later, an extract was isolated from bovine hypothalami by Chang and Leeman (1970), which caused salivation (rat bioassay) and was later found to be SP.

Much of the early work (pre-1970) on the distribution and actions of SP was based on bioassay. Unfortunately, preparations or standards used were often contaminated with other biologically active substances, such as bradykinin. Figure 9.1 shows the amino acid sequence of SP, the structurally related peptide bombesin and bradykinin.

Table 9.1 summarises the distribution, pharmacological actions and potential physiological roles of SP. It is currently believed to be a sensory neurotransmitter involved in pain sensation (Iversen, 1982). It is also thought to be involved in the control of gastrointestinal motility and exocrine gland function, including the pancreas (Sundler, Håkanson & Leander, 1980).

SP-like immunoreactivity (SPi) has been reported in human and dog plasma (Nilsson, Pernow, Fisher & Folkers, 1975; Powell, Cannon, Skrabanek & Kirrane, 1978), but levels vary considerably presumably due to variations in antibody specificity and assay sensitivity. It appears that exogenous SP has a shorter half-life than endogenous SP-like immunoreactivity (SPLI) and there is evidence to suggest that SP

Substance P

Arg - Pro - Lys - Pro - Gln - Gln - Phe - Phe - Gly - Leu - Met - NH_2

Mol. Wt. 1348

Bombesin

pGlu - Gln - Arg - Leu - Gly - Asn - Gln - Trp - Ala - Val - Gly - His - Leu - Met - NH_2

Mol. wt. 1620

Bradykinin

Arg - Pro - Pro - Gly - Phe - Ser - Pro - Phe - Arg

Mol. wt. 1060

Fig. 9.1.

Amino acid sequences and molecular weights of substance P, bradykinin and bombesin.

SUBSTANCE P

DISTRIBUTION

Throughout body, highest concentrations in gut and nervous system:

- brain (basal ganglia and hypothalamus)
- spinal cord (dorsal horn and dorsal root ganglia)
- myenteric plexus
- gut endocrine (enterochromaffin) cells

PHARMACOLOGICAL EFFECTS

Vasodilatation and hypotension

Non-vascular smooth muscle contraction

Salivation (rat bioassay)

Histamine release

POTENTIAL PHYSIOLOGICAL ROLES

? sensory neurotransmitter (nociception)

? exocrine secretion, pancreas and salivary glands

? gut motility, direct or indirect

Table 9.1.

Distribution, actions and possible functions of substance P (see text for details).

circulates as part of a larger, less readily degraded molecule (Powell et al., 1978). Whether or not this form is physiologically active is unknown. Levels are not known to change during feeding, but high blood levels have been reported in patients with carcinoid syndrome and medullary thyroid carcinomas (Powell et al., 1978; Skrabanek, Cannon, Dempsey, Kirrane, Nelligan & Powell, 1979). Others have also reported raised levels of SPLI, but this did not co-chromatograph with SP on gel permeation analysis (McGregor, 1982). In the carcinoid syndrome, SP is thought to be responsible for the flushing, hypotension, bronchoconstriction and diarrhoea (Powell & Skrabanek, 1981).

Although found within nerve fibres of the renal cortex (Hökfelt et al., 1978; Forsmann et al., 1982), the role of SP in the regulation of renal function is unclear. The kidney is involved in the metabolism of SP (Campbell & Ward, 1979) and is a rich source of inactivating enzymes (Ward & Johnson, 1978). Macfarlane, Mills & Ward (1974) reported that infusion of SP into the renal artery of the anaesthetised dog produced a marked diuresis and natriuresis. This was associated with increases in inulin and PAH clearances, although at one dose, renal sodium excretion rose independently. Subsequently, several workers have observed broadly similar responses within a comparable dose range, although there have been differences between intravenous versus intrarenal administration and associated haemodynamic changes (Table 9.2). Most studies have been in the anaesthetised dog, but a recent study in the conscious rat (Kramer, Klingmuller, Flachskampf & Düsing, 1983) suggests that SP-induced increases in renal electrolyte excretion and urine flow, are independent of detectable haemodynamic changes, although only one dose of SP was used.

Table 9.2. Results from some studies of the renal effects of SP (see text for discussion).

REFERENCE	SPECIES	DOSE	ROUTE	BP	RBF	GFR	$\dot{V}$	$U_{Na}V$
Macfarlane et al., 1974	Dog (a)	1-*100 ng $kg^{-1}min^{-1}$	i.a.	-	*↑	*↑	*↑	*↑
Mills et al., 1974	Dog (a)	1-*100 ng $kg^{-1}min^{-1}$	i.a.	-	-	-	*↑	*↑
Hallberg & Pernow, 1975	Dog (a)	1.2 ng kg^{-1} min^{-1}	i.v.	→	↓	-	-	-
"	"	> 2.5 ng $kg^{-1}min^{-1}$	i.v.	↓	↑	-	-	-
Arendhorst et al., 1976	Rat (a)	50 pg min^{-1}	i.a.	-	→	→	↑	↑
Gullner et al., 1978	Dog (a)	5 ng kg^{-1} min^{-1}	i.a.	↓	↑	↓	↑	↑
"	"	0.5-*50 ng $kg^{-1}min^{-1}$	i.v.	*↓	*→/↓	*↓	*↓	→
Gullner & Bartter, 1979	Dog (a)	0.2 ng kg^{-1} min^{-1}	i.a.	→	→	→	→	→
Kramer et al., 1983	Rat (c)	1 ng kg^{-1} min^{-1}	i.v.	→	→	→	↑	↑

Abbreviations: a, anaesthetised; c, conscious; i.a., intrarenal; i.v., intravenous; BP, mean blood pressure; RBF, renal blood flow or estimate; GFR, glomerular filtration rate; $\dot{V}$, urine flow, $U_{Na}V$, renal sodium excretion; ↑, increase; ↓, decrease; →, no change; -, no record.

In view of the presence of SP within the kidney and the uncertainty surrounding its renal actions, its effects were studied in the conscious rabbit.

RESULTS

Systemic blood pressure and heart rate (Fig. 9.2)

There was no significant alteration in these parameters.

Renal haemodynamics

Figure 9.3 shows the small dose-related fall in ERPF and GFR and the 25% reduction in ERPF during the high dose infusion (200 pmol kg^{-1} min^{-1}). Filtration fraction also rose significantly at this dose.

Urine composition (Table 9.3)

Excretion of sodium, potassium, chloride and phosphate did not change significantly at any dose. Calcium excretion fell significantly during the middle (20 pmol kg^{-1} min^{-1}) and high doses of SP infusion. Figure 9.4 shows the dose-dependent changes in fractional sodium and calcium excretion, together with the rise in urinary sodium:calcium (Na/Ca) ratio. Although not statistically significant, fractional sodium excretion did tend to increase at the high dose. There were dose-related falls in urine flow and free water clearance, but no significant change in osmolar clearance. There was no change in urine pH at any dose.

Plasma composition and haematocrit (Table 9.4)

Plasma potassium and phosphate concentrations fell slightly during the high dose infusion and plasma chloride concentration decreased during infusion of the middle dose of SP. While there seemed to be a dose-dependent rise in haematocrit, plasma solids and PGPS, these changes did not reach statistical significance. Figure

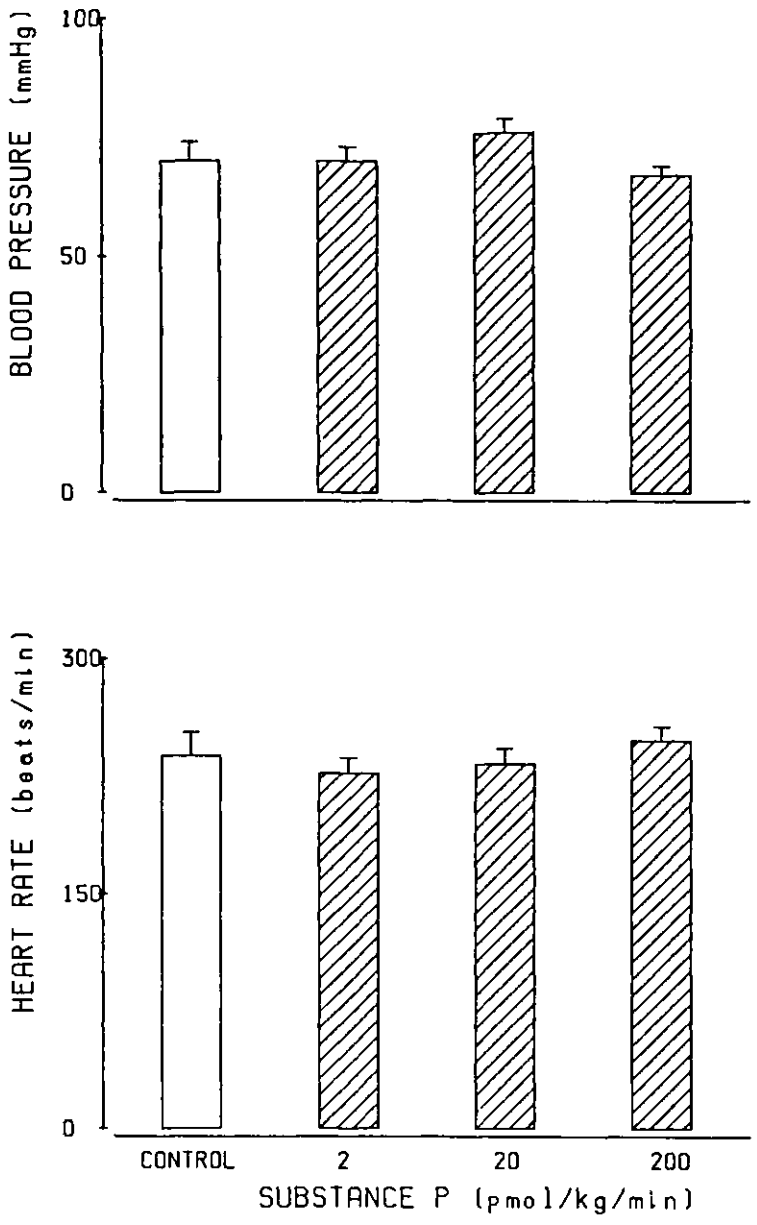


Fig. 9.2.

Effect of increasing doses of SP on heart rate and mean blood pressure. Results are expressed as mean ± S.E. of mean, n = 12 for each treatment group.

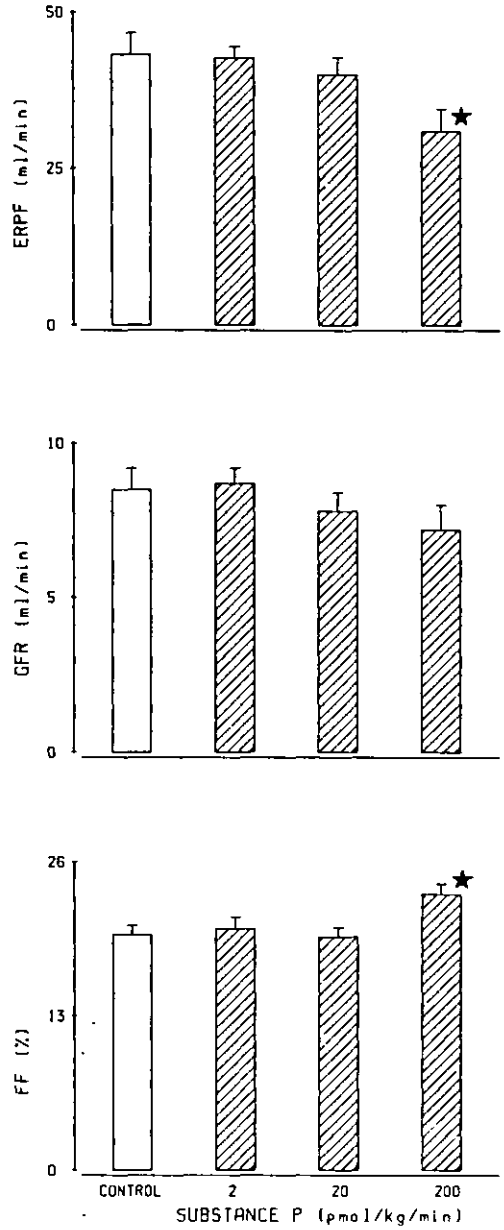


Fig. 9.3.

Effect of increasing doses of SP on effective renal plasma flow (ERPF), glomerular filtration rate (GFR) and filtration fraction (FF). Results are expressed as mean ± S.E. of mean, n= 12 for each treatment group. * indicates significant difference ($p < 0.05$) from control value as determined by Dunnett's test after analysis of variance.

Table 9.3.

Effects of substance P on renal electrolyte excretion, urine flow, solute excretion and urine pH.

	Control (vehicle only)	Substance P in pmol kg^{-1} min^{-1} 2 (low)	20 (middle)	200 (high)	n
$^{+}U_{Na}V$ (µmol min^{-1})	3.0 ± 0.7	3.2 ± 0.5	2.3 ± 0.5	3.9 ± 0.6	12
$^{+}U_{K}V$ (µmol min^{-1})	5.5 ± 0.5	6.0 ± 0.5	5.7 ± 0.8	4.1 ± 0.6	12
$^{+}U_{Cl}V$ (µmol min^{-1})	6.0 ± 0.6	5.9 ± 0.7	4.2 ± 0.6	5.3 ± 1.0	12
$^{+}U_{Ca}V$ (µmol min^{-1})	0.65 ± 0.14	0.78 ± 0.18	**0.35 ± 0.07	**0.25 ± 0.04	12
$^{+}U_{Pi}V$ (µmol min^{-1})	0.70 ± 0.12	1.01 ± 0.08	0.78 ± 0.07	0.79 ± 0.14	12
$^{+}\dot{V}$ (µl min^{-1})	309.9 ± 57.1	294.9 ± 35.7	227.1 ± 65.5	**99.4 ± 23.9	12
$^{+}C_{Osm}$ (µl min^{-1})	158.9 ± 13.3	156.4 ± 8.6	128.8 ± 15.1	113.1 ± 17.1	12
C_{H_2O} (µl min^{-1})	192.1 ± 40.6	159.1 ± 31.2	178.6 ± 44.3	17.7 ± 38.6	12
pH	6.62 ± 0.20	6.38 ± 0.12	6.92 ± 0.14	6.90 ± 0.14	12

Values are expressed as mean ± S.E. of mean. $^{+}$Transformed variable, in which case the geometric mean and approximate S.E. of mean are given. n = number of observations. * and ** signify $P < 0.05$ and < 0.01 respectively, when compared with the control value by Dunnett's test after analysis of variance. Abbreviations are as follows:- $U_{Na}V$, $U_{K}V$ etc., renal excretion; $\dot{V}$, urine flow; C_{Osm}, osmolar clearance; C_{H_2O}, free water clearance.

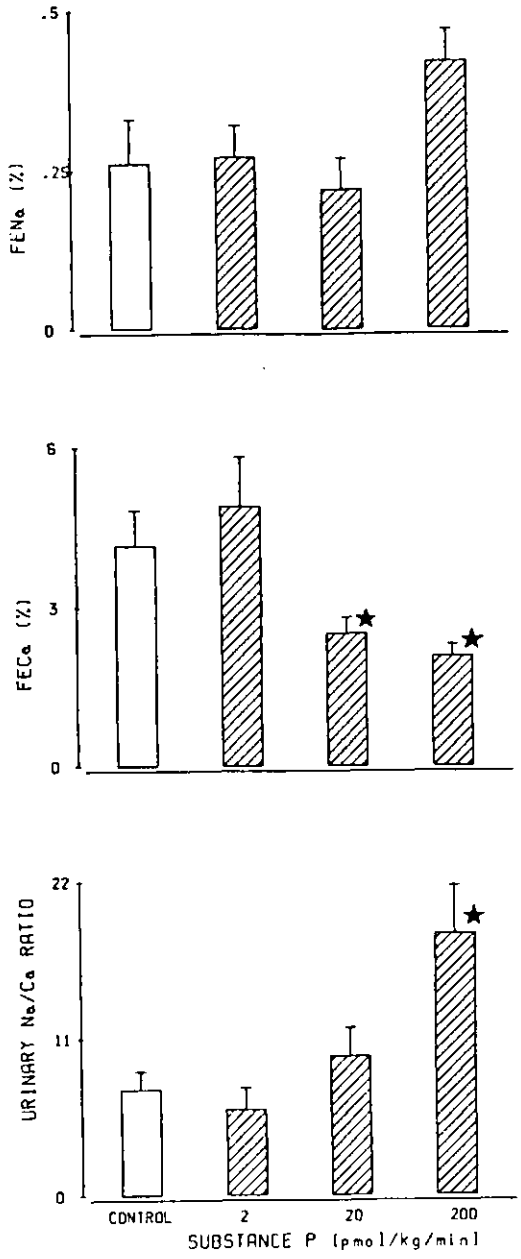


Fig. 9.4.

