REVIEW

Structure-activity relationship of vasoactive intestinal peptide (VIP): potent agonists and potential clinical applications

Satomi Onoue · Shingen Misaka · Shizuo Yamada

Abstract Vasoactive intestinal peptide (VIP) has been identified as one of major peptide transmitters in the central and peripheral nervous systems, being involved in a wide range of biological functions. The general physiologic effects of VIP include vasodilation, anti-inflammatory actions, cell proliferation, hormonal secretion, regulation of gastric motility, and smooth muscle relaxation; therefore, VIP has emerged as a promising drug candidate for the treatment of several diseases. A number of clinical applications of VIP or its derivatives have been developed; however, VIP-based drugs are not yet in clinical use, possibly because of mainly two serious problems: (1) poor metabolic stability and (2) poor penetration to the desired site of action. To overcome these shortcomings, the development of efficacious VIP analogues and several drug delivery systems has been attempted on the basis of numerous structure–activity relationships (SAR) studies and pharmacological experiments. Combination of the use of potent VIP analogues and an appropriate drug delivery system might be advantageous for the VIP-based therapy. We review in this paper SAR studies of VIP for the identification of potent therapeutic agents, describe the development of selective and/or metabolically stable VIP receptor agonists/antagonists, and discuss the potential application for clinical treatment using drug delivery systems.

Keywords VIP · PAC1 · VPAC1 · VPAC2 · Structure–activity relationship · DDS

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Introduction

Vasoactive intestinal peptide (VIP), an octacosapeptide, was first isolated and characterized from porcine duodenum in the early 1970s by Said and Mutt (1970). Because of the high similarity of primary and secondary structures, VIP has been identified as a member of the glucagons–secretin family (Table 1), which includes pituitary adenylate cyclase-activating polypeptide (PACAP) 27 and 38 (Miyata et al. 1989; Miyata et al. 1990), glucagon (Unger et al. 1978), secretin (Jorpes 1968), glucagon-like peptide-1 (Mojsov et al. 1987), growth-hormone-releasing factor (GHRF)-(1–29) (Rivier et al. 1982) and helodermin (Hoshino et al. 1984). VIP is widely distributed in cholinergic presynaptic neurons of the central nervous system and peripheral peptidergic neurons innervating diverse tissues, such as the heart, lung, digestive and genitourinary tract, eye, skin, ovaries, and thyroid gland (Vaudry et al. 2000). It has been well identified that VIP acts as one of major peptide transmitters in the central and peripheral nervous systems, being involved in a wide range of biological functions in organisms (Abad et al. 2006), including metabolic processes, exocrine and endocrine secretions, cell differentiation, and relaxation of smooth muscle (Said 1991), secretion of regulatory hormones (Onoue et al. 2001a), and regulation of immune response (Delgado et al. 1999b). On the basis of its numerous biological actions, VIP has been considered a promising candidate for pharmaceutical agents for several diseases, including diabetes, asthma, impotence, and rheumatism (Pozo 2003; Gozes and Furman 2004; Abad et al. 2006).

A number of structure–activity relationship (SAR) studies of VIP were carried out with different techniques to identify the portions of peptides involved in interactions with VIP receptors (Stiuso et al. 2006). These SAR investigations enabled successful development of effica-

Table 1 Primary and secondary structures of VIP-related peptides

	Homology	5	10	15	20	25	30
VIP	–	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					
PACAP27	68%	– – – G I – – – **S – S – Y – – – – – – – – – – A A** V –					
Secretin	32%	– – – G I – **– S E L S – –** – E G **A R L Q R L – Q G** L V					
Glucagon	21%	– – Q G T – **S D – S K Y L D S R R A Q D F V Q W L M** – T					
GLP-1-(7–36)	21%	– A E G T – **S – V S S Y L** E G Q **– A – E F I A W L V K** G R					
GHRF-(1–29)	25%	Y A – – I **– – N S – R K V** L G **Q L S A R – L – Q D I M S** R					
Helodermin	54%	– – – – I – – Q **Q – S K – L A K L – L Q – – –** A – – – G S R T S P P P					

All peptides were amidated at C terminus, except for glucagon. The deduced α-helical region in each neuropeptide was shaded.

cious VIP-receptor agonists with high specificity and/or stability, clinical applications of which were also proposed. We will review various new developments of VIP derivatives in the context of previous findings, representing a glimpse of the increasing volume of literature. In addition, recent researches on a drug delivery system (DDS) for potential VIP-based therapy will also be discussed.

