Vasoactive Intestinal Peptide

Advances in Peptide Hormone
Research Series

Volume Editor

Sami I. Said, M.D.

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Sami I. Said, M.D.
Department of Medicine
University of Oklahoma
Health Sciences Center
and Veterans Administration Cente
Oklahoma City, Oklahoma

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Preface

The discovery of the vasoactive intestinal peptide and subsequent research on its chemical and biological properties and clinical and physiological significance make up a fascinating story that has not yet been fully told.

In 1967, my associates and I found that extracts of mammalian lungs were highly vasoactive, and we could not explain this vasoactivity (systemic vasodilation, hypotension) solely on the basis of the histamine and prostaglandin content of lung tissue. Concluding that we were dealing with one or more vasoactive peptides, I decided to seek expert biochemical help with the isolation of these peptides.

On the advice of Sune Bergström, then Rector of Karolinska Institute, Stockholm, I went to Viktor Mutt's laboratory where I was accepted for a sabbatical year (despite my considerable inexperience in peptide biochemistry). Together, we extracted and partially purified a vasodilator peptide from porcine lung. On the premise that the same peptide might occur in other organs (including, we hoped, the intestine), we then turned our search to intestinal extracts which were far more readily available to us. Using the same bioassay that guided our extraction of the lung peptide (measuring the increase in peripheral blood flow and the fall in arterial blood pressure of dogs), we discovered that peptide fractions from porcine duodenum indeed contained a vasodilator component* which we purified to homogeneity and named vasoactive intestinal peptide (VIP).

During the more than 10 years that have since elapsed, much has been learned about this peptide. It has been chemically characterized, synthesized, identified as a neural peptide, and found to occur widely but selectively in the central and peripheral nervous systems of human subjects and many animal species. Besides dilating blood vessels, VIP is now known to relax tracheobronchial and most gastrointestinal smooth muscle, stimulate intestinal water and electrolyte secretion, promote hepatic glycogenolysis, cause neuronal excitation, and release prolactin, growth hormone, renin, and other hormones. VIP is also a potent stimulant of adenylate cyclase activity in many tissues, and this action probably underlies many of its biological effects.

Some tumors of endocrine (especially pancreatic islet-cell) or neurogenic origin (e.g., pheochromocytoma, ganglioneuroma) may secrete large amounts of VIP, resulting in the clinical syndrome of "pancreatic cholera" or "Verner-Morrison Syndrome."

Although the physiological role(s) of VIP remains to be established, the peptide

* The presence of a vasodepressor principle in intestinal extracts had actually been noted by Bayliss and Starling, almost 70 years earlier, during their experiments leading to the discovery of secretin.

is released by physiological stimuli including depolarizing concentrations of K^+, electrical stimulation of the vagus and chorda tympani, and field stimulation of isolated segments of the trachea and gut. Evidence points to VIP as a likely mediator of the nonadrenergic, noncholinergic inhibitory system, at least in some organs. In promoting blood flow, mediating smooth muscle relaxation or modulating other physiological phenomena, VIP probably acts as a "neurohumor," "neurotransmitter," or "neuromodulator" rather than as a "classical hormone."

In this volume, an international group of investigators present and critically review their work on this peptide. The authors include biochemists, physiologists, pharmacologists, morphologists, histochemists, pathologists, gastroenterologists, endocrinologists, and other biologists and medical scientists. Their chapters deal with these aspects of VIP: isolation, structure and synthesis, measurement and histochemical localization, biological actions, receptors, metabolism, physiological role, and clinical significance. The result is an up-to-date, comprehensive reference source on VIP, a peptide that at first seemed like another "candidate hormone" of the gastrointestinal tract, but is today viewed as a major neuropeptide with likely regulatory influence on gastrointestinal, cardiovascular, genitourinary, neural, endocrine, and neuroendocrine function.

This preface gives me the privilege of presenting this monograph on VIP to its readers on behalf of the many colleagues and associates who contributed to its creation. My thanks to them, and to Raven Press, for making it possible.