Effect of increasing doses of SP on fractional excretion of sodium (FENa), calcium (FECa) and urinary sodium : calcium ratio (Na/Ca). FENa and FECa were found to be lognormally distributed and therefore results are expressed as geometric mean ± approximate S.E. of mean, n = 12 for each treatment group. * indicates significant difference ($p < 0.05$) from control value as determined by Dunnett's test after analysis of variance.

Table 9.4. Effects of substance P on plasma composition and haematocrit.

	Control (vehicle only)	Substance P in pmol kg^{-1} min^{-1} 2 (low)	20 (middle)	200 (high)	n
P_{Na} (mmol l^{-1})	139.4 ± 0.8	139.4 ± 0.8	139.1 ± 1.1	136.8 ± 0.5	12
P_{K} (mmol l^{-1})	4.07 ± 0.07	4.08 ± 0.05	3.91 ± 0.11	**3.61 ± 0.09	12
P_{Cl} (mmol l^{-1})	105.8 ± 0.7	108.1 ± 0.6	*102.2 ± 1.0	104.3 ± 1.1	12
P_{Ca} (mmol l^{-1})	3.18 ± 0.04	3.14 ± 0.06	3.12 ± 0.07	3.08 ± 0.05	12
P_{Pi} (mmol l^{-1})	1.67 ± 0.02	1.68 ± 0.04	1.54 ± 0.04	*1.50 ± 0.10	12
P_{Glu} (mmol l^{-1})	9.2 ± 0.2	9.1 ± 0.3	10.2 ± 0.5	9.3 ± 0.2	8
P_{Osm} (m Osm kg^{-1})	275 ± 1	275 ± 1	276 ± 1	273 ± 2	12
PS (%)	6.19 ± 0.19	6.12 ± 0.19	6.18 ± 0.23	6.76 ± 0.30	8
Hct (%)	33 ± 2	30 ± 3	35 ± 1	38 ± 1	8
+PGPS (%)	7.70 ± 0.24	7.60 ± 0.18	7.58 ± 0.28	8.67 ± 0.40	8

Values are expressed as mean ± S.E. of mean. +Transformed variable, in which case the geometric mean and approximate S.E. of mean are given. n = number of observations. * and ** signify $P < 0.05$ and < 0.01 respectively, when compared with the control value by Dunnett's test after analysis of variance. Abbreviations are as follows:- P_{Na}, P_{K}, etc., plasma concentrations; PS, plasma solids; Hct, haematocrit; PGPS, post-glomerular plasma solids.

9.5 shows the small dose-related rise in plasma renin activity (PRA).

DISCUSSION

SP had no significant effects on pulse and mean arterial blood pressure, even at the highest dose of 200 pmol kg^{-1} min^{-1}. In anaesthetised dogs, several workers have reported dose-related falls in mean blood pressure during intravenous infusion of between 2 and 25 ng kg^{-1} min^{-1} (approximately 2 to 40 pmol kg^{-1} min^{-1}) of SP (Hallberg & Pernow, 1975; Gullner et al., 1978). However, when SP was infused in man, hypotension and tachycardia were transient, with rapid development of tachyphylaxis (Powell et al., 1978).

Small doses of SP (< 2 pmol kg^{-1} min^{-1}) infused into the renal artery of the anaesthetised dog and rat have been consistently shown to increase renal sodium and water excretion, without changes in blood pressure and renal perfusion (Macfarlane et al., 1974; Mills, Macfarlane & Ward, 1974; Arendhorst, Cook & Mills, 1976; Gullner et al., 1978). The effects of intravenous SP are less well documented. Two studies in different species, with and without anaesthetic, have reported quite different results. Gullner et al. (1978) did not observe any increase in fractional sodium excretion in the anaesthetised dog, even during 50 ng kg^{-1} min^{-1} (approximately 40 pmol kg^{-1} min^{-1}) of SP, but did record a fall in blood pressure, renal blood flow, creatinine clearance and urine flow. In contrast, Kramer's group (1983) observed a 4-fold rise in renal sodium excretion and doubling of urine flow in the conscious rat, but no change in renal haemodynamics during infusion of approximately 1 ng kg^{-1} min^{-1} (approximately 1 pmol kg^{-1} min^{-1}) of SP. In the rabbit, renal sodium excretion did not change significantly, but fractional

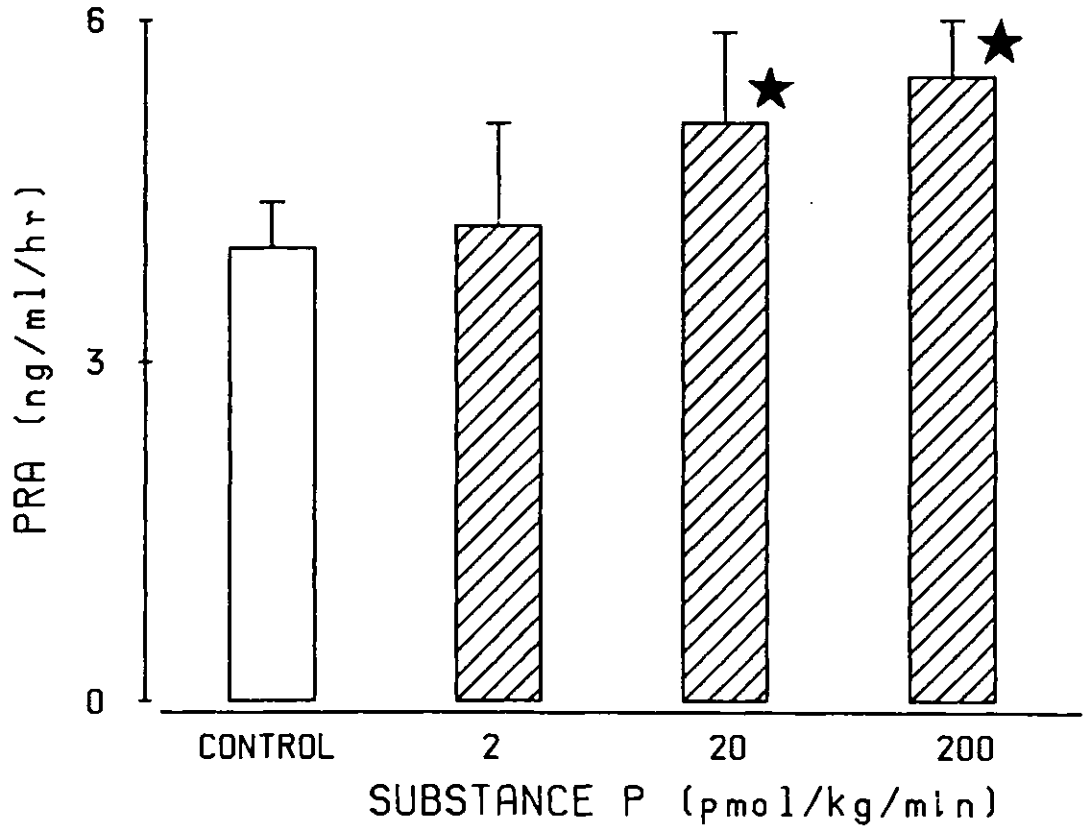


Fig. 9.5.

Effect of increasing doses of SP on plasma renin activity (PRA). This variable was found to be lognormally distributed and therefore results are expressed as geometric mean ± approximate S.E. of mean, n = 8 for each treatment group. * indicates significant difference ($p < 0.05$) from control value as determined by Dunnett's test after analysis of variance.

sodium excretion did appear to rise during the high dose infusion. At this dose, ERPF fell, GFR did not change and filtration fraction increased. Arendhorst et al. (1976) and Kramer et al. (1983) have also recorded a small rise in filtration fraction.

High doses of SP given intravenously in anaesthetised dogs, have been shown to decrease plasma volume and increase capillary permeability to protein (Macfarlane, Ward & Mills, 1974). Under these circumstances, the natriuretic effect of SP does not occur, although it has been suggested that increased peritubular capillary permeability to protein, by reducing oncotic pressure, would tend to decrease proximal tubular reabsorption of sodium and water, and thus explain the natriuretic effect of SP. However, in the rabbit there appears to have been very little change in plasma protein concentration as estimated by plasma solids.

The fall in renal and fractional calcium excretion, apparently independent of changes in sodium excretion, suggests a distal tubular site of action of SP. This may be parathormone-like, or related to slight inhibition of distal tubular sodium reabsorption (c.f. thiazide diuretics: Suki, 1979). It is unlikely to reflect changes in peritubular capillary physical factors, as no other electrolyte was affected. With regard to a parathormone-like effect, results from a study of similar design in the rabbit (Peart et al., 1981), showed that the fall in renal calcium excretion produced by parathormone was associated with a marked natriuresis (Fig. 9.6). This contrasts with the specific reduction of renal calcium excretion induced by SP in the absence of any significant change in renal sodium excretion.

When infused into the renal artery, SP produces an increase in renal blood flow, even in the presence of a decrease in blood

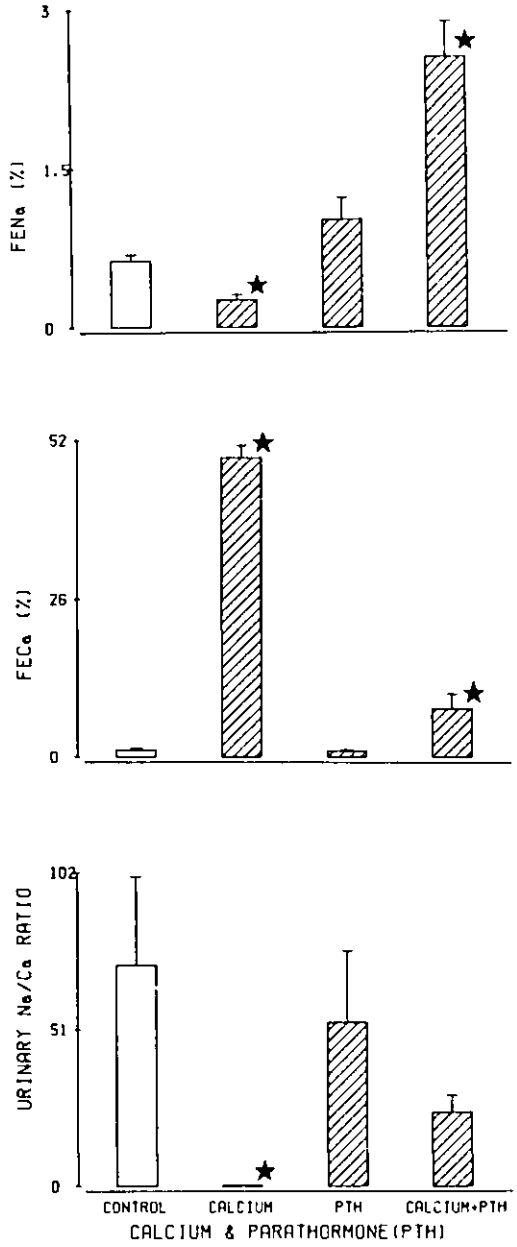


Fig. 9.6 (legend overleaf).

Fig. 9.6.

Effect of parathormone (PTH; 0.15 μg kg^{-1} min^{-1}) and calcium chloride (1.0 mg kg^{-1} min^{-1}) on fractional excretion of sodium (FENa), calcium (FECa) and urinary sodium : calcium ratio (Na/Ca) in the conscious rabbit. FENa and FECa were found to be lognormally distributed and therefore results are expressed as geometric mean ± approximate S.E. of mean, n = 8 for each treatment group. * indicates significant difference ($p < 0.05$) from control value as determined by Dunnett's test after analysis of variance. The important comparisons to be made are "calcium" alone with "calcium + PTH": A large increase in FENa occurred in the presence of PTH, FECa fell and the Na/Ca ratio increased.

pressure, but when large doses are infused systemically, renal blood flow and blood pressure fall (Gullner et al., 1978). Hallberg & Pernow (1975) explored the effects of systemically infused SP on various vascular beds in the anaesthetised dog. They found the splanchnic circulation exquisitely sensitive to SP-induced vasodilatation. In contrast, low doses of SP appeared to reduce renal blood flow slightly, but with no change in blood pressure. The data of Arendhorst et al. (1976) and Kramer et al. (1983) also show a slight reduction in renal blood flow during SP infusion. In the rabbit, a small dose-related fall in ERPF and GFR did occur, but during the high dose infusion, the reduction in ERPF was much greater. These findings suggest an effect on the post-glomerular circulation with efferent arteriole vasoconstriction and increased post-glomerular renal vascular resistance, similar to the action of angiotensin-II (Freeman & Davis, 1979). A direct vasoconstrictor action of SP does seem unlikely in view of its well documented vasodilating effect, although this is still a possibility. Generalised vasodilatation, particularly splanchnic, may lead to increased reflex sympathetic activity to maintain blood pressure and produce renal vasoconstriction. However, in this study there was no indirect evidence of sympathetic stimulation, such as increased heart rate. As SP did increase PRA, local intrarenal release of angiotensin-II is also possible. These renal vascular effects of SP are similar to those of VIP (Chapter 6.1).

SP has been reported to suppress renin release in the presence and in the absence of natriuresis and renal vasodilatation (Gullner et al., 1978; Gullner & Bartter, 1979). Doses observed to increase renin release also produced hypotension and may therefore have

stimulated renin through reflex sympathetic activation (Pettinger et al., 1973). It is unusual for a vasodilator to inhibit renin release, although a similar effect has been seen with CCK8 (Chapter 4.1) and cannot be explained easily by known regulatory mechanisms (Davis & Freeman, 1976; Peart, 1978). In the rabbit, basal PRA levels were low, making it difficult to detect suppression of renin release. As stated above, the increases in PRA produced by the middle and high doses of SP cannot be explained readily in terms of the generally accepted stimuli to renin release, but the changes are small.

Little is known of the physiological stimuli to SP release, although it has been reported to rise on standing and fall during sodium deprivation (Kramer, Düsing, Stelkens, Heinrich, Kipnowski & Glänzer, 1980); it is therefore difficult to postulate any contribution to the regulation of renal function. The presence of peptidergic nerves within the renal cortex close to blood vessels (Hökfelt et al., 1978; Forsmann et al., 1982) suggests involvement in the control of intrarenal blood flow and even renin release, particularly in view of its effect on renal calcium excretion, as this ion probably plays a central part in the control of renin release (Peart, 1978). This may in turn alter renal electrolyte handling through changes in physical factors, although a direct tubular action of SP is still possible.

From this study, it does not appear that SP is important in renal salt and water handling, if its presence in the circulation is required. A different approach is needed to define a possible intrarenal role.

SUMMARY TO CHAPTER 9

1. Substance P (SP) was the first "gut-brain" peptide to be discovered and its pharmacological actions include hypotension, vasodilatation and smooth muscle contraction. Although found in plasma, it is not thought to be a circulating hormone, but rather a neurotransmitter. Peptidergic nerves containing SP-like immunoreactivity have been found within the kidney.

