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# Vasoactive intestinal peptide (VIP)*

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In 1969, Said and Mutt reported the extraction from normal lung tissue of a peptide which was capable of causing gradual but prolonged peripheral vasodilation. This finding led them to search for similar vasoactive substances in extracts of other metabolically active organs and, in 1970, they described the isolation of a potent peripheral and splanchnic vasodilatory peptide from hog small intestine, which they named Vasoactive Intestinal Peptide (VIP) (Said and Mutt, 1970 a, b). Subsequent purification of VIP allowed determination of its amino-acid sequence, which showed it to be a straight chain of 28 amino-acid residues with basic properties because of a predominance of arginine and lysine residues (Said and Mutt, 1972). Comparison of the amino-acid sequence of VIP with those of the classical hormones secretin and pancreatic glucagon showed a considerable degree of homology (Fig. 1). Thus, these three peptides, together with the more recently discovered glucose-dependent insulin releasing polypeptide (GIP), were thought to be related and it was suggested that they were perhaps derived from a common ancestral peptide. Further weight was added to these suggestions by the fact that the many biological effects of VIP, which include systemic vasodilation, glycogenolysis (Kerins and Said, 1973), lipolysis (Frandsen and Moody, 1973), inhibition of gastric acid production (Makhlouf and Said, 1975), stimulation of myocardial contractility (Said *et al.*, 1972), insulin secretion, increased flow of alkaline pancreatic juice and small intestinal juice with increased cyclic AMP content (Makhlouf and Said, 1975), overlap markedly with the actions of the other three peptides. Thus VIP was included as a member of the secretin family of hormonal peptides and, in 1974, it appeared in a list of 'candidate hormones of the gut' compiled by Grossman *et al.* (1974).

*Serum is unsuitable for VIP estimations as the level falls during the time taken for the clot to form and retract. Plasma should be frozen immediately after collection—Ed.

In the eight or so years since its discovery, a great deal of information has been amassed about VIP. With the development of highly sensitive and specific radioimmunoassays for VIP, it has been possible to study its distribution in the body and its mode of release. However, with this growth in knowledge controversy developed as to whether VIP fulfilled the classical criteria for a hormone or whether it acted locally as a paracrine substance or even as a neurotransmitter or neuromodulatory substance. This has now been resolved in favour of the latter, as discussed below.

Perhaps one of the first indications that VIP might not be a hormone was obtained from its distribution in the gut. Unlike the other members of the secretin family, which are located in discrete regions of the upper small intestine (secretin) and pancreas (glucagon), VIP appeared to have a much wider distribution. High concentrations of VIP were found throughout the length of the gut from the oesophagus to the rectum, including the pancreas (Bloom *et al.*, 1975; Said, 1975) (Fig. 2). A similar distribution of VIP-producing endocrine cells was also observed by some authors (Polak *et al.*, 1974; Buffa *et al.*, 1977) (Fig. 2). Others, however, (Larsson

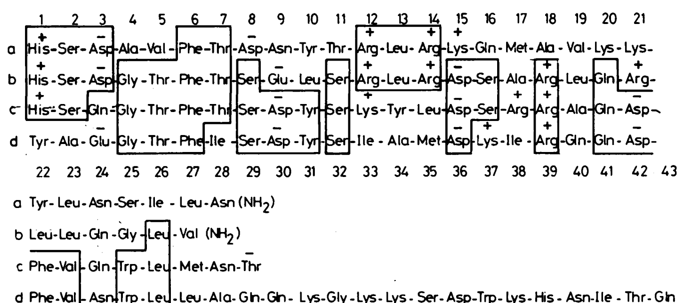


Fig. 1 *Amino-acid sequences of (a) VIP, (b) secretin, (c) pancreatic glucagon, (d) GIP (all porcine).*

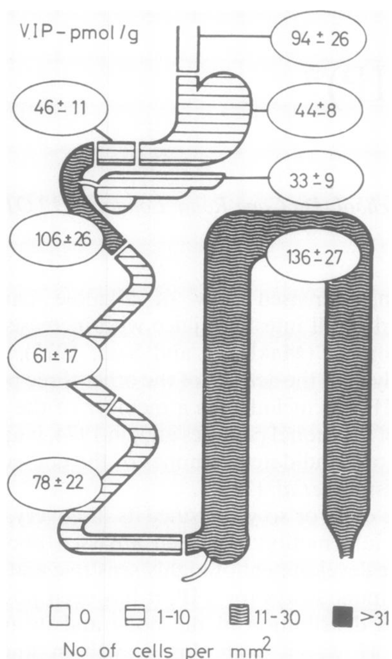


Fig. 2 *Distribution of VIP cells and VIP concentration in the human gastrointestinal tract.*

*et al.*, 1976b) were able to find VIP endocrine cells only in the antral region of the cat and in no other species examined.