Sami I. Said

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Isolation and Structure of Vasoactive Intestinal Polypeptide from Various Species

Viktor Mutt

Department of Biochemistry II, Karolinska Institutet, S-104 01 Stockholm, Sweden

It may be that Bayliss and Starling, in connection with their discovery of secretin, were the first to observe also vasoactive intestinal polypeptide (VIP) by way of one of its actions, but this is not certain. What they did observe was that secretin in acidic extracts of the duodenal and jejunal mucosa was associated with another substance which caused a fall in blood pressure (2). As VIP could have been present in those extracts, and as VIP on intravenous injection causes a fall in blood pressure (39), the action they observed may have been due to VIP. Furthermore, they found that although secretin could be extracted from the epithelial cells the depressor substance occurred in more deeply lying structures, possibly the muscularis mucosae (3). This too fits, at least roughly, with what is now known concerning the distribution of secretin (23,34) and VIP (13) in intestinal tissue. On the other hand, they claimed that the depressor substance could be removed from the mucosa by extraction with absolute alcohol in a Soxhlet apparatus, leaving the secretin unextracted (3). It is highly improbable that VIP would have been extracted under such conditions, although the actual experiment has not been carried out. It is more likely that the alcohol-soluble depressor substance was histamine (4) rather than VIP. Furthermore, Bayliss and Starling originally claimed that the depressor substance could be extracted by aqueous acid from the lower end of the ileum, and that such extracts had no stimulatory action on the pancreas (2). As VIP does, although rather weakly, stimulate the pancreas (40), this suggests that the ileal extracts did not contain VIP but did contain the depressor substance. Perhaps significantly, however, Bayliss and Starling in their full paper of 1902, when discussing the effects of ileal extracts, stated that these had "either no secretory effect at all, or a very feeble one." This is in contrast to the definite claim in their preliminary communication that the extracts had "no excitatory influence on the pancreas" (2,3).

Regardless of who first observed VIP, it is clear that VIP was first isolated by Said and Mutt from hog upper intestinal tissue (38–40). Said had been interested in the occurrence of vasoactive peptides in lung tissue and had come to work on such peptides in a laboratory where large amounts of porcine upper intestinal tissue were routinely being processed. It was a logical next step to

determine if extracts of intestinal tissue, like lung tissue, contained vasoactive peptides. Not only did they do so, but at a considerably higher concentration than those in the lung extracts. It therefore seemed reasonable to isolate at least one vasoactive peptide from intestinal tissue before continuing with the work on lung tissue.

PORCINE VIP

Isolation of Porcine VIP

The isolation of porcine VIP was based on its vasodilatory action as determined in anesthetized dogs (39). The starting material for the purification was a side fraction from the isolation of secretin. This side fraction, called "postsecretin," was obtained briefly as follows: Hog intestines were heat-coagulated to destroy proteolytic enzymes and then extracted in the cold with dilute aqueous acetic acid. Peptides were adsorbed to alginic acid, eluted with 0.2 M HCl, and precipitated from the eluate with NaCl. This precipitate was dissolved in 66% ethanol, and a fraction that precipitated on neutralization of the solution was removed and discarded. The soluble peptides were recovered in aqueous solution and precipitated at pH 4 with NaCl. This precipitate was extracted with methanol. A precipitate that formed when the methanolic solution was adjusted to pH 7.5 was removed, and the soluble peptides were precipitated with ether at pH 2.5, dissolved in water, and reprecipitated with NaCl. The precipitated material was chromatographed at pH 8 on carboxymethyl cellulose, and the effluent was separated into three fractions, one appearing before the bulk of the secretin activity and called "presecretin," the second containing the bulk of the secretin activity, and the third, displaced from the column either by increasing the ionic strength of the eluant or by eluting with 0.2 M HCl, termed "postsecretin." Up to this point the method was essentially that originally worked out by Jorpes and Mutt for the preparation of secretin (21,27). All three fractions were vasodilatory in the dog (38; and *unpublished*), but the "postsecretin" was definitely the strongest. For the isolation of VIP the "postsecretin" peptides were first chromatographed on Sephadex G-25 in 0.2 M acetic acid and the active fraction purified further by chromatography on carboxymethyl cellulose in 0.1 M NH_4HCO_3. This was followed by countercurrent distribution in the system 1-butanol/0.1 M NH_4HCO_3, resulting in a highly purified form of VIP. For elucidation of the amino acid sequence of VIP the material was nevertheless purified (slightly) further by additional chromatography on Sephadex G-25 (40).