2. The renal effects of SP were studied in 4 conscious rabbits by infusing intravenously 3 different doses of 2, 20 and 200 pmol kg^{-1} min^{-1}.

3. SP produced a small dose-related rise in PRA and fall in renal calcium excretion. Filtration fraction rose and ERPF fell during infusion of the highest dose. There were no changes in blood pressure, heart rate, plasma solids, plasma calcium concentration and sodium excretion.

4. The haemodynamic changes indicate a rise in renal vascular resistance affecting mainly the efferent arteriole. These results do not support the view that SP is a potent natriuretic agent, but do suggest effects on the post-glomerular circulation and tubular handling of calcium. The latter action appears to be independent of changes in renal sodium excretion.

CHAPTER 10

PEPTIDES WITH NO OBVIOUS RENAL ACTION AND THOSE WITH DISCRETE OR INCONSISTENT EFFECTS IN THE CONSCIOUS RABBIT

Peptides with no obvious renal action and those with discrete or inconsistent effects in the conscious rabbit.

INTRODUCTION

The following peptides produced either no changes in renal function and plasma composition, or effects of doubtful significance: Motilin, Met-enkephalin, Pancreatic polypeptide, Somatostatin and Bombesin.

Each peptide is briefly considered under the following sub-headings: Isolation, Structure, Location, Plasma levels, Actions, Results, then Discussion, if appropriate.

MOTILIN.

Isolation: Brown, Mutt & Dryburgh (1971), from hog intestine.

Structure: This is shown in Figure 10.1.

Location: Specific endocrine cells of the upper intestine.

Plasma levels: Reported meal response rising from 48 ± 6 to 73 ± 10 pmol l^{-1} (Christofides & Bloom, 1981).

Actions: Plasma concentrations correlate with changes in gastrointestinal motility (Christofides & Bloom, 1981).

Results: These and the doses given are shown in Tables 10.1, 10.2 and 10.3. The more stable synthetic analogue 13 nle-motilin was used. No significant changes were observed.

METHIONINE (MET-) ENKEPHALIN.

Isolation: Hughes, Smith, Kosterlitz, Fothergill, Morgan & Morris (1975), from brain.

MOTILIN

Phe - Val - Pro - Ile - Phe - Thr - Tyr - Gly - Glu - Leu - Glu - Arg - Met - Glu - Glu - Lys - Glu - Arg - Asn - Lys - Gly - Gln

(Nle)

Fig. 10.1.

Amino acid sequence of motilin and site of substitution in 13nle-motilin.

Structure: This is shown in Figure 10.2.

Location: Found in brain and nerves of the gastrointestinal tract. Also in gut endocrine cells (Larsson, 1980). Adrenal medulla a rich source (Clement-Jones, Lowry, Rees & Besser, 1980b).

Plasma levels: 14-140 pg ml^{-1} (Clement-Jones et al., 1980a). No rise on feeding (Dr. P. Price, personal communication).

Actions: An endogenous opiate agonist with particular affinity for so-called δ-receptors (Kosterlitz & Paterson, 1980), which may influence osmotically controlled vasopressin release (Grossman, Besser, Milles & Baylis, 1980). Met-enkephalin inhibits vasopressin release (Aziz, Forsling & Woolf, 1981). May be involved in the control of gut motility (c.f. morphine) (Sundler et al., 1980). Related peptides produce vasodilatation and hypotension when given systemically (Said, 1983).

Results: These and the doses given are shown in Tables 10.4, 10.5 and 10.6. No consistent effects were observed.

Discussion: The failure to demonstrate any change in vasopressin concentration may be due to the water-loaded state of the animals and therefore maximal inhibition of vasopressin release.

PANCREATIC POLYPEPTIDE.

Isolation: Lin & Chance (1974), from pancreas.

Structure: This is shown in Figure 10.3.

Location: Pancreas.

MET-ENKEPHALIN

Tyr - Gly - Gly - Phe - Met

LEU-ENKEPHALIN

Tyr - Gly - Gly - Phe - Leu

Fig. 10.2.

Amino acid sequences of met- and leu-enkephalin. Met-enkephalin is the N-terminal pentapeptide of beta-endorphin. All are ACTH-related peptides.

PANCREATIC POLYPEPTIDE

Ala - Pro - Leu - Glu - Pro - Val (Glu) - Tyr - Pro - Gly - Asp - Asn (Asp) - Ala

- Thr - Pro - Glu - Gln - Met - Ala - Gln - Tyr - Ala - Ala - Asp (Glu) -

Leu - Arg - Arg - Tyr - Ile - Asn - Met - Leu - Thr - Arg - Pro - Arg

- Tyr - NH_2

Fig. 10.3.

Amino acid sequence of human pancreatic polypeptide. Amino acid substituents of bovine pancreatic polypeptide are shown in brackets.

Plasma levels: Basal concentration of around 20 pmol l^{-1}, rising to between 200 and 400 pmol l^{-1} after a mixed meal (Adrian, Greenberg, Besterman & Bloom, 1978).

Actions: Inhibition of pancreatic enzyme secretion, gallbladder contraction (or active relaxation) and intestinal motility (Floyd, 1980). No effect on renal salt and water excretion (Lin & Chance, 1978).

Results: These and the doses given are shown in Tables 10.7, 10.8 and 10.9. Human pancreatic polypeptide was used, which differs by only 3 amino acids from bovine. The plasma levels achieved during the high dose infusion are comparable with those found in patients with pancreatic endocrine tumours (Adrian, 1982). There was a significant dose-related fall in renal calcium excretion. Phosphate excretion also fell at the highest dose infused, in spite of a rise in plasma phosphate concentration.

Discussion: There is no ready explanation for the changes in calcium and phosphate excretion. Pancreatic polypeptide could have a specific tubular action of its own, or influence parathormone release. The lack of change in sodium excretion makes any small change in GFR and RPF an unlikely factor.

SOMATOSTATIN.

Isolation: Brazeau, Vale, Burgus, Ling, Butcher, Rivier & Guillemin (1973), from hypothalamus.

Structure: This is shown in Figure 10.4.

Location: Central and peripheral nervous systems, including gut and kidney autonomic nerves and endocrine cells

SOMATOSTATIN

Ala - Gly - Cys - Lys - Asn - Phe - Phe - Trp - Lys - Thr - Phe -

Thr - Ser - Cys

Fig. 10.4.

Amino acid sequence of the cyclic tetradecapeptide somatostatin (-14). Two N-terminally extended forms exist, somatostatin -28 and -25.

throughout the gastrointestinal tract and pancreas (Patel et al., 1981; Forssmann et al., 1982).

Plasma levels: Larger molecular forms exist (see Fig. 10.4) and all probably circulate bound to larger molecular weight components (Arnold & Lankisch, 1980). Reported concentrations of somatostatin-like immunoreactivity range between < 5 to 150 pmol l^{-1} with a meal rise of < 10 pmol l^{-1} (O'Shaughnessy, 1982).

Actions: These are summarized in Table 10.10. In both the anaesthetized and conscious dog, large doses of somatostatin-14, infused intravenously and directly into the renal artery, have produced diuresis and increased free water clearance apparently by inhibition of vasopressin at its renal site of action (Reid & Rose, 1977; Brautbar, Levine, Coburn & Kleeman, 1979).

Results: These and the doses given are shown in Tables 10.11, 10.12 and 10.13. Plasma concentrations achieved during the middle dose are probably supraphysiological and levels observed during the high dose are found in patients with somatostatinomas (O'Shaughnessy, 1982).

The most consistent observation was the increase in plasma calcium concentration at all doses of somatostatin. This change appeared even at the lowest dose. Plasma glucose concentration rose during infusion of the high dose. Plasma renin activity (PRA) did not change.

Discussion: Plasma solids did not change significantly and was unrelated to the increase in plasma calcium concentration (see

General Comment at the end of this chapter). Somatostatin is known to inhibit calcitonin release, but not to affect parathormone (Patel et al., 1981; Brautbar et al., 1979). The rise in plasma glucose concentration is probably due to impaired insulin release in the presence of a glucose challenge i.e., infusion of 0.28 M glucose (Arnold & Lankisch, 1980).

The changes in urinary pH are difficult to interpret in the face of directional changes at different doses. Phosphate reabsorption is influenced by urinary pH: luminal acidosis causing inhibition (Sutton & Dirks, 1981). This, together with the rise in plasma phosphate concentration may explain the phosphaturia at the low dose of somatostatin. In the rabbit, changes in phosphate excretion cannot be attributed to alterations in circulating parathormone (Berndt et al., 1978, 1980; Fig. 10.5).

The role of somatostatin-containing renal nerves cannot be surmised from these results.

BOMBESIN.

Isolation: Erspamer, Falconieri Erspamer, Inselvini & Negri (1972) from frog skin.

Structure: This is shown in Figure 10.6, amphibian and porcine (mammalian) bombesins.

Location: High concentrations of bombesin-like immunoreactivity are found in gut, brain and lung (Ghatei, 1982). In the mammalian gastrointestinal tract, it seems largely confined to nerves of the mucosa and myenteric plexus (Walsh, Reeve & Vigna, 1981).

Plasma levels: Attempts to measure endogenous bombesin-like

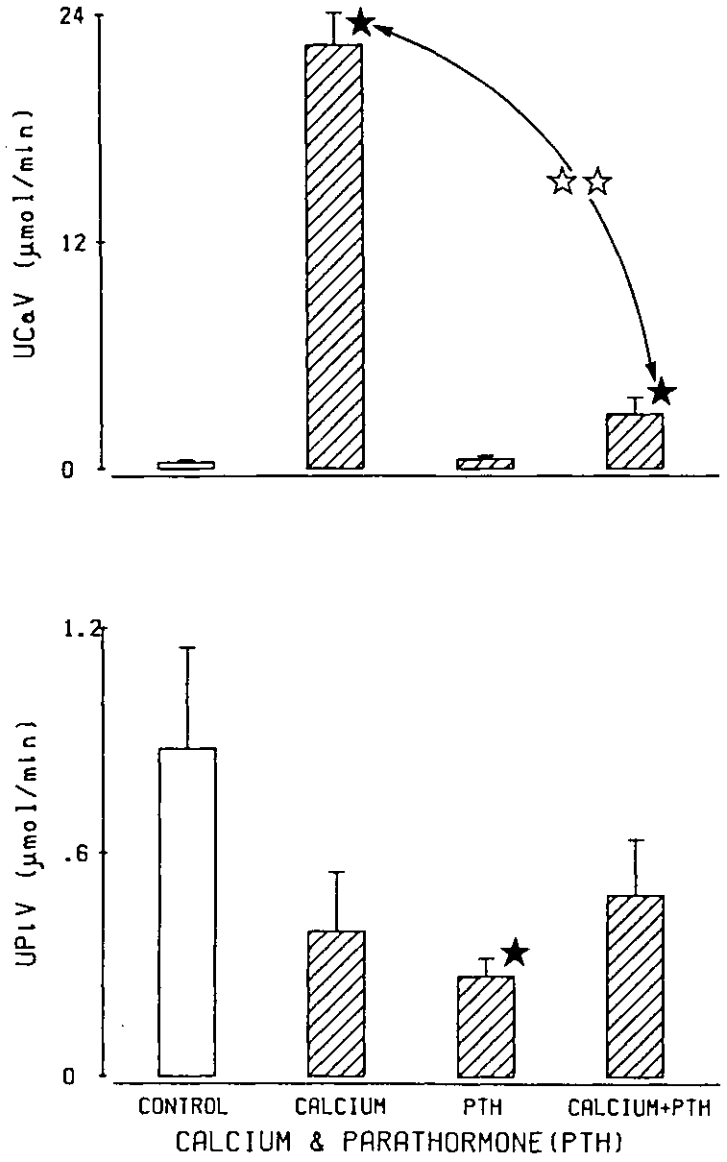


Fig.10.5 (legend overleaf).

Fig. 10.5.

The effects of intravenous calcium (calcium chloride; 1.0 mg kg^{-1} min^{-1}) and parathormone (PTH; 0.15 µg kg^{-1} min^{-1}) on renal calcium and phosphate excretion ($U_{Ca}V$ and $U_{Pi}V$ respectively) in the conscious rabbit. These variables were found to be lognormally distributed and therefore results are expressed as geometric mean ± approximate S.E. of mean, n = 8 for each treatment group. * indicates significant difference from control value as determined by Dunnett's test after analysis of variance. The important points to note are the fall in $U_{Ca}V$ when PTH is added to calcium (open stars) and the absence of phosphaturia. In fact, $U_{Pi}V$ is significantly less than the control value during PTH (alone) infusion.

AMPHIBIAN BOMBESIN

pGlu - Gln - Arg - Leu - Gly - Asn - Gln - Trp - Ala - Val - Gly - His - Leu - Met - NH_2

MAMMALIAN BOMBESIN (GRP)

Ala - Pro - Val - Ser - Val - Gly - Gly - Gly - Thr - Val - Leu - Ala - Lys - Met - Tyr - Pro - Arg - Gly - Asn - His - Trp - Ala - Val - Gly - His - Leu - Met - NH_2

Fig. 10.6.

Amino acid sequences of amphibian bombesin (-14) and mammalian bombesin (-27), also known as Gastrin-Releasing Peptide (GRP).

immunoreactivity in human plasma have proved unsuccessful and even with prior extraction, no rise has been detected after a meal (Walsh et al., 1981).

Actions: These are summarized in Table 10.14. Renal experiments in anaesthetised dogs have shown afferent arteriole vasoconstriction, reduced filtration fraction and increased renin release (Erspamer, Melchiorri & Sopranzi, 1973).

Results: These and the doses used are shown in Tables 10.15, 10.16 and 10.17. The 14 amino acid form was infused. The plasma levels recorded during the highest dose are comparable with those reported during infusion of 2.5 pmol kg^{-1} min^{-1} (4 ng kg^{-1} min^{-1}) in man (Melchiorri, 1978).

Like somatostatin, the most consistent effect was an increase in plasma calcium concentration at all doses of bombesin. Filtration fraction rose during infusion of the high dose, probably due to the slight increase in GFR. Urine flow also fell at this dose, but the change was not dose-related. Plasma renin activity did not change.

Discussion: Plasma solids did not increase significantly and did not contribute to the rise in plasma calcium concentration (see General Comment at the end of this chapter). Bombesin stimulates release of many hormones, including calcitonin, but inhibits parathormone secretion (Ghatei, 1982). This action would be expected to produce hypo- and not hypercalcaemia.