In classical endocrine theory, a hormone is produced from a discrete gland in response to a stimulus and is secreted into the circulatory system in which it is carried to a distant target organ where it exerts its effect. In normal subjects, VIP does circulate but only at very low concentrations (Mitchell and Bloom, 1978). Circulating plasma VIP concentrations in 110 healthy fasting subjects, showing a skew distribution, are given in Table 1. So far, however, there has been no convincing demonstration of a significant change in peripheral plasma VIP concentration after ingestion of a mixed meal. This lack of response to such a physiological stimulus may well point to a non-endocrine role for VIP but it is by no means conclusive. Indeed, there have been several reports of the stimulation of VIP release into the peripheral circulation by other agents. Instillation of hydrochloric acid into the duodenum in sufficient quantity to stimulate the release of secretin also results in a significant increase in plasma VIP concentrations from a basal level of 1·7 to a peak at 6 minutes of 6·6 pmol/l, a rise which is sustained for at least 30 minutes (Ebeid *et al.*, 1977a; Bloom *et al.*, 1978). Similarly, the intraduodenal administration of hypertonic saline (6%, 3%) and phenylamine in the dog also results in the release of VIP (Ebeid *et al.*, 1977a). An intravenous infusion of calcium gluceptate (Ebeid *et al.*, 1977b), and, in pigs, electrical stimulation of the vagus are other procedures which result in a considerable release of VIP (Schaffalitzky de Muckadell *et al.*, 1977). It is particularly interesting that in the latter much higher VIP concentrations were attained in portal blood than in systemic blood, perhaps confirming previous reports that the liver is one of the major sites of degradation of VIP. Similarly, in the pig, higher systemic levels are produced by an infusion of VIP into the systemic veins than when the same solution is infused into the portal vein (Fig. 3). Degradation of VIP in the liver suggests that its actions may be limited to the gastrointestinal tract and portal circulation. Perhaps another indication that VIP does not have an important role as a peripheral circulatory hormone is its rapid clearance from plasma. The half life of porcine VIP has been reported as 0·85 minutes in the pig and 1·03 to 1·22 minutes in man (Domschke *et al.*, 1978). This is far more rapid than that of the classical hormones gastrin, pancreatic glucagon, and secretin.

Table 1 *Plasma VIP concentrations (pmol/l) in 110 healthy fasting subjects.*

| | |
|---|---|
| Range | 0·5–21 |
| Mean | 2·1 |
| Mode | 1·5 |
| Median | 1·7 |

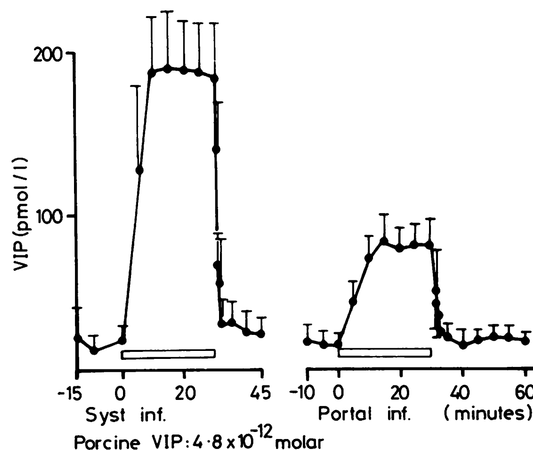


Fig. 3 *Peripheral plasma VIP concentrations attained with an infusion of VIP (4·8 pmol/l) given into a systemic or portal vein. (From the data of Modlin et al., 1978.)*