Amino Acid Sequence of Porcine VIP

Quite unintentionally, the amino acid sequence of porcine VIP was determined without application of the commonly used Edman method (16) for determination of amino acid sequences in proteins and peptides. Amino acid analysis showed

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that the peptide was composed of 28 amino acid residues; in acid hydrolyzates of the peptide, 14 amino acids were found in the proportions Ala_2, Asp_5, Arg_2, Glu_1, His_2, Ile_1, Leu_3, Lys_3, Met_1, Phe_1, Ser_2, Thr_2, Tyr_2, Val_2. Of the acids often occurring in peptides, cysteine, glycine, proline, and tryptophan were absent. Because the peptide was strongly basic, as evidenced by its properties on electrophoresis and ion-exchange chromatography, it was obvious that several of the side chain carboxyls of its acidic amino acids had to be amidated in the intact peptide. The only histidine residue was found to be situated at the N-terminal, which suggested that VIP might be related to secretin and to glucagon; this possibility was supported by the finding that dipeptidyl aminopeptidase I removed a histidyl-serine residue from the N-terminal of VIP (40), as it had been shown to do with glucagon (26). Cleavage of the peptide at its only methionine residue by the method of Gross and Witkop (19) resulted in two peptides, an N-terminal heptadecapeptide and a C-terminal undecapeptide. The sequence of these was established by a series of enzymatic degradations and determinations of amino acid compositions, N-terminal residues, and electrophoretic mobilities of the smaller peptides obtained. Interestingly, porcine pancreatic kallikrein (a gift from Prof. E. Werle, Munich) was found to cleave the N-terminal heptadecapeptide at only one of its three residues that were susceptible to cleavage by trypsin; this of course facilitated the sequence determination. The results of the work unequivocally showed that porcine VIP has the sequence: His-Ser-Asp-Ala-Val-Phe-Thr-Asp-Asn-Tyr-Thr-Arg-Leu-Arg-Lys-Gln-Met-Ala-Val-Lys-Lys-Tyr-Leu-Asn-Ser-Ile-Leu-Asn-NH_2 (31). A notable feature is that as in many other but by no means all hormonal peptides the C-terminal carboxyl is amidated.

Other Forms of Porcine VIP

It is well known that peptide hormones are usually not biosynthesized as such from their component amino acids but are formed by the cleavage of longer precursor forms. Such cleavage may take place either extracellularly as for angiotensin (42) or intracellularly as for insulin (44). A consequence of this is that various intermediate cleavage forms may be present in tissues and/or the circulating blood. Bioactive gastrin, for instance, has been isolated in forms of three chain lengths in which each of the longer forms is an N-terminal extension of the preceding shorter one. In gastrin each of these three forms of different length in turn occurs in two forms with different electrical charge depending on whether the phenolic group of its single tyrosine residue is esterified with sulfuric acid. There is strong evidence for the occurrence of still other forms of gastrin (18). In the case of VIP no other form than the octacosapeptide has yet been isolated, but there is no reason to believe that none occurs. Indeed Dimaline and Dockray presented evidence for the presence in human colonic mucosa of immunoreactive VIP of a smaller molecular size than the octacosapeptide (14); and Yamaguchi and co-workers (52), in extracts of certain endocrine

tumours of the pancreas, detected large amounts of a VIP immunoreactive peptide of about 15 K molecular weight (i.e., about 4.5 times that of the octacosapeptide). As VIP has a C-terminal α-amide structure and there is reason to believe that such structures in peptides are formed by the cleavage of C-terminal extended precursor forms of the peptides (43), it is probable that such a precursor form of VIP remains to be isolated.