The vascular effects of bombesin have been compared with those of angiotensin-II (Erspamer, Melchiorri & Sopranzi, 1972). Although filtration fraction rose in the conscious rabbit, unlike angiotensin-

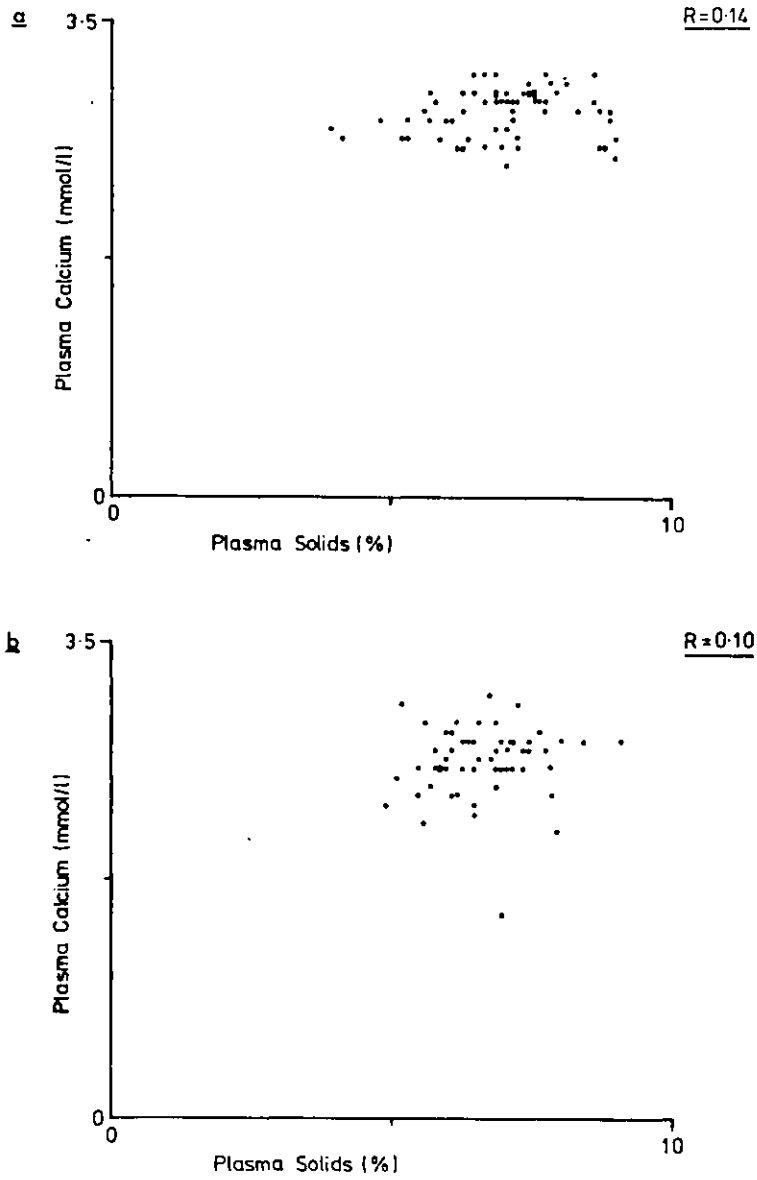
11, this was due to a small increase in GFR rather than a fall in ERPF (Freeman & Davis, 1979).

These results do not support the view of Erspamer et al. (1972, 1973), that bombesin is a potential hormone regulator of the circulation and kidneys.

General Comment

Although no significant increase in plasma solids occurred, the hypercalcaemic actions of somatostatin and bombesin may have been due to the overall effect of individual increments in plasma solids. Figures 10.7a and 10.7b are scattergrams of the relationship between plasma calcium concentration (ordinate) and plasma solids (abcissa), for all observations made during the somatostatin and bombesin studies, respectively. In both studies, the relationship was weak, with statistically insignificant correlation coefficients of < 0.2. This makes it unlikely that changes in plasma solids would explain the rise in plasma calcium concentration during infusion of these peptides.

Although somatostatin and bombesin are of similar chain length and molecular weight, there are no homologous sequences in their primary structure which might give a clue to their common hypercalcaemic action, nor do they bear any obvious structural resemblance to parathormone (Fig. 10.8).



Figs. 10.7a (somatostatin) and 10.7b (bombesin).

See text for details.

PARATHORMONE (PTH)

(Ser) (Leu)
Ala - Val - Ser - Glu - Ile - Gln - Phe - Met - His - Asn - Leu -

(Asn)
Gly - Lys - His - Leu - Ser - Ser - Met - Glu - Arg - Val - Glu -

Trp - Leu - Arg - Lys - Lys - Leu - Gln - Asp - Val - His - Asn -

Phe

Fig. 10.8.

Amino acid sequence of bovine parathormone (1-34). This is the active fragment of PTH (1-84) for its hypercalcaemic action (Potts, 1976). Amino acid substituents of the human counterpart are shown in brackets.

LEGENDS TO TABLES.

Legend to tables of plasma composition and haematocrit:

Values are expressed as mean ± S.E. of mean. $^{+}$Transformed variable, in which case the geometric mean and approximate S.E. of mean are given. n = number of observations. * and ** signify $P < 0.05$ and < 0.01 respectively, when compared with the control value by Dunnett's test after analysis of variance. Abbreviations are as follows:- P_{Na}, P_K, etc., plasma concentration; PS, plasma solids; Hct, haematocrit; PGPS, post-glomerular plasma solids; PRA, plasma renin activity.

Legend to tables of systemic and renal haemodynamics:

Values are expressed as mean ± S.E. of mean. n = number of observations. * and ** signify $P < 0.05$ and < 0.01 respectively, when compared with the control value by Dunnett's test after analysis of variance. Abbreviations are as follows:- BP, mean arterial blood pressure; HR, heart rate; ERPF, effective renal plasma flow; GFR, glomerular filtration rate; FF, filtration fraction.

Legend to tables of urine composition:

Values are expressed as mean ± S.E. of mean. $^{+}$Transformed variable, in which case the geometric mean and approximate S.E. of mean are given. n = number of observations. * and ** signify $P < 0.05$ and < 0.01 respectively, when compared with the control value by Dunnett's test after analysis of variance. Abbreviations are as follows:- U_{NaV}, U_KV, etc., renal excretion; $\dot{V}$, urine flow; C_{Osm}, osmolar clearance; C_{H_2O}, free water clearance.

Table 10.1. Effects of motilin on plasma composition and haematocrit.

	Control (vehicle only)	Motilin in pmol kg^{-1} min^{-1} 1 (low)	10 (middle)	100 (high)	n
Motilin (pmol l^{-1})	< 10	107 - 440	1280 - 1760	6700 - 15000	4
P_{Na} (mmol l^{-1})	135.7 ± 1.2	133.8 ± 1.0	137.1 ± 0.5	134.2 ± 1.1	12
P_{K} (mmol l^{-1})	4.03 ± 0.08	3.88 ± 0.08	4.00 ± 0.08	**3.56 ± 0.03	12
P_{Cl} (mmol l^{-1})	97.7 ± 0.6	99.7 ± 0.8	98.6 ± 0.7	95.8 ± 0.8	12
P_{Ca} (mmol l^{-1})	3.11 ± 0.06	3.09 ± 0.06	3.24 ± 0.02	3.11 ± 0.05	12
P_{Pi} (mmol l^{-1})	1.10 ± 0.02	1.10 ± 0.08	*1.27 ± 0.08	1.05 ± 0.04	12
P_{Glu} (mmol l^{-1})	8.1 ± 0.2	8.0 ± 0.5	8.4 ± 0.2	8.0 ± 0.3	8
P_{Osm} (m Osm kg^{-1})	284 ± 3	285 ± 1	285 ± 2	282 ± 2	12
PS (%)	6.56 ± 0.27	**5.92 ± 0.08	6.39 ± 0.11	*6.24 ± 0.11	8
Hct (%)	34 ± 1	35 ± 1	33 ± 1	33 ± 1	8
$^{+}$PGPS (%)	8.49 ± 0.33	**7.55 ± 0.14	8.07 ± 0.16	7.89 ± 0.21	8
$^{+}$PRA (ng ml^{-1} hr^{-1})	36.0 ± 5.5	33.8 ± 3.9	25.4 ± 3.1	35.0 ± 4.9	8

Table 10.2. Effects of motilin on mean arterial blood pressure, heart rate and renal haemodynamics.

	Control (vehicle only)	Motilin in pmol kg^{-1} min^{-1} 1 (low)	10 (middle)	100 (high)	n
BP (mm Hg)	75 ± 3	78 ± 4	75 ± 3	84 ± 5	12
HR (beats min^{-1})	270 ± 9	268 ± 13	250 ± 5	272 ± 5	12
ERPF (ml min^{-1})	40.6 ± 4.0	41.2 ± 3.9	48.4 ± 4.8	38.1 ± 3	12
GFR (ml min^{-1})	9.2 ± 0.8	9.2 ± 1.1	10.1 ± 2.9	8.1 ± 2.3	12
FF (%)	22.7 ± 0.6	21.9 ± 0.7	21.0 ± 0.4	20.7 ± 0.8	12

Table 10.3. Effects of motilin on renal electrolyte excretion, urine flow, solute excretion and pH.

	Control (vehicle only)	Motilin in pmol kg^{-1} min^{-1} 1 (low)	10 (middle)	100 (high)	n
$^{+}U_{Na}V$ (µmol min^{-1})	4.6 ± 1.6	6.3 ± 1.3	5.3 ± 1.2	5.4 ± 0.7	12
$^{+}U_{K}V$ (µmol min^{-1})	7.9 ± 0.8	9.3 ± 1.2	9.3 ± 1.0	5.9 ± 0.6	12
$^{+}U_{Cl}V$ (µmol min^{-1})	8.0 ± 1.3	7.2 ± 1.0	6.8 ± 1.5	4.7 ± 1.0	12
$^{+}U_{Ca}V$ (µmol min^{-1})	0.45 ± 0.21	0.60 ± 0.24	0.81 ± 0.24	0.25 ± 0.05	12
$^{+}U_{Pi}V$ (µmol min^{-1})	0.94 ± 0.20	0.84 ± 0.10	1.16 ± 0.16	0.57 ± 0.14	12
$^{+}\dot{V}$ (µl min^{-1})	99.3 ± 15.9	126.9 ± 29.4	119.5 ± 24.4	77.6 ± 13.0	12
$^{+}C_{Osm}$ (µl min^{-1})	196.0 ± 11.2	197.8 ± 26.4	203.0 ± 23.4	180.9 ± 14.8	12
C_{H_2O} (µl min^{-1})	-82.8 ± 23.2	-49.4 ± 20.9	-66.1 ± 19.5	-96.4 ± 13.4	12
pH	7.17 ± 0.23	7.62 ± 0.24	7.57 ± 0.19	7.56 ± 0.24	12

Table 10.4. Effects of met-enkephalin on plasma composition and haematocrit.

	Control (vehicle only)	Met-enkephalin in ng kg^{-1} min^{-1} 10 (low)	100 (middle)	1000 (high)	n
Met-enkephalin (pg ml^{-1})	21 - 36	30 - 81	58 - 159	165 - 233	3
P_{Na} (mmol l^{-1})	135.3 ± 0.8	133.8 ± 0.4	133.8 ± 0.8	135.1 ± 0.6	12
P_{K} (mmol l^{-1})	3.56 ± 0.06	3.40 ± 0.14	3.42 ± 0.07	3.57 ± 0.06	12
P_{Cl} (mmol l^{-1})	102.4 ± 1.0	102.6 ± 0.9	102.0 ± 0.5	101.3 ± 0.6	12
P_{Ca} (mmol l^{-1})	3.02 ± 0.09	2.97 ± 0.06	2.96 ± 0.08	2.81 ± 0.04	12
P_{Pi} (mmol l^{-1})	0.92 ± 0.07	0.91 ± 0.11	0.92 ± 0.08	0.95 ± 0.09	9
P_{Glu} (mmol l^{-1})	8.4 ± 0.7	9.5 ± 0.6	9.3 ± 0.3	9.8 ± 0.6	4
P_{Osm} (m Osm kg^{-1})	282 ± 3	277 ± 3	282 ± 1	277 ± 2	12
PS (%)	4.84 ± 0.23	5.42 ± 0.17	**6.13 ± 0.30	5.40 ± 0.25	8
Hct (%)	34 ± 2	31 ± 2	31 ± 1	32 ± 1	4
$^{+}$PGPS (%)	5.81 ± 0.32	6.84 ± 0.19	**7.68 ± 0.42	6.64 ± 0.33	8
$^{+}$PRA (ng ml^{-1} hr^{-1})	22.3 ± 4.3	16.7 ± 1.9	15.2 ± 1.1	21.0 ± 2.4	8
$^{+}$AVP (pmol l^{-1})	37.6 ± 8.8	18.3 ± 10.1	29.6 ± 10.9	23.7 ± 8.1	7

Note:- Higher AVP levels were recorded in this study (c.f. pentagastrin and somatostatin).

Table 10.5. Effects of met-enkephalin on mean arterial blood pressure, heart rate and renal haemodynamics.

	Control (vehicle only)	Met-enkephalin in ng kg^{-1} min^{-1} 10 (low)	100 (middle)	1000 (high)	n
BP (mm Hg)	80 ± 2	84 ± 3	86 ± 4	84 ± 3	12
HR (beats min^{-1})	273 ± 12	292 ± 15	273 ± 9	288 ± 8	12
ERPF (ml min^{-1})	46.2 ± 5.5	46.7 ± 4.1	47.2 ± 3.3	46.6 ± 3.8	12
GFR (ml min^{-1})	8.9 ± 1.3	10.0 ± 1.1	10.3 ± 0.8	9.0 ± 0.7	12
FF (%)	18.2 ± 1.3	*21.3 ± 0.8	**22.1 ± 1.5	19.6 ± 1.1	12

Table 10.6. Effects of met-enkephalin on renal electrolyte excretion, urine flow, solute excretion.

	Control (vehicle only)	Met-enkephalin in ng kg^{-1} min^{-1} 10 (low)	100 (middle)	1000 (high)	n
$^{+}U_{Na}V$ (µmol min^{-1})	4.6 ± 1.6	3.1 ± 1.1	4.7 ± 1.5	2.7 ± 1.1	12
$^{+}U_{K}V$ (µmol min^{-1})	6.9 ± 2.2	6.0 ± 1.2	11.0 ± 2.1	7.2 ± 1.3	12
$^{+}U_{Cl}V$ (µmol min^{-1})	4.3 ± 1.6	4.4 ± 1.5	6.5 ± 1.2	4.1 ± 1.4	12
$^{+}U_{Ca}V$ (µmol min^{-1})	0.38 ± 0.1	0.62 ± 0.09	0.34 ± 0.07	0.61 ± 0.12	12
$^{+}U_{Pi}V$ (µmol min^{-1})	0.44 ± 0.12	0.48 ± 0.14	0.36 ± 0.06	0.45 ± 0.15	12
$^{+}\dot{V}$ (µl min^{-1})	169.4 ± 45.7	191.2 ± 53.8	134.0 ± 18.1	128.5 ± 39.8	12
$^{+}C_{Osm}$ (µl min^{-1})	126.2 ± 51.6	151.0 ± 37.1	187.9 ± 24.1	161.0 ± 26.1	12
C_{H_2O} (µl min^{-1})	33.2 ± 27.2	85.2 ± 44.9	-57.9 ± 30.4	35.9 ± 46.2	12

Table 10.7. Effects of human pancreatic polypeptide on plasma composition and haematocrit.

	Control (vehicle only)	Pancreatic polypeptide in pmol kg^{-1} min^{-1} 1 (low)	10 (middle)	25 (high)	n
Pancreatic polypeptide (pmol l^{-1})	5 - 38	20 - 60	360 - 400	840 - 1180	4
P_{Na} (mmol l^{-1})	135.0 ± 0.8	136.6 ± 1.0	136.6 ± 0.5	137.2 ± 0.7	12
P_{K} (mmol l^{-1})	3.81 ± 0.08	3.83 ± 0.11	3.61 ± 0.09	3.82 ± 1.03	12
P_{Cl} (mmol l^{-1})	99.3 ± 1.1	98.0 ± 1.1	99.1 ± 1.4	98.3 ± 0.9	12
P_{Ca} (mmol l^{-1})	3.00 ± 0.05	2.98 ± 0.03	3.02 ± 0.03	2.97 ± 0.05	12
P_{Pi} (mmol l^{-1})	1.33 ± 0.04	**1.49 ± 0.05	1.42 ± 0.04	**1.49 ± 0.04	12
P_{Glu} (mmol l^{-1})	8.1 ± 0.2	8.1 ± 0.3	9.0 ± 0.3	8.1 ± 0.2	8
P_{Osm} (m Osm kg^{-1})	281 ± 3	280 ± 2	280 ± 2	278 ± 4	12
PS (%)	5.71 ± 0.19	5.81 ± 0.15	5.74 ± 0.12	6.04 ± 0.24	8
Hct (%)	32 ± 1	31 ± 1	33 ± 1	31 ± 1	8
$^{+}$PGPS (%)	7.34 ± 0.27	7.44 ± 0.18	7.48 ± 0.18	7.75 ± 0.23	8
$^{+}$PRA (ng ml^{-1} hr^{-1})	9.8 ± 3.2	10.9 ± 3.1	12.6 ± 4.0	9.5 ± 3.3	8

Table 10.8. Effects of pancreatic polypeptide on mean arterial blood pressure, heart rate and renal haemodynamics.