In 1973, Bloom and colleagues found that patients with the Verner-Morrison or Watery Diarrhoea Syndrome (see contribution by Professor Welbourn, page 85) who presented with profuse watery diarrhoea (several litres daily), hypokalaemia, and hypo- or achlorhydria had extremely high circulating VIP concentrations, often in excess of 300 pmol/l (Fig. 4). The known biological actions of this peptide fitted very well with a causative role of VIP for the symptoms of the disease. This finding was later confirmed by Said and Faloona (1975). In many cases, the source of these high concentrations of VIP was a pancreatic tumour composed of VIP-containing endocrine-type cells (Fig. 4). However, in a few cases (Fig. 4) the source of VIP was not an endocrine-like tumour but an abdominal ganglioneuroblastoma. This led to a search for the presence of VIP in a wide range of both animal and human tissue. High concentrations of VIP were found in the brains of the rat, cat, pig, and man (Bryant *et al.*, 1976; Larsson *et al.*, 1976b; Said and Rosenberg, 1976) and also in other organs such as the adrenals, urinary bladder, gallbladder, and salivary glands (Bryant *et al.*, 1976) (Table 2, 3). The bulk of VIP extracted from pig brain and gallbladder behaved on gel permeation chromatography identically to the pure intestinal VIP originally extracted by Said and Mutt, indicating considerable similarity (Fig. 5). In the brain extract there was a small peak of VIP immunoreactivity of apparently higher molecular weight, but its significance is unknown.

Immunofluorescent studies of human and animal intestine have demonstrated VIP in many tiny nerve fibres in the mucosa and muscle layers (Bryant *et al.*, 1976). Similarly, VIP-containing nerve fibres were

| | |
|---|---|
| Cerebral hemisphere | 27·5 ± 6·4 |
| Brain-stem | 4·0 ± 1·0 |
| Cerebellum | 0·7 ± 0·03 |

Table 2 *VIP concentrations in rat brain (pmol/g) (mean ± SEM)*

| *Pig* | | | *Man* | | |
|---|---|---|---|---|---|
| *Organ* | *1* | *2* | *Organ* | *1* | *2* |
| Hemisphere | 12·4 | 10·1 | Cerebral grey | 12·1 | 23·2 |
| Cerebellum | 1·0 | 0·8 | Cerebellum | 0·2 | 0·7 |
| Medulla | 4·5 | 7·4 | Medulla | 1·1 | 2·9 |
| Pituitary | 0·9 | 1·3 | Occipital lobes | 6·5 | 23·0 |
| Adrenal | 10·7 | 13·6 | Frontal lobes | 14·4 | 46·6 |
| Pancreas | 58·2 | 60·0 | Hypothalamus | 10·7 | 21·9 |
| Large bowel | 92·6 | 90·2 | Corpus callosum | 0·2 | 0·3 |
| Bladder | 18·2 | 11·6 | Midbrain | 3·3 | 5·8 |
| Gallbladder | 25·0 | 14·0 | Pons | 0·6 | 0·2 |
| Salivary gland | 20·8 | 12·0 | | | |
| Liver | <0·08 | <0·08 | | | |
| Plasma | 7·0 | 24·0 | | | |

Tissue concentrations in pmol/g wet tissue weight.
Plasma concentrations in pmol/l.

Table 3 *Representative VIP concentrations in the tissues of two human subjects and two pigs.*

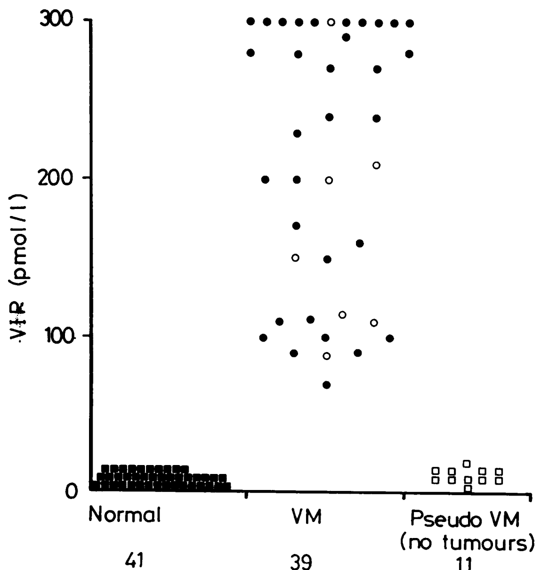


Fig. 4 *Plasma VIP concentrations in normal subjects, patients with the Verner-Morrison syndrome (VM), and patients with the pseudo VM syndrome. The solid and open circles indicate pancreatic tumours and neuroectodermal tumours, respectively.*