Perhaps more interesting is the finding of Dimaline and Dockray that, whereas immunoreactive VIP from human colonic muscle appeared to be of the same molecular size as the porcine octacosapeptide, colonic mucosal VIP occurred in no fewer than four forms: ordinary VIP, the apparently smaller form of VIP mentioned above, and two forms of apparently the same molecular size as "ordinary" VIP but less basic (14). Dimaline and Dockray at first considered the possibility that the ordinary VIP could be the VIP that occurs in muscle, whereas the more acidic forms occur in mucosal endocrine cells; however, their own later work on VIP in various species and intestinal segments showed that there is no simple explanation for the occurrence of the different forms (15). One could speculate on various possibilities that would explain why some derivative or analog of VIP would differ from it in electrical charge(s), but as the variant form(s) will presumably soon be isolated and analyzed there seems to be no reason for such speculation. It may be mentioned that in our laboratory Tatemoto isolated a variant form of porcine secretin which is less basic than the "ordinary" secretin, and he localized the difference to the N-terminal tryptic peptides of the two forms (47).

CHICKEN VIP

The second species from which VIP was isolated was the chicken (32). The isolation method was similar but not identical to that used for the isolation of porcine VIP, the main difference being that the chromatography of the methanol-soluble peptides on carboxymethyl cellulose was not carried out in 0.1 M NH_4HCO_3 but in 0.0225 M phosphate buffer pH 6.4 with a linear gradient of NaCl to 0.3 M in the same buffer. The sequence determination also was similar to that used for porcine VIP. Here the main difference was that the cleavage with kallikrein was carried out before the cleavage with CNBr, instead of after. The sequence of chicken VIP is: His-Ser-Asp-Ala-Val-Phe-Thr-Asp-Asn-Tyr-Ser-Arg-Phe-Arg-Lys-Gln-Met-Ala-Val-Lys-Lys-Tyr-Leu-Asn-Ser-Val-Leu-Thr-NH_2. This differs from the sequence of porcine VIP in positions 11, 13, 26, and 28.

BOVINE VIP

The second mammalian species from which VIP was isolated was the cow. The method used differed in several respects from the methods used earlier for the isolation of porcine and chicken VIP. Chromatography on carboxymethyl

cellulose was again carried out with ammonium bicarbonate, but with a stepwise change of its concentration in the eluant. Final purification was achieved by reversed-phase high-performance liquid chromatography on Bondapak C_{18} in the solvent system 0.005 M CH_3COONH_4 in 36% ethanol. The amino acid sequence of bovine VIP was determined in collaboration with Jörnvall (at the Department of Chemistry of this institute) using automatic sequencer assisted (17) methodology. This greatly facilitated and speeded up the work. The sequence was found to be identical to that of porcine VIP (10).

HUMAN VIP

Using a method similar to that worked out for the isolation of bovine VIP from upper intestinal tissue, Carlquist and McDonald in our laboratory recently, in collaboration with Professor V.L.W. Go at the Mayo Clinic, Rochester, Minnesota, isolated VIP, or at any rate one form of VIP, from human colonic tissue. This VIP has, like porcine/bovine, and chicken VIP, N-terminal histidine; and acid-catalyzed hydrolyzates of it have an amino acid composition identical to that of corresponding hydrolyzates of porcine/bovine VIP.

DISCUSSION

The original isolation of VIP was straightforward and did not present particular difficulties. To a certain extent, this is illusory, as the isolation did not start from intestinal tissue but, because of earlier work with secretin and cholecystokinin, from a small fraction of intestinal peptides to which the activity was localized. VIP, whether porcine/bovine (10,31) or chicken (32) shows amino acid sequence similarities to secretin [whether porcine (30) or chicken (33)] and to glucagon [whether mammalian (7,46), duck (45), or chicken/turkey (35).