	Control (vehicle only)	Pancreatic polypeptide in pmol kg^{-1} min^{-1} 1 (low)	10 (middle)	25 (high)	n
BP (mm Hg)	77 ± 4	75 ± 4	77 ± 4	71 ± 4	12
HR (beats min^{-1})	246 ± 10	*221 ± 7	233 ± 10	**212 ± 11	12
ERPF (ml min^{-1})	52.3 ± 7.6	51.2 ± 5.7	46.9 ± 7.6	54.9 ± 10.3	12
GFR (ml min^{-1})	11.9 ± 1.9	11.1 ± 1.1	11.0 ± 1.8	11.8 ± 2.0	12
FF (%)	22.2 ± 0.8	21.9 ± 0.6	22.8 ± 1.0	21.9 ± 0.7	12

Table 10.9. Effects of pancreatic polypeptide on renal electrolyte excretion, urine flow, solute excretion and pH.

	Control (vehicle only)	Pancreatic polypeptide in pmol kg^{-1} min^{-1} 1 (low)	10 (middle)	25 (high)	n
$^{+}U_{Na}V$ (µmol min^{-1})	7.5 ± 1.5	6.2 ± 1.8	5.2 ± 1.7	4.5 ± 1.9	12
$^{+}U_{K}V$ (µmol min^{-1})	7.4 ± 1.1	5.6 ± 0.7	4.2 ± 0.6	5.2 ± 1.4	12
$^{+}U_{Cl}V$ (µmol min^{-1})	8.6 ± 1.1	7.0 ± 1.4	4.5 ± 1.3	4.5 ± 1.5	12
$^{+}U_{Ca}V$ (µmol min^{-1})	1.19 ± 0.31	0.88 ± 0.23	*0.58 ± 0.13	**0.43 ± 0.16	12
$^{+}U_{Pi}V$ (µmol min^{-1})	0.74 ± 0.28	1.17 ± 0.31	0.55 ± 0.17	**0.24 ± 0.10	12
$^{+}\dot{V}$ (µl min^{-1})	225.0 ± 49.3	298.0 ± 81.1	145.8 ± 3.34	143.9 ± 29.7	12
$^{+}C_{Osm}$ (µl min^{-1})	203 ± 22.3	196.0 ± 16.9	147.4 ± 22.9	182.5 ± 35.6	12
C_{H_2O} (µl min^{-1})	65.3 ± 51.4	189.4 ± 56.3	14.8 ± 20.8	-49.5 ± 40.9	12
pH	7.72 ± 0.28	7.58 ± 0.22	7.79 ± 0.14	8.05 ± 0.14	12

1. Inhibition of hormone release:	Gastrointestinal	Insulin Glucagon Pancreatic polypeptide Gastrin Secretin Cholecystokinin Motilin GIP VIP
	Pituitary	Growth hormone Prolactin TSH ACTH
	Others	Renin Aldosterone Calcitonin

2. Inhibition of gastric, pancreatic, biliary and salivary secretion.

3. Inhibition of gastric and biliary motility and reduced splanchnic blood flow.

4. Inhibition of central and peripheral neuronal activity.

5. Stimulation of NaCl absorption by rabbit intestine.

6. Diuretic.

Table 10.10. Some reported actions of somatostatin (based on articles by Patel et al., 1981; O'Shaughnessy, 1982).

Table 10.11. Effects of somatostatin on plasma composition and haematocrit.

	Control (vehicle only)	Somatostatin in pmol kg^{-1} min^{-1} 3 (low)	30 (middle)	250 (high)	n
Somatostatin (pmol l^{-1})	< 4	8 - 16	68 - 120	550 - 770	4
P_{Na} (mmol l^{-1})	136.3 ± 0.9	136.0 ± 1.1	137.8 ± 0.8	136.7 ± 0.9	12
P_{K} (mmol l^{-1})	3.87 ± 0.29	3.86 ± 0.04	4.03 ± 0.06	3.96 ± 0.13	12
P_{Cl} (mmol l^{-1})	100.2 ± 1.0	98.6 ± 0.9	**95.3 ± 0.6	97.8 ± 0.9	12
P_{Ca} (mmol l^{-1})	2.77 ± 0.03	**2.89 ± 0.05	**2.99 ± 0.03	**2.87 ± 0.08	12
P_{Pi} (mmol l^{-1})	1.07 ± 0.05	**1.25 ± 0.06	1.11 ± 0.03	*1.22 ± 0.08	12
P_{Glu} (mmol l^{-1})	9.6 ± 0.7	8.8 ± 0.6	9.4 ± 0.4	**11.5 ± 0.4	8
P_{Osm} (m Osm kg^{-1})	280 ± 2	284 ± 3	277 ± 2	281 ± 3	12
PS (%)	6.51 ± 0.35	7.40 ± 0.29	6.79 ± 0.25	6.91 ± 0.64	8
Hct (%)	33 ± 1	31 ± 2	33 ± 2	32 ± 2	8
$^{+}$PGPS (%)	8.16 ± 0.51	9.42 ± 0.39	8.61 ± 0.35	8.38 ± 0.86	8
$^{+}$PRA (ng ml^{-1} hr^{-1})	11.6 ± 2.2	9.7 ± 1.7	11.3 ± 1.6	13.5 ± 4.2	8
$^{+}$AVP (pmol l^{-1})	1.37 ± 0.60	2.6 ± 1.6	2.2 ± 1.1	2.1 ± 1.3	8

Table 10.12. Effects of somatostatin on mean arterial blood pressure, heart rate and renal haemodynamics.

	Control (vehicle only)	Somatostatin in pmol kg^{-1} min^{-1} 3 (low)	30 (middle)	250 (high)	n
BP (mm Hg)	71 ± 5	76 ± 4	79 ± 3	71 ± 3	12
HR (beats min^{-1})	244 ± 11	254 ± 16	229 ± 7	238 ± 11	12
ERPF (ml min^{-1})	40.9 ± 5.7	51.3 ± 5.4	42.7 ± 4.0	53.4 ± 7.7	12
GFR (ml min^{-1})	8.7 ± 1.3	11.0 ± 1.1	9.4 ± 1.1	10.9 ± 1.5	12
FF (%)	20.6 ± 0.9	21.4 ± 0.6	21.6 ± 0.7	20.6 ± 0.5	12

Table 10.13. Effects of somatostatin on renal electrolyte excretion, urine flow, solute excretion and pH.

	Control (vehicle only)	Somatostatin in pmol kg^{-1} min^{-1} 3 (low)	30 (middle)	250 (high)	n
$^{+}U_{Na}V$ (µmol min^{-1})	3.9 ± 1.1	5.8 ± 0.9	5.7 ± 1.0	4.9 ± 1.3	12
$^{+}U_{K}V$ (µmol min^{-1})	5.1 ± 0.8	5.6 ± 0.5	**9.3 ± 2.2	*7.9 ± 1.1	12
$^{+}U_{Cl}V$ (µmol min^{-1})	5.0 ± 1.4	4.9 ± 0.7	6.1 ± 0.8	5.1 ± 0.9	12
$^{+}U_{Ca}V$ (µmol min^{-1})	0.75 ± 0.17	0.69 ± 0.21	0.65 ± 0.15	0.48 ± 0.13	12
$^{+}U_{Pi}V$ (µmol min^{-1})	0.73 ± 0.21	**1.37 ± 0.47	0.53 ± 0.20	0.84 ± 0.22	12
$^{+}\dot{V}$ (µl min^{-1})	132.9 ± 44.1	*367.0 ± 112.5	125.5 ± 33.7	248.2 ± 79.9	12
$^{+}C_{Osm}$ (µl min^{-1})	148.6 ± 23.2	190.5 ± 30.3	195.7 ± 21.3	168.4 ± 26.0	12
C_{H_2O} (µl min^{-1})	90.7 ± 97.0	310.9 ± 73.7	-13.4 ± 58.1	201.9 ± 76.5	12
pH	6.99 ± 0.25	*6.27 ± 0.12	*7.67 ± 0.25	7.38 ± 0.20	12

CLAIMED PHARMACOLOGICAL ACTIONS	Stimulation of intestinal, biliary, uterine and urinary smooth muscle
	Renal vasoconstriction and systemic hypertension
	Stimulation of gastric and pancreatic secretion
	Release of gastrin, PP, insulin, GIP and glucagon
	Satiety
? PHYSIOLOGICAL ROLE	? satiety
	? gastrin release and gastric secretion

Table 10.14. Biological effects of bombesin and GRP.

Table 10.15. Effects of bombesin on plasma composition and haematocrit.

	Control (vehicle only)	Bombesin in pmol kg^{-1} min^{-1} 0.06 (low)	0.6 (middle)	6 (high)	n
Bombesin (pmol l^{-1})	140 - 300	180 - 330	230 - 350	440 - 570	4
P_{Na} (mmol l^{-1})	136.1 ± 0.9	137.3 ± 0.4	137.3 ± 1.2	134.5 ± 0.8	12
P_{K} (mmol l^{-1})	3.75 ± 0.11	3.68 ± 0.06	3.79 ± 0.03	3.54 ± 0.09	12
P_{Cl} (mmol l^{-1})	100.8 ± 1.2	99.9 ± 0.6	101.9 ± 0.8	*98.7 ± 1.2	12
P_{Ca} (mmol l^{-1})	2.49 ± 0.09	**2.73 ± 0.04	**2.80 ± 0.05	**2.73 ± 0.06	11
P_{Pi} (mmol l^{-1})	1.43 ± 0.07	1.37 ± 0.09	1.29 ± 0.12	1.18 ± 0.11	11
P_{Glu} (mmol l^{-1})	10.4 ± 0.7	9.4 ± 0.4	8.9 ± 1.0	12.4 ± 1.1	8
P_{Osm} (m Osm kg^{-1})	276 ± 2	270 ± 3	273 ± 3	273 ± 3	12
PS (%)	6.28 ± 0.35	6.40 ± 0.27	6.51 ± 0.19	7.08 ± 0.43	8
Hct (%)	34 ± 1	35 ± 1	36 ± 1	35 ± 1	8
$^{+}$PGPS (%)	8.26 ± 0.51	8.59 ± 0.47	8.93 ± 0.25	9.92 ± 0.60	8
$^{+}$PRA (ng ml^{-1} hr^{-1})	18.2 ± 2.2	18.7 ± 3.7	18.5 ± 2.3	19.0 ± 2.5	8

Table 10.16. Effects of bombesin on mean arterial blood pressure, heart rate and renal haemodynamics.

	Control (vehicle only)	Bombesin in pmol kg^{-1} min^{-1} 0.06 (low)	0.6 (middle)	6 (high)	n
BP (mm Hg)	86 ± 2	91 ± 5	88 ± 4	84 ± 5	11
HR (beats min^{-1})	274 ± 5	278 ± 4	269 ± 4	291 ± 6	11
ERPF (ml min^{-1})	53.3 ± 3.0	53.4 ± 3.8	48.9 ± 2.1	55.1 ± 5.4	12
GFR (ml min^{-1})	13.1 ± 0.8	13.7 ± 1.1	13.3 ± 0.7	16.5 ± 1.8	12
FF (%)	24.5 ± 1.1	25.7 ± 1.2	27.1 ± 1.0	**29.6 ± 0.7	12

Table 10.17. Effects of bombesin on renal electrolyte excretion, urine flow, solute excretion and pH.

	Control (vehicle only)	Bombesin in pmol kg^{-1} min^{-1} 0.06 (low)	0.6 (middle)	6 (high)	n
$^{+}U_{Na}V$ (µmol min^{-1})	14.9 ± 3.1	8.6 ± 3.1	[1]3.5 ± 3.1	14.7 ± 4.3	12
$^{+}U_{K}V$ (µmol min^{-1})	6.2 ± 0.3	8.1 ± 1.4	7.3 ± 0.6	6.3 ± 0.8	12
$^{+}U_{Cl}V$ (µmol min^{-1})	15.5 ± 2.9	11.6 ± 3.2	12.3 ± 3.8	11.8 ± 4.1	12
$^{+}U_{Ca}V$ (µmol min^{-1})	0.64 ± 0.14	0.38 ± 0.13	0.56 ± 0.20	0.97 ± 0.45	12
$^{+}U_{Pi}V$ (µmol min^{-1})	0.80 ± 0.25	0.50 ± 0.11	0.72 ± 0.13	1.12 ± 0.41	12
$^{+}\dot{V}$ (µl min^{-1})	175.5 ± 42.8	92.0 ± 20.3	160.1 ± 32.8	*90.0 ± 16.9	12
$^{+}C_{Osm}$ (µl min^{-1})	254.8 ± 32.2	246.4 ± 48.1	266.5 ± 26.7	255.4 ± 53.1	12
C_{H_2O} (µl min^{-1})	-37.0 ± 41.8	-172.3 ± 56.5	-81.2 ± 29.5	-211.9 ± 56.2	12
pH	6.94 ± 0.34	8.00 ± 0.28	6.99 ± 0.46	7.27 ± 0.29	12

CHAPTER 11

GENERAL DISCUSSION

General Discussion.

".....in this molecular world, structure always underlies function." (Homer Smith, 1951b).

The preceding 8 chapters have each described and discussed the renal effects of particular gastrointestinal hormones. Originally, the term hormone (from the Greek hormao, "I arouse or excite") could be applied to any compound (chemical messenger) which acted locally or generally. With the passage of time, its use has tended to become restricted to those substances which are released into the circulation and produce an effect on some target organ at a distance. Not all gut hormones have been found to circulate and thus the more neutral term peptide is often used to describe them. Moreover, terms such as paracrine and neurocrine are used to describe substances released from specialised cells and acting locally; perhaps only one step removed from a neurotransmitter. Thus, the distinctions become blurred and even false, particularly if substances produce effects by several different modes of release. Such distinctions must be dynamic, as for example improvements in assay techniques may reveal hitherto unsuspected and physiologically significant changes in circulating levels.

Where possible in these studies, actions have been related to changes in plasma concentration of the relevant peptide, especially after food, bearing in mind the evidence that many are putative neurotransmitters, which are not known to circulate under normal conditions and may also be present within the kidney i.e., local hormone (e.g. VIP and Substance P; Hökfelt et al., 1978; Forsmann et al., 1982). Others such as neurotensin and perhaps CCK8, may have a

dual role as neurotransmitter and circulating hormone, in which case supraphysiological plasma concentrations may simulate the effects of local release from nerve endings within renal tissue. However, the problem with this sort of extrapolation is that it takes no account of wider systemic effects and likely interactions with other hormones: stimulation, potentiation and antagonism. Examples include VIP and reflex sympathetic stimulation, CCK8 and calcitonin release, glucagon and insulin release and antagonism, cholecystokinin and secretin release and potentiation, amongst others; all discussed in some detail in the relevant chapters. It is perhaps less surprising that gut peptides should interact in this way, as gastrointestinal function is so diverse and complex in higher species, involving as it does, digestion absorption and motility within specialised segments of bowel, that fine regulation is essential. To use teleological reasoning, this requirement is easier to accomplish if several peptides with specific functions are employed, rather than a single peptide with multiple actions. In evolutionary terms, it is possible or even likely, that a unique and large peptide molecule in a more primitive animal has acquired new actions or given rise to fragments with specific functions. Thus, a pre-existing hormone may be modified when required to perform a new or additional function, perhaps through a process of natural selection. A good example is prolactin which in lower species is involved in salt and water metabolism (Katz & Lindheimer, 1977). Prolactin from amphibia has mammotrophic activity when tested in appropriate bioassays (Bern & Nicoll, 1968). Hence, the prolactin molecule had the capacity for a particular function, before evolution of the relevant receptive tissue. In such cases it is difficult to know whether the hormone

elaborated new or more hormonal actions as necessary, or if new receptor systems developed in new tissues. Again, teleologically speaking, it may be that "Nature" takes advantage of the structure-activity relations of molecules as they occur and that evolutionary pressures force them in various directions. It is intriguing to know why noradrenaline and serotonin are found in the middle of green bananas and why serotonin and heparin are present in nettle stings (Douglas, 1975). Some structural feature is obviously important and may involve the ability to transfer electrons. The structural relationships amongst many of the gut peptides and the biological actions shared by some structurally unrelated peptides, indicate the importance of peptide homologies, evolution and structure-activity relations.