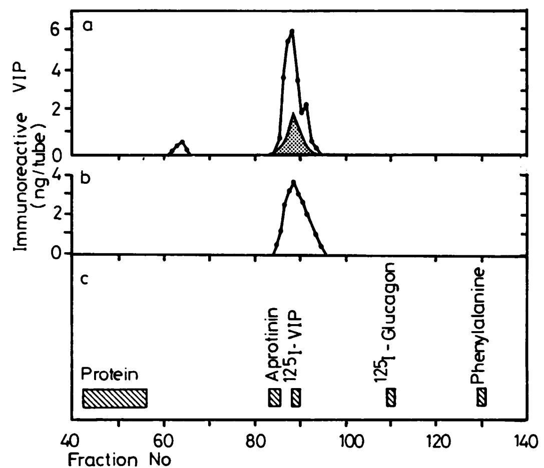


Fig. 5 *Gel permeation chromatography elution profile of immunoreactive VIP from (a) porcine brain, (b) porcine gallbladder. The stippled area in (a) indicates pure VIP.*

demonstrated in the salivary gland and in the endocrine and exocrine pancreas (Bishop *et al.*, Wharton *et al.*, in preparation). Within several months of the original demonstration of VIP in nervous tissues various independent groups using different antisera reported similar data. In 1977, Fuxe and co-workers showed by immunocytochemical techniques the presence of VIP in nerve terminals in the cerebral cortex, hypothalamus, amygdaloid nucleus, and corpus striatum. Giachetti and colleagues (1977) also demonstrated very elegantly by subcellular fractionation that in the cerebral cortex and hypothalamus the highest concentrations of VIP were in the synaptosome fraction.

Since the initial localisation of VIP in nerve fibres in the central nervous system, it has been found in the superior and inferior mesenteric ganglia (Hökfelt *et al.*, 1977), submucous and myenteric plexi of the intestinal wall (Bryant *et al.*, 1976; Larsson *et al.*, 1976b), cerebral vascular nerves (Larsson *et al.*, 1976a), and nerves of the female and male genital organs (L. I. Larsson *et al.*, unpublished observations).

The very wide distribution of VIP in nervous tissue, its questionable presence in true endocrine cells, its wide spectrum of actions, and its rapid clearance time from the circulation thus differentiate this peptide from the other members of the secretin family and obviously challenge the original belief that VIP is a simple gastrointestinal hormone.

The nerve endings of the autonomic nervous system are classically divided into two types, adrenergic and cholinergic, each type containing small secretory granules. Recently, ultrastructural studies have indicated a system of nerve terminals within the gut with much larger, more electron dense granules resembling those of the peptidergic system of the neurohypophysis, and evidence exists that these consist, in part at least, of VIP-containing nerves (see contribution by Dr Polak, page 68). It is possible then that VIP may be acting as a neurotransmitter substance within this new division of the autonomic nervous system. Recently, it has been shown that very high concentrations of VIP occur in nerve fibres in the neurohypophysis so that it is possible that VIP might affect the release of vasopressin or oxytocin and may have an osmoregulatory role (Van Noorden *et al.*, 1979).

In summary, VIP immunoreactivity has been demonstrated in association with nerves throughout the central nervous system. However, the evidence for a neurotransmitter role is still very circumstantial. An endocrine role cannot be excluded, as while it would seem likely that it does not play an important part in the regulation of the peripheral circulation, it might well act within the portal system or locally on blood vessels within the gut as a paracrine substance. Certainly, in the case of the Verner-Morrison syndrome, endocrine effects of VIP are apparent, but it must be stressed that this is a pathological situation.

Until it is shown that VIP is released in response to a stimulus at the presynaptic junction and elicits an action potential at the postsynaptic junction, and that this action can be blocked by application of specific antagonists, we can still only speculate as to the functional role of VIP.

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