VIP/PACAP receptors

The effects of VIP are mediated through interaction with two receptor subclasses named VPAC1 and VPAC2 receptors, and the effects of PACAP are also mediated through interaction with the same receptors, as well as through a selective receptor named PAC1 (Table 2; Rawlings and

Hezareh 1996). VIP/PACAP receptors are members of a large family of G protein-coupled receptors, often referred as the GPCR-B family, which includes the receptors of glucagons–secretin family peptides, calcitonin, and parathyroid hormone (Laburthe et al. 1996). These are widely distributed glycoproteins with a large hydrophilic extracellular domain followed by seven hydrophobic transmembrane helices (Vaudry et al. 2000). They share several specific properties, the most important of which is the presence of large N-terminal extracellular domains that contain ten highly conserved amino acids, including six cysteines, putative N-terminal leader sequences, and several potential N-glycosylation sites.

Several different splice variants of PAC1 receptor with different signaling properties were identified, which can be generated by alternative splicing of the exon encoding the

Table 2 Characteristics of PACAP/VIP receptors

Subtypes	Splice variants	Ligand affinity (Kd)	Signaling	Distribution
PAC1	Short Very short, Hip Hop1, Hop2 Hiphop1 Hiphop2 TM4	PACAP27≒PACAP38: 0.5 nM VIP: >500 nM	cAMP PLC Ca^{2+}	CNS (wide-ranging), Retina, Ganglion cells, Pancreatic beta islet, Adrenal glands, Vasculature, Liver
VPAC1	–	PACAP27≒PACAP38≒VIP: 1 nM	cAMP PLC Ca^{2+}	CNS (partial), Adrenal gland, Liver, Respiratory system, Kidney, Intestine, Thymus
VPAC2	–	PACAP27≒PACAP38≒VIP: 1 nM	cAMP Ca^{2+}	CNS (wide-ranging), Pancreatic beta islet, Respiratory system, Stomach, Spleen, Tymus, Testis

third intracellular loop of the receptor. In contrast, no splice variants seem to exist for VPAC1 and 2 receptors. PACAP and VIP have approximately equal affinity for VPAC1 and VPAC2 receptors (which are 49% identical in amino acid sequence); however, PACAP has about 1,000 times higher binding activity than VIP toward PAC1 receptors. These VIP/PACAP receptors are encoded by different genes and expressed in different cell populations in both the central nervous system and peripheral tissues (Solano et al. 2001). PAC1 receptor is mainly localized in the central nervous system (CNS) and the anterior pituitary, whereas VPAC receptors are generally concentrated in peripheral tissues such as the intestine, adrenal gland, liver, and kidney (Shivers et al. 1991). Although the expression of PAC1 receptor messenger RNA (mRNA) in the CNS was found to be much higher than those of VPAC1 and VPAC2 receptor mRNA (Basille et al. 2000), the expression of VPAC2 receptor mRNA has been confirmed in brain tissues, including the hypothalamus and hippocampus (Hashimoto et al. 1993; Usdin et al. 1994). VPAC1 receptor mRNA has been found to be abundant in lung and T lymphocytes, and VPAC2 receptor is found predominantly in the smooth muscle layers and the basal part of the mucosal epithelium in the lung.

Signal transduction of VIP/PACAP receptors

VIP/PACAP receptors are preferentially coupled to $G_{\alpha s}$ proteins that stimulate adenylate cyclase activity. It has been shown that VIP induces the translocation and degradation of the α subunit of Gs protein (Yajima et al. 1998). PAC1 and VPAC1 receptors can stimulate, in addition, inositol triphosphate synthesis and calcium mobilization (Solano et al. 2001). Thus, VIP and PACAP exert their biological functions in the central and peripheral nervous systems via the stimulation of various protein kinases, including the phospholipase C/protein kinase C (PKC), the mitogen-activated protein (MAP) kinase, and the cGMP-dependent protein kinases pathways, as well as the adenylate cyclase/protein kinase A (PKA) pathway (Vaudry et al. 2000). There appears to be some differences in the activating signaling pathway among ligands, depending on which receptor has been activated. PAC1 receptor activation has been found to induce the above-mentioned signaling transduction mechanisms to various degrees (Filipsson et al. 2001). VPAC1