An attempt will now be made to draw together some of the apparently disparate observations made in this series of experiments, and to discuss in more detail common actions. These aspects will be considered under the following sub-headings: Structure-function relations, Pathophysiology, Concluding remarks.

1. Structure-function relations

Several procedures may be used to investigate such relationships.
(i) If possible, the first step is to synthesise analogues, test their biological actions against the parent molecule and proceed to substitute each amino acid in turn, thus determining the active site of a peptide. This is the ideal, but depends on the size of the active site and can only be carried out with relatively small peptides such as angiotensin-II (Regoli, Park & Rioux, 1974).
(ii) Chemical modification of certain functional groups or group with a specific agent e.g., oxidation of methionine residues. The

problem here is that such reactions are never specific and a change in function may result not from alteration of a functional group, but because of a change in secondary or tertiary structure, although of course, these cannot be readily separated. It is therefore necessary to look for changes in molecular size and shape. An example of this type of study is determination of the amino acid sequence responsible for the vascular effects of parathormone (Pang, Yong, Kenny & Tenner, 1982).

(iii) The actions of proteolytic enzymes may give a clue as to the importance of wholeness (active structures) of a peptide and which fragments have biological actions. Gastrin and CCK are good examples. The larger forms may be biosynthetic precursors (Rehfeld, 1980). They possess C-terminal homology, but the biochemical profile of CCK is modified by sulphation of the C-terminal tyrosine residue and N-terminal extension. It seems that sulphated tyrosine and N-terminal extension are required to reduce the ratio of acid-stimulating to gallbladder contracting activity (Grossman, 1976).

(iv) Perhaps the easiest approach is to simply compare the amino acid sequences (primary structure) of various peptides with similar actions, looking for areas of homology e.g., pentagastrin and arginine vasopressin, in view of the observations reported in Chapter 3.1. Even if the positions of some amino acids do correspond, the problem here is that the amino acid sequence does not always imply the secondary and tertiary structures, which may be greatly modified by the groups either side of an homologous segment. Comparison with other species may help, looking for amino acid substitutions in related peptides and comparing biological potency. When this phenomenon of sequential isomerism is observed and biological activity is preserved, it may be assumed that the sequence in which

the modification occurs is not essential. For example, alterations in the N-terminus of the gastrin, CCK, caerulein family of peptides, but with preservation of the C-terminal tetrapeptide, affects potency, but not action (Rehfeld, 1980).

It is known that peptides may exhibit more than one biological action and the question arises as to whether or not these are located at the same active site of the molecule. In the case of parathormone, the N-terminal dipeptide is necessary for the hypercalcaemic effect, but not the vascular action (Pang et al., 1982).

As peptides are antigenic and measured almost exclusively by radioimmunoassay, it is important to correlate chemical structure with immunological potency as well as biological activity. The immunological determinant of a hormone is not necessarily identical with its active site and for many of the gut peptides, the active site appears to be the C-terminal part, but most antisera are N-terminally directed. Neurotensin is an example as discussed in Chapter 8. Discrepancies between the effects of exogenous infusions simulating postprandial levels and the responses during feeding, may be due to circulation of inactive N-terminal fragments after food. Fragments of this peptide are now available which would enable detailed study of structure-activity relations and a clearer appreciation of neurotensin physiology.

a) Effects on calcium homeostasis

Primary structure homologies are helpful and important in the case of gastrin and CCK. The C-terminal octapeptide of CCK (CCK8) has all the actions and is more potent (on a molar basis) than the parent molecule, CCK33 (Rehfeld, 1980). This larger molecule has an

homologous sequence with calcitonin, but this does not include the C-terminal octapeptide sequence (Fig. 11.1). Moreover, the hypocalcaemic action of calcitonin requires the intact molecule, any modification being associated with loss of activity (Potts, 1976). CCK8-induced hypocalcaemia may depend upon calcitonin release, but CCK8 at a cellular level increases calcium influx and intracellular calcium levels (Christophe & Waelbroeck, 1981). The N-terminal hexapeptide of CCK8 is not a calcitonin secretagogue and oxidation of the 2 methionine residues also leads to loss of calcitonin-stimulating activity (Care & Bruce, 1971). The effect of these modifications on renal calcium handling and plasma calcium and renin levels would be of considerable interest. Glucagon is also a calcitonin secretogogue and was observed to depress plasma calcium concentration in the rabbit, but unlike CCK8, stimulated renin release. The glucagon-related peptides VIP and secretin are not known to release calcitonin, but share long amino acid sequences (Fig. 6.1). An important difference may be the C-terminal pentapeptide sequence of glucagon which like gastrin (previously shown to be a calcitonin secretagogue, but much less potent than CCK8) and CCK8 contains adjacent methionine and aspartate residues (Fig. 11.2). Could this dipeptide sequence be essential for this biological effect, or could the steric presentation of the methionine residue be the key? Another example of interspecies homology is met-enkephalin and the N-terminal hexapeptide of CCK8 (Fig. 11.1). Met-enkephalin produced no signficant effects on renal function or plasma calcium, serving to emphasise the functional importance of the C-terminal dipeptide of CCK8 and gastrin. A difficulty when trying to equate homologous sequences (intra- and interspecies) is that potency may differ so much that relationships can be missed because doses are

GASTRIN (1-17) : Glp-Gly-Pro-Trp-Leu-Glu-Glu-Glu-Glu-Glu-Ala-Tyr(SO_3H)-Gly-Trp-Met-Asp-Phe·NH_2

CCK (16-33) : -Leu-Asp-Pro-Ser-His-Arg-Ile-Ser-Asp-Arg-Asp-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-Phe·NH_2

β-LPH (58-65) : -Asp-Lys-Arg-Tyr-Gly-Gly-Phe-Met-

CT (16-23) : -Leu-Asn-Asn-Phe-His-Arg-Phe-Ser-

Fig. 11.1.

Intraspecies homology between human gastrin, porcine cholecystokinin (CCK), beta-lipotropin (β-LPH) including met-enkephalin, and porcine calcitonin (CT); taken from Rehfeld, 1980.

Glucagon

- Trp - Leu - Met - Asp - Thr

Gastrin and CCK

- Gly - Trp - Met - Asp - Phe - NH_2

Pentagastrin

BocAla - Trp - Met - Asp - Phe - NH_2

Fig. 11.2.

C-terminal pentapeptide amino acid sequences of glucagon, gastrin, cholecystokinin (CCK) and pentagastrin, with the homologous dipeptide underlined.

too low or even too high.

Peptides with differing chemical structures may possess the same biological activity e.g., the hypercalcaemic action of bombesin and somatostatin. These peptides do not have any obvious structural relationship with one another or parathormone. Bombesin is related to substance P, which did reduce renal calcium excretion (Chapter 9). Pancreatic polypeptide also had this effect but is unrelated structurally (Chapter 10). However, a C-terminal methionine residue recurs throughout and just may be the link in respect of calcium homeostasis. In the case of somatostatin, inhibition of calcitonin release may be sufficient explanation.

b) Effects on renin release and renal sodium excretion

As briefly discussed in Chapter 6, three major mechanisms exist for the regulation of renin release. These are:-

i) The intrarenal baroreceptor reacting to afferent arteriole stretch or wall tension, increased tension inhibiting and decreased tension stimulating renin release (Peart, 1978).

ii) The controversial macula densa receptor. Although the relationship between sodium balance and renin release is not disputed i.e., sodium loading inhibiting and depletion stimulating, the exact nature of the macula densa stimulus is. The original studies of Vander and Miller (1964) suggested that an increase in sodium load to the macula densa might inhibit renin release, while the work of Thurau et al. (1972) indicated the opposite, but made no distinction between increased renin activation and secretion. Teleologically, Thurau's postulate (1964) seems more likely in an effort to limit sodium loss by the proximal tubule, but most workers support the original view that a decrease rather than an increase in sodium delivery

stimulates renin release (Davis & Freeman, 1976). It is also uncertain as to whether it is sodium or chloride which is the stimulus and if it is load, concentration or rate of transport that matters (Davis & Freeman, 1976). It may therefore be argued that substances which produce a natriuresis, like CCK8 and pentagastrin, would inhibit renin release. It has been suggested that the natriuretic response to VIP accounts for the rise in renin observed (Porter & Ganong, 1982) and this explanation could also be applied to glucagon.

iii) Renal sympathetic nerves and circulating catecholamines. To this may be added peptidergic nerves (? VIP-containing) and some circulating peptides (? CCK and VIP particularly in disease states associated with high circulating levels).

These mechanisms are not mutually exclusive and the effects of a given substance may affect one more than another. Vasodilating peptides like VIP and glucagon may reflexly activate the sympathetic nervous system and thus stimulate renin release via the beta-receptor and also activate the intrarenal baroreceptor, mediated perhaps by prostaglandins. CCK8 also observed to produce vasodilatation, may inhibit renin through macula densa stimulation as mentioned above. However, the final common pathway for all these mechanisms may be changes in juxta-glomerular cell cytosolic calcium (Peart, 1978; see Discussion, Chapter 4.1). With regard to the actions of CCK8 and pentagastrin, an attractive hypothesis is that these substances may increase cytosolic calcium directly (shown to date only in pancreatic acinar cells; Christophe & Waelbroeck, 1981; Petersen, 1982) or via

inhibition of the contraluminal sodium pump (see Fig. 11.3). This in turn would inhibit sodium reabsorption (natriuresis) and in the juxta-glomerular cell, inhibit renin release. It may also be relevant here that the C-terminal dipeptide of CCK and gastrin contracts smooth muscle in vitro and in vivo (Grossman, 1976): contraction of vascular smooth muscle adjacent to juxta-glomerular cells may increase membrane permeability to calcium by stretch, thus raising intracellular calcium and inhibiting renin secretion (Fray, 1980; see Discussion, Chapter 4.1).

These studies were begun in the hope of finding an antinatriuretic gut peptide which circulated in blood and was effective at physiological concentrations. Bearing in mind the points made earlier concerning immunogenic and biologically active sites, neurotensin has come closest to fulfilling these criteria. However, in man neurotensin consistently reduced chloride excretion, but not sodium. This difference compared with the rabbit may be fortuitous, yet provide a clue as to its tubular site of action. Until recently, all reabsorption of sodium chloride was thought to depend on active sodium transport. However, it is now known that primary active chloride transport occurs in the thick ascending limb of the loop of Henle (Burg & Stoner, 1976). In this segment of the nephron, Na^+, K^+ and Cl^- co-transport exists with a complex stoichiometry ($2Na^+$:1K:$3Cl^-$) (Ellory, Dunham & Logue, 1982). Thus it is conceivable that stimulation of this transport system by neurotensin may account for the changes in excretion of these electrolytes in man and rabbit, although dosage may be critical. With regard to species differences, it may be relevant that the rabbit diet is relatively rich in potassium and low in sodium. This might explain the

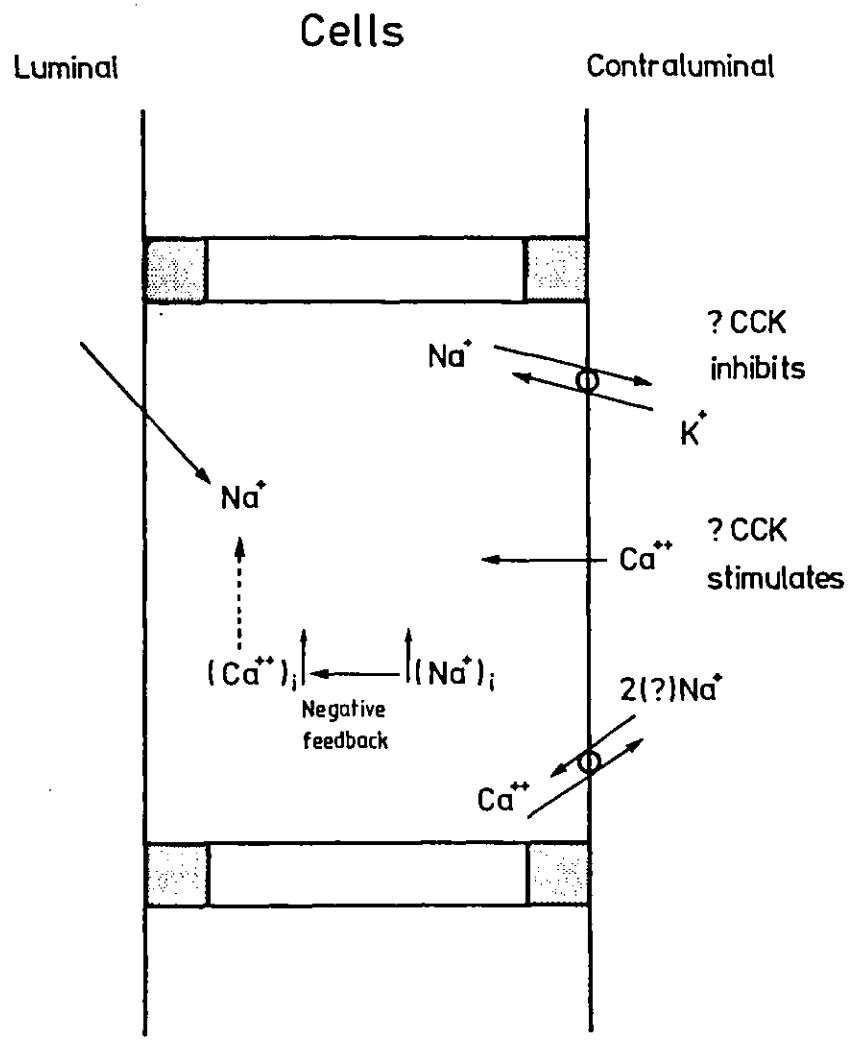


Fig. 11.3.

Proposed model of epithelial cell indicating potential role of calcium ions in negative feedback mechanism and influence of CCK-like peptides either directly on intracellular calcium by increasing influx, or indirectly by decreasing sodium pump rate. The latter would lead to a fall in the rate of calcium efflux across the contraluminal cell membrane and, thus, an increase in both cytosolic calcium and calcium ion activity. This in turn may induce a decrease in rate of sodium entry across the luminal cell membrane (see text for details; based on Taylor & Windhager, 1979).

proportionate differences in the individual electrolytes, but encouragingly, directional changes in these studies were similar. (Chapter 8: Table 8.3 and Tables 8.4 - 8.6a,b).

The natriuretic effect of pentagastrin and CCK8 may be explained as discussed above, the site being proximal or distal and the potential relationship with amiloride and triamterene worth pursuing.

2. Pathophysiology

The effects reported in these studies have been largely observed at pharmacological doses of the relevant peptide. Such effects may be more important in disease states associated with high circulating levels. Several gastrointestinal endocrine tumours exist which may secrete insulin, gastrin, glucagon, somatostatin, pancreatic polypeptide and VIP, separately or together (Welbourn et al., 1981). VIP has the most widespread vascular and renal effects of these gut peptides. It, together with several other gut peptides, has been implicated aetiologically in the watery-diarrhoea syndrome (Friesen, 1982), but as with some of the other putative candidates (secretin, gastrin and pancreatic polypeptide) diarrhoea was never observed in the present series of experiments. However, prolonged exposure to high circulating levels of VIP or other peptide may be necessary (Modlin, Bloom & Mitchell, 1977). VIP could be important in disorders of salt and water balance. Increased levels have been found in patients with cirrhosis and hepatic failure, heart failure and in animals with experimentally-induced endotoxic shock (Hunt et al., 1979; Sullivan, Chase, Christofides, Bloom & Williams, 1981; Clark, Adrian, McMichael & Bloom, 1983; Freund, Ebeid & Fischer, 1981). Increased levels of VIP may be in response to tissue

ischaemia (Modlin, Bloom & Mitchell, 1978), with VIP playing a protective role by its vasodilating action. However, its effect on renal function and capillary permeability may be detrimental and responsible for some of the changes observed in these disorders.