TABLE 1. *Amino acid sequences of VIP, secretin, and glucagon*

VIP	
Porcine/bovine	H S D A V F T D N Y T R L R K Q M A V K K Y L N S I L N [a]
Chicken	H S D A V F T D N Y S R F R K Q M A V K K Y L N S V L T [a]
Secretin	
Porcine	H S D G T F T S E L S R L R D S A R L Q R L L Q G L V [a]
Chicken	H S D G L F T S E Y S K M R G N A Q V Q K F I Q N L M [a]
Glucagon	
Mammalian	H S Q G T F T S D Y S K Y L D S R R A Q D F V Q W L M N T
Turkey/chicken	H S Q G T F T S D Y S K Y L D S R R A Q D F V Q W L M S T
Duck	H S Q G T F T S D Y S K Y L D T R R A Q D F V Q W L M S T

The one-letter notation for amino acid residues (*Eur. J. Biochem.* 5:151–153, 1968) is used.

[a] The C-terminal amino acid residue is in amide form.

References to the sequences are given in the text.

It may be seen from Table 1 that all these peptides have N-terminal histidylseryl sequences which are separated by three amino acid residues from a phenylalanylthreonyl sequence. These four identities are the only ones that are common to all of the peptides and, taken by themselves, would, although interesting, not be particularly impressive. However, various similarities between the peptides become much more prominent if, in addition to positions with identical amino acid residues, those with chemically similar residues are also taken into consideration, and if not all the peptides are included in every comparison. For instance in position 11 all seven peptides have a hydroxy amino acid; this is serine in all cases except porcine/bovine VIP where it is threonine. In position 12 all peptides have a basic amino acid, either arginine or lysine. In position 22 there is an aromatic amino acid (either phenylalanine or tyrosine) in all cases except in porcine secretin where there is leucine; and in positions 23 and 26 there is in all cases a hydrophobic amino acid, either leucine, isoleucine, or valine. In position 24 there is a residue of either asparagine or glutamine.

Although it is the similarities between peptides that often attract attention and suggest evolutionary relationships, it is the differences that make peptides individual. As secretin and VIP stimulate the secretion of water and bicarbonate from the pancreas, and glucagon does not, it was suggested that the amino acid residues that are, in a particular position, identical in VIP and secretin but not in glucagon might be of particular importance for the stimulation of pancreatic secretion; on the other hand, the residues that are identical in VIP and glucagon but not in secretin might be of importance for stimulation of glycogenolysis, as VIP and glucagon stimulate glycogenolysis but secretin does not (31). Indeed, in position 3 there is a residue of aspartic acid in secretin and VIP but not in glucagon, and this residue has been found to be very important for the stimulation of pancreatic secretion by secretin (5,51). Although VIP and secretin stimulate pancreatic secretion, they do not do so in an identical fashion. On intravenous injection the effect of VIP sets in briskly but wears off much more quickly than does the effect of secretin (40).

Bodanszky (6) pointed out that in position 15 VIP has a basic amino acid residue, lysine, whereas porcine secretin has an acidic one, aspartic acid. They synthesized analogs of secretin 5-27 in which Asp-15 was replaced by asparagine or lysine and found that these analogs reacted more strongly with VIP receptors and more weakly with secretin receptors on pancreatic acinar cells than did secretin 5-27 itself. This led them to suggest that the negative charge in position 15 of porcine secretin might be of importance for the reaction of porcine secretin with its receptors and the positive charge of VIP in this position for the reaction of VIP with *its* receptors (6).