As stated in Chapter 1, the kidney is an important site of metabolism and clearance for many of the gut peptides and fragments. High circulating levels of gastrin, glucagon, pancreatic polypeptide and motilin have been found by radioimmunoassay in anephric patients and those with chronic renal failure (Trimble & Buchanan, 1976; Owyang, Miller, DiMagno, Brennan & Go, 1979; Borody, Byrnes & Henderson, 1980), but many of these are biologically inactive fragments (Bloom & Polak, 1982). However, in the light of the observation that pentagastrin in man is natriuretic, high levels of gastrin-like immunoreactivity found in patients with renal failure may contribute to the excessive sodium loss found in those cases of so called salt-losing nephropathy. Perhaps more important, any vasopressin-like effect would exacerbate the more commonly found salt and water retention.

Concluding remarks

The differences observed between rabbit and man in the renal response to some of these peptides illustrates the problem of species variation. As already mentioned, differences in dietary composition (herbivore versus carnivore, sodium:potassium ratio) may significantly affect responses, presumably for adaptive reasons. The antidiuretic response to pentagastrin observed in the rabbit, but not in man, may indicate differing sensitivity to changes in plasma arginine vasopressin (AVP) or AVP-like action. It is notable that water intoxication is easily produced in the rabbit, which seems unable to readily excrete a water load. This is one important

difficulty encountered when using rabbits in the study of renal function (Kaplan & Smith, 1935).

While the original question considered in Chapter 1, of whether one or other gastrointestinal peptide might be involved in the antinatriuretic response to sodium deprivation, has not been fully answered, the wider issue of the effects of circulating gut peptides on renal function in general, has given rise to a number of potentially significant observations. The contribution of these peptides to alterations in renal function in health and disease may be important and is certainly worthy of further study.

Hormonal influences on the kidney seem even more complex than originally believed. A large number of structurally diverse peptides appear able to affect renal function and together with aldosterone may control short and long term regulation of renal sodium excretion, respectively. A so-called natriuretic factor has been implicated in the genesis of hypertension (de Wardener & Macgregor, 1980). To date, this substance has not been isolated, but is believed to be ouabain-like. It may be that renal actions of gut peptides such as gastrin and CCK are relevant in this context.

It is hoped that this small beginning will lead to more work on the hypothetical "gut-renal endocrine axis", for it may offer new insights into renal physiology and pathophysiology. Many examples have been briefly mentioned in the relevant chapters, including some suggestions as to areas worthy of future study.

APPENDIX

A1: Attempt to measure changes in plasma pentagastrin concentration during infusion in four conscious rabbits.

These measurements were kindly carried out by Professor G. Dockray, Dept. of Physiology, University of Liverpool.

Tables A.1 and A.2 show the changes in plasma gastrin-like immunoreactivity in rabbit plasma during step-up infusions of pentagastrn (0.1, 1.0 and 10 μg kg^{-1} min^{-1}), using two different antisera, L48 and L112 respectively. Each dose of pentagastrin was infused intravenously for 60 minutes and samples taken at the times indicated.

Antiserum L48 cross-reacts equally with G17 and CCK8, but has approximately 50-fold less affinity for G5. This can only be approximate as relative potencies were not checked against actual pentagastrin (beta-alanine substituted for glycine). Antiserum L112 cross-reacts equally with all peptides containing the G4 sequence and extended at the N-terminus, including G17, CCK8 and presumably pentagastrin. CCK8 was used as a standard for both assays. The concentration of CCK8 needed for 50% inhibition of L48 binding was about 3 pmol l^{-1}, while with L112 it was 160 pmol l^{-1}. Non-specific effects of plasma were therefore more likely to significantly increase estimates of "basal" concentrations with L112 compared with L48.

Results are erratic, but overall, plasma levels barely changed during infusion of 0.1 μg kg^{-1} min^{-1}, but at 1.0 and 10 μg kg^{-1} min^{-1}, concentrations increased approximately 10- and 100-fold, respectively. "True" plasma levels in molar terms are probably quite close to those given by antiserum L112. The metabolic clearance rate

Dose	Time (min)	Rabbit			
		A	B	C	D
		Plasma levels (pmol l^{-1})			
0	0	216	< 20	88	< 20
0.1	+ 30	60	210	32	44
0.1	+ 60	34	50	44	24
1.0	+ 30	320	280	264	360
1.0	+ 60	(4,150)	360	296	1,950
10	+ 30	1,700	-	1,650	2,450
10	+ 60	2,400	1,060	1,490	(400)
Off	+ 5	168	62	80	160
	+ 10	64	60	48	800
	+ 20	32	68	< 20	40

Table A.1. Changes in plasma gastrin-like immunoreactivity during step-up infusion of pentagastrin in μg kg^{-1} min^{-1}, using antiserum L48. Values in brackets are suspect.

Dose	Time (min)	Rabbit			
		A	B	C	D
		Plasma levels (pmol l^{-1})			
0	0	800	< 1,000	2,800	< 1,000
0.1	+ 30	1,600	7,200	1,200	< 1,000
0.1	+ 60	3,200	5,200	< 1,000	< 1,000
1.0	+ 30	12,500	12,500	9,000	9,000
1.0	+ 60	90,000	(6,000)	(7,200)	60,000
10	+ 30	56,000	-	-	60,000
10	+ 60	52,000	47,000	42,000	(8,000)
Off	+ 5	6,400	7,200	4,800	10,000
	+ 10	5,200	6,800	2,240	(>40,000)
	+ 20	3,000	7,000	2,240	4,000

Table A.2. Changes in plasma gastrin-like immunoreactivity during step-up infusion of pentagastrin in μg kg^{-1} min^{-1}, using antiserum L112. Values in brackets are suspect.

of pentagastrin is considerable as levels were almost undetectable 5 minutes after stopping the infusion. In fact, the plasma half-life of pentagastrin in the anaesthetised cat has been reported to be 1.5 ± 0.8 minutes (Blair, Farra, Richardson & Steinbok, 1970).

A2: Gut hormone release in response to a standard meal with and without salt.

These gut hormone radioimmunoassays were kindly performed by Professor S.R. Bloom and colleagues, Dept. of Medicine, Royal Postgraduate Medical School, Hammersmith Hospital, London. This work was completed in collaboration with Dr. S. Moss.

INTRODUCTION

The aim of this study, based on the tenet proposed in Chapter 1, was to determine whether the plasma gut hormone profile in response to a standard breakfast meal (so-called Hammersmith Test Meal; HTM, Fig. A.1) would be altered by the presence or absence of salt (50 mmol sodium).

A single-blind crossover study was performed in 11 healthy male and female (9:2 respectively) subjects aged between 22 and 36 years. The female subjects were not on the oral contraceptive pill. The protocol was as follows:

During an equilibrium period of 3 days, each subject ate a 2,500 Calorie fixed diet containing almost no salt. To this was added 150 mmol sodium per day as capsules containing 12.5 mmol sodium each. These were swallowed and not opened (and sprinkled on food). The distribution was 25 mmol sodium at breakfast, 50 mmol at lunch and 75 mmol at supper. Fluid intake was between 1.5 and 2 litres per 24 hours and kept constant for each individual. No alcohol or undue physical exertion were allowed.

On days 4 and 5, 10 ml fasting blood samples were taken and placed in tubes containing lithium heparin and aprotinin (400 KIU ml^{-1}). Samples were immediately spun and the plasma frozen and stored at

2 medium-size eggs, boiled to taste

60 g salt-free bread as toast

35 g marmalade or jam

10 g salt-free butter

150 ml unsweetened orange juice

18 g protein

66 g carbohydrate

22 g fat

530 Calories

Fig. A.1.

Composition of the Hammersmith Test Meal (HTM).

-20°C until thawed for assay. Each subject later received the HTM as lunch, with or without 50 mmol sodium. The order was randomised for each subject e.g., salt on day 4, no salt on day 5 or vice versa. The overall sodium and calorie intake on these days remained 150 mmol and 2,500 Calories respectively. No fluid was allowed for 1 hour before the HTM.

Each subject rested supine for 1 hour before a plastic cannula (Chapter 2) was inserted in an antecubital vein. While seated, blood samples were withdrawn 15 and 5 minutes before the start of the meal. Thereafter blood samples were taken at 15, 30, 60 and 180 minutes and treated as described earlier. During the meal, salt (50 mmol sodium) or placebo (lactose) were taken orally in gelatin capsules.

Samples were assayed (by radioimmunoassay; Chapter 2) for gastrin, pancreatic glucagon, enteroglucagon (Ghatei, 1982), VIP, neurotensin, motilin and pancreatic polypeptide.

RESULTS

Figures A.2 to A.9 show the changes in plasma glucose concentration and levels of the above gastrointestinal peptides in response to the "test meal". Closed circles represent the response to the meal plus sodium and open circles, the meal without sodium. The arrow indicates the start of the meal (mean value of pooled basal estimates). There was no alteration in the pattern of the plasma level response of any of these gut peptides. VIP levels did not change during the meal and pancreatic glucagon levels fell, although enteroglucagon levels rose.

DISCUSSION

The presence or absence of salt did not affect plasma

concentrations of the above peptides. Although these results do not support the hypothesis that changes in dietary sodium may alter gut peptide release, further experiments are warranted. The pattern of response under conditions of severe sodium deprivation might be more relevant.

This particular meal is not an equally potent stimulus to release of all gut peptides. The greater responses were seen with gastrin and pancreatic polypeptide. The lack of rise in VIP supports the view that it is probably not a circulating hormone. The fall in pancreatic glucagon is of interest as changes in both directions, particularly in relation to enteroglucagon, may enable quite subtle effects, including renal actions, but contrasts with the results of Holst (1978).

Legend to Figs. A.2 to A.9.

Plasma glucose and gut hormone responses to the HTM. The arrow indicates the start (time, 0) of the meal and the basal value is based on the mean of 3 estimates (fasting, -15 and -5 minutes; see text) in each individual subject. Closed circles represent values obtained during and after the meal with 50 mmol sodium, and open circles without. Results are expressed as mean ± S.E. of mean, n = 11.

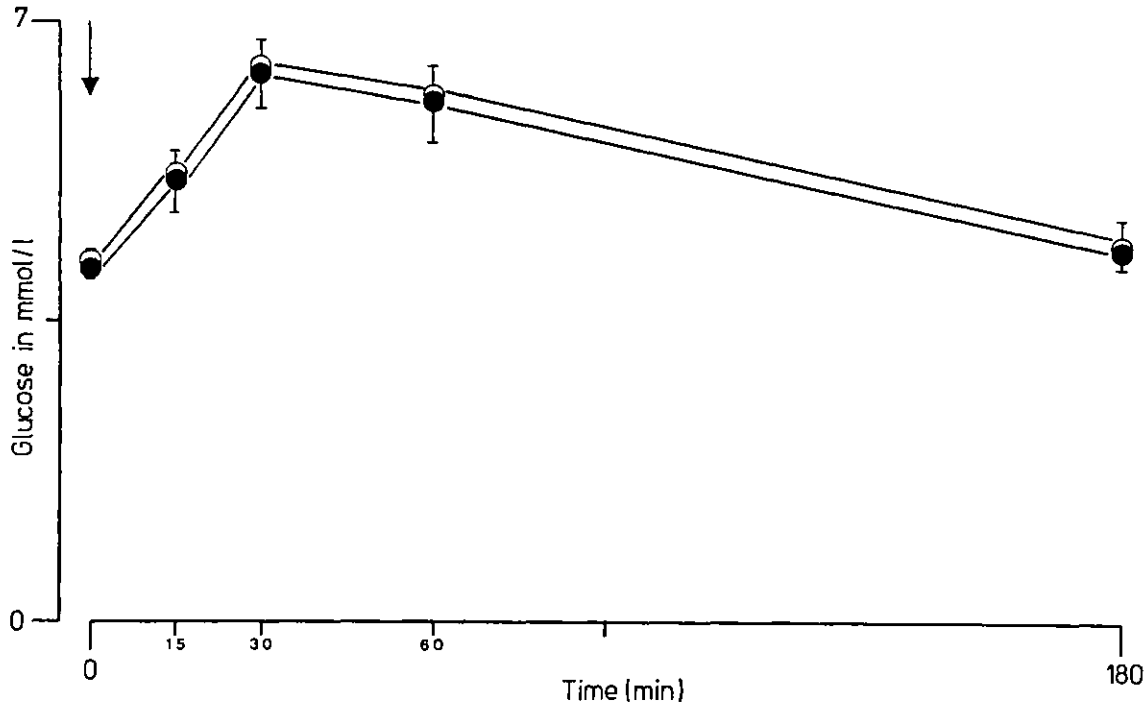


Fig. A.2.

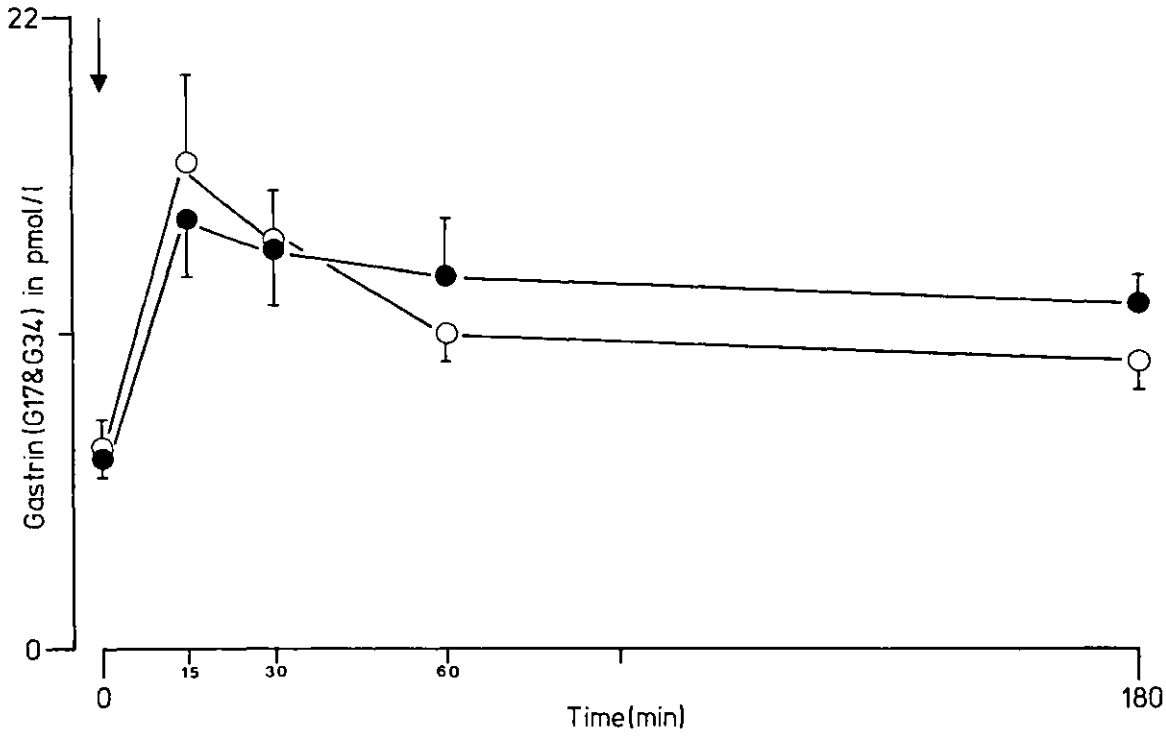


Fig. A.3.