In its brisk but brief stimulating action on the secretion of pancreatic juice in the anesthetized cat, chicken secretin seems to be distinctly more VIP-like than porcine secretin (33), and it is interesting to note that in position 15 it has a residue of the neutral glycine instead of the aspartic acid residue of porcine secretin. It also has, like VIP but unlike porcine secretin, a residue of tyrosine

in position 10, a residue of valine in position 19, and a residue of lysine in position 21. Moreover, chicken secretin and porcine/chicken VIP have, in distinction from porcine secretin, (nonidentical) nonpolar amino acid residues in positions 5 and 26, residues with side chain amides in position 16, and aromatic amino acid residues in position 22. On the other hand, it must be admitted that in a few other positions the residues are more alike in porcine secretin and VIP than in chicken secretin and VIP. Thus in position 12 porcine secretin and VIP have a residue of arginine, but chicken secretin has a residue of lysine; and in position 23 porcine secretin and VIP have residues of leucine, but chicken secretin has a residue of isoleucine.

VIP is a basic peptide, more basic than secretin, which in turn is more basic than glucagon. In many peptides oxidation is known to lead to inactivation because of conversion of methionine residues to residues of methionine sulfoxide (12). It was shown several years ago that cholecystokinin (CCK) may be oxidized with essentially complete loss of activity, and that the activity could be recovered on reduction (28). VIP has a single residue of methionine, but if VIP is oxidized with dilute hydrogen peroxide under the conditions described for CCK no loss of activity takes place. However, on thin-layer chromatography, on either silica gel or cellulose in the 1-butanol-acetic acid-pyridine water (30:6:20:24) system of Waley and Watson (50), the chromatographic properties of the oxidized VIP differ from those of VIP, suggesting that the conversion to the methionine sulfoxide analog has taken place on oxidation *(unpublished).*

Peptide hormones vary widely in the extent to which they show species variations. Porcine and human calcitonin differ in no fewer than 18 of their 32 amino acid residue positions (36), whereas porcine and human glucagon are identical throughout their 29 residues. Indeed no differences have been found in any of the half-dozen mammalian glucagons that have been isolated (46); of the three avian glucagons isolated, the two from the chicken and the turkey are identical. They differ from the mammalian form in only one residue position whereas the one from the duck differs from the mammalian form in two positions of which one is the same as for chicken/turkey glucagon (45).

Too little information is available concerning VIP to make a definite statement, but the identity of bovine and porcine VIP and, judging from the amino acid composition and N-terminal residue the probable similarity of it to human VIP, seems to suggest that VIP, like glucagon, may have been conserved during evolution in mammals. The sequence differences between porcine/bovine VIP and chicken VIP are of a fairly conservative nature; although these differences are slightly more numerous than those between mammalian glucagon and the avian glucagons, they are far fewer than the differences between porcine secretin and chicken secretin. VIP, of course, shows structural similarities to peptides other than glucagon and secretin. Its similarities to the gastric inhibitory peptide (GIP) (8,31), bombesin (1,49), and the heptacosapeptide PHI recently isolated from porcine intestinal tissue (48) are fairly extensive; its similarities (20) to neurotensin (11), the "pancreatic polypeptide" (22,25), and CCK (29) are less

extensive but not necessarily less interesting. This also applies to the similarities between VIP and the *Escherichia coli* ribosomal protein S-7 (37), evident if the methionine residue of VIP is aligned with methionine-114 of S-7.

CONCLUSIONS

VIP has been a peptide with many surprises. Discovered on the basis of its vasoactive action, it was unexpectedly found to be structurally closely related to the earlier known hormones secretin and glucagon, and to exhibit, at least pharmacologically, their physiological actions to some extent. The unexpected discovery of immunoreactive VIP in nerves (9,24,41) greatly increased the interest in peptides in the central nervous system and tissues of the gastrointestinal tract. It is therefore somewhat astonishing that VIP has not yet been isolated from tissues of the central nervous system.

Other problems of direct chemical interest are isolation of the variant forms of VIP that have been described and the isolation of VIP from additional, preferably distant, species. Perhaps it is also not too much to be expected that the large amount of work that has been, and continues to be, carried out on the biological properties of VIP will reveal which of its many functions are of physiological importance and which are perhaps merely pharmacological curiosities.

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