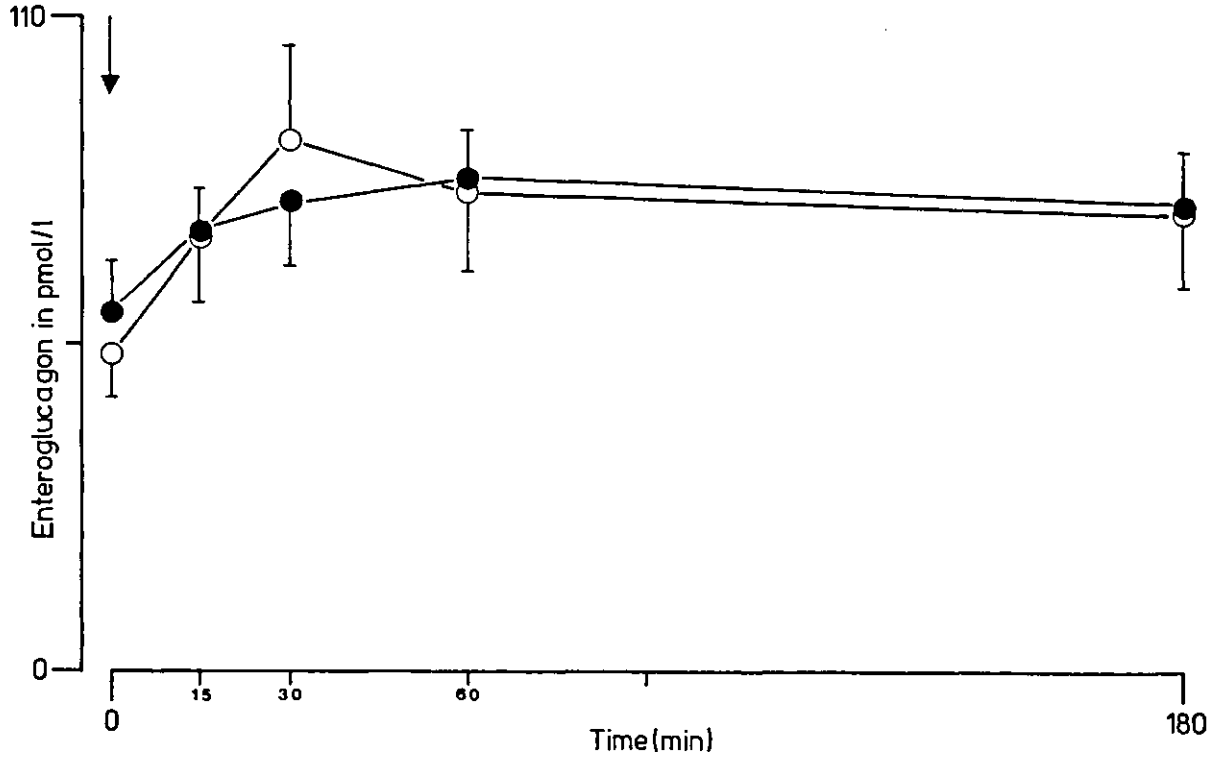


Fig. A.4.

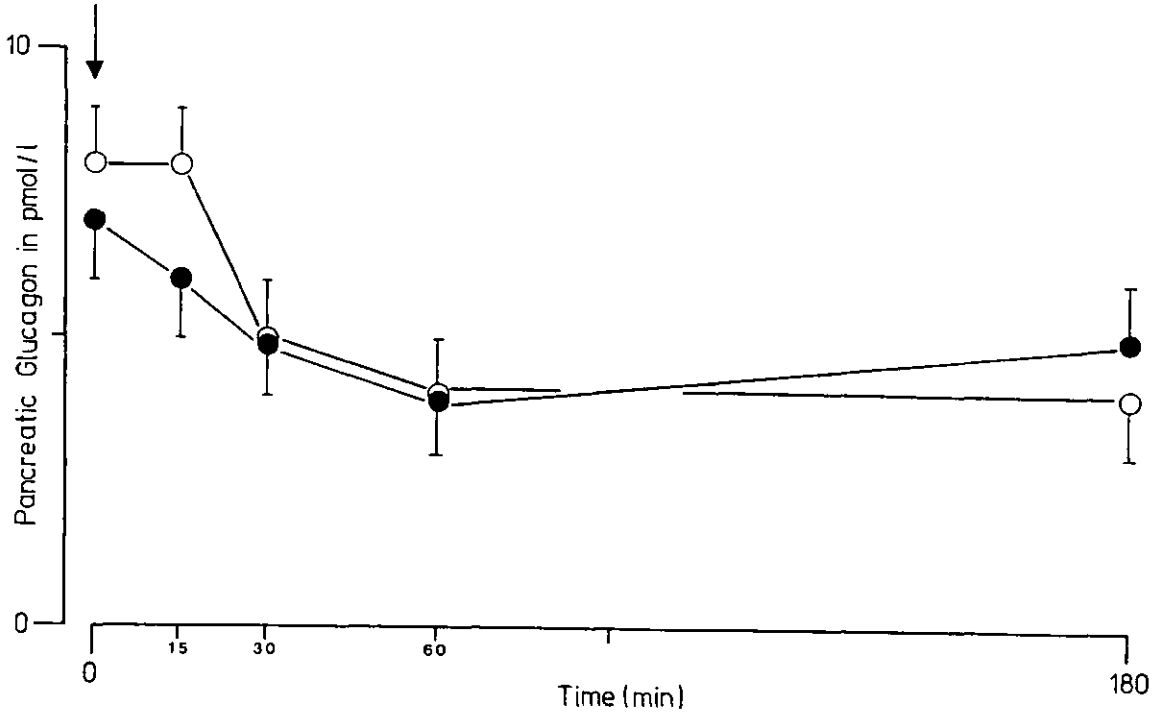


Fig. A.5.

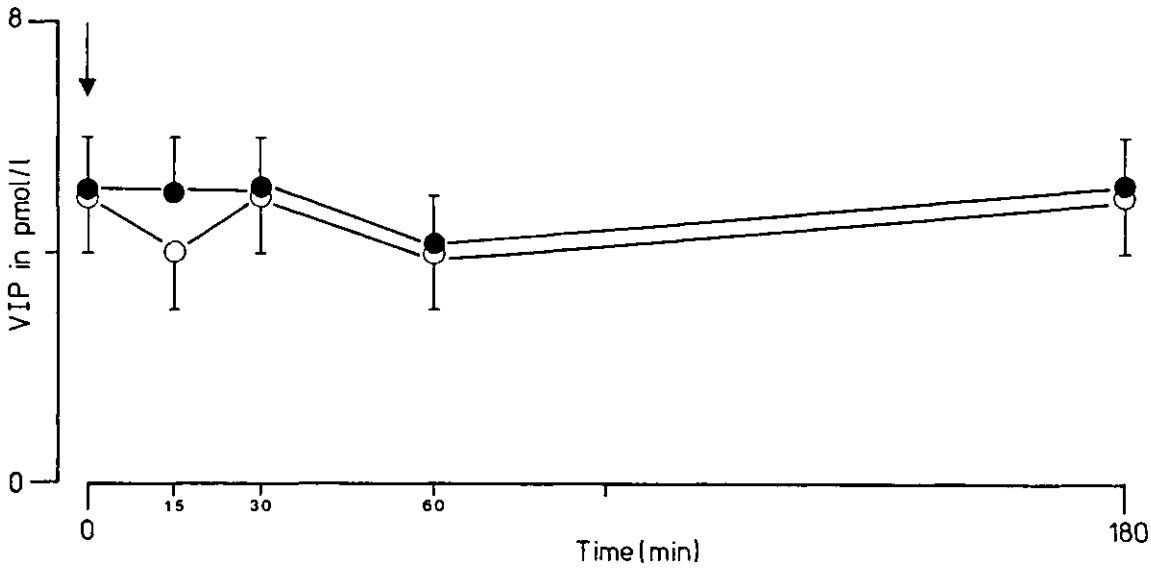


Fig. A.6.

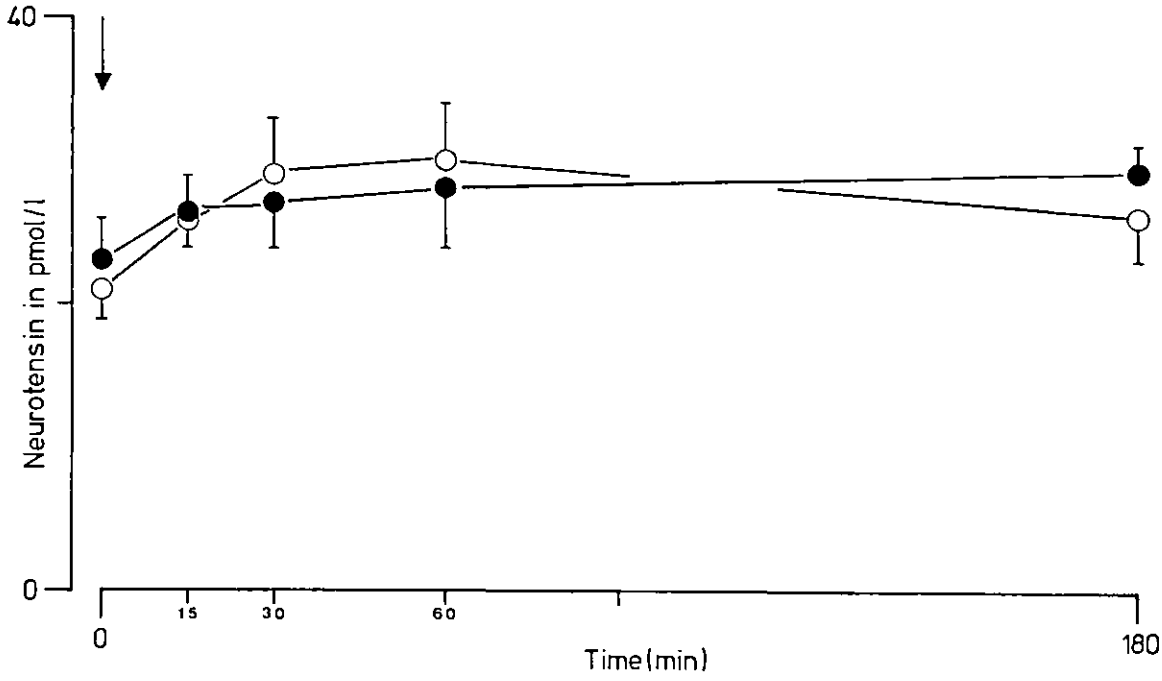


Fig. A.7.

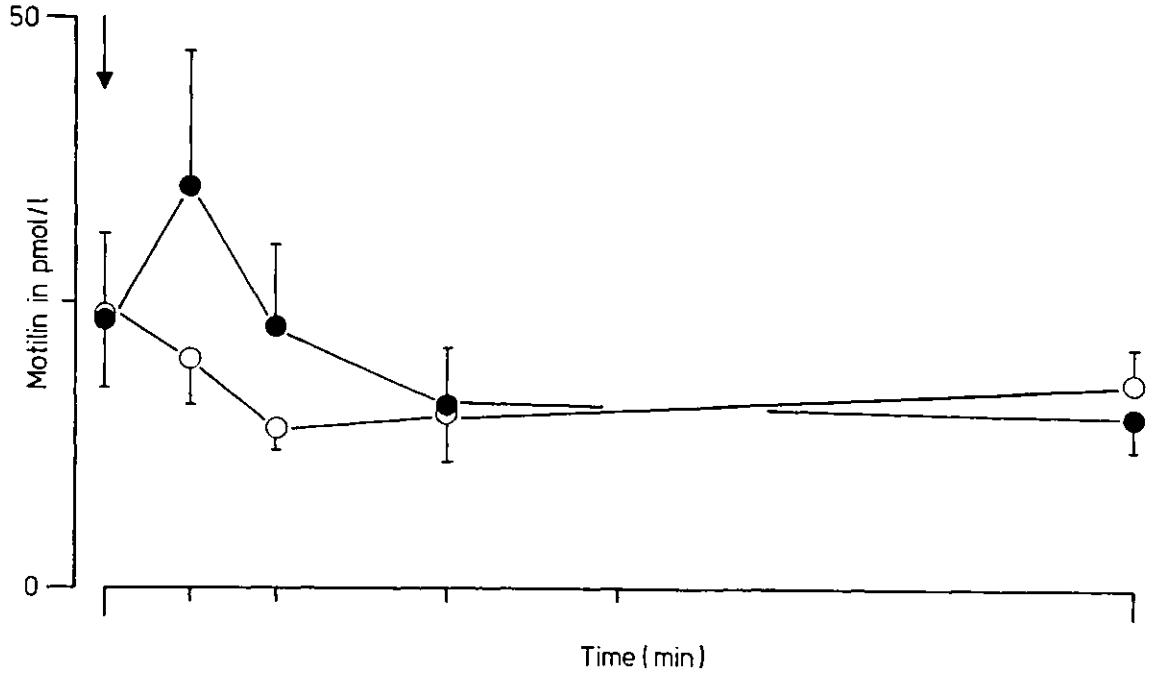


Fig. A.8.

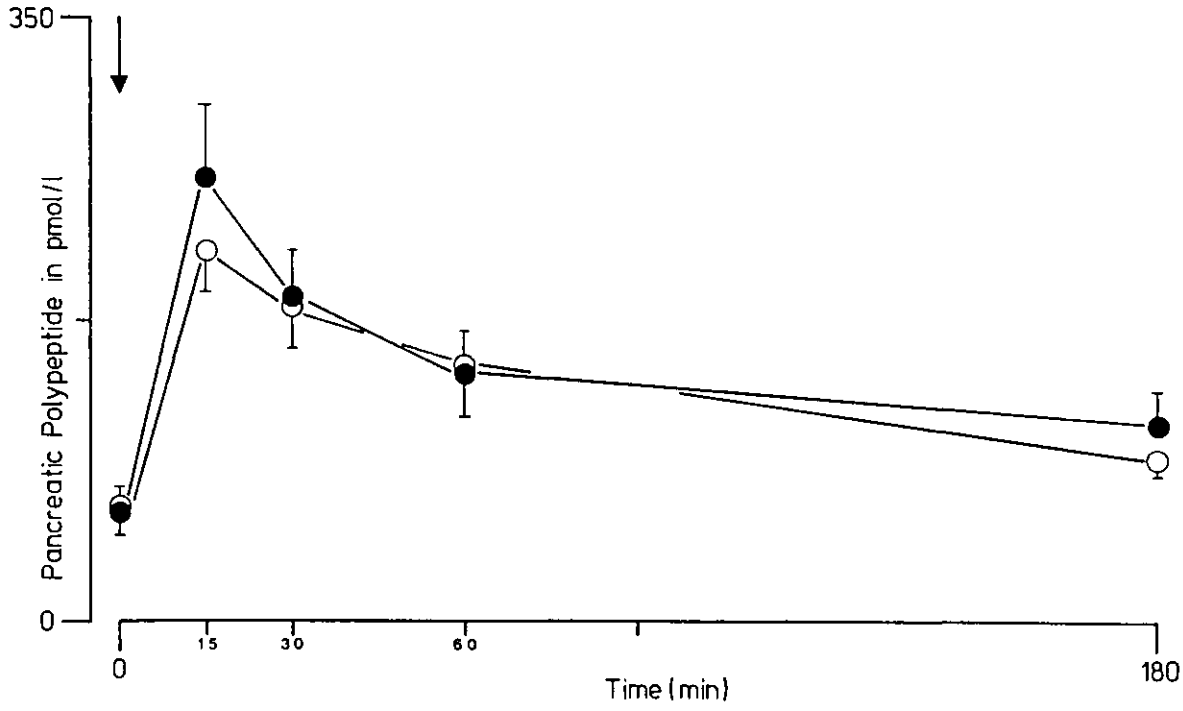


Fig. A.9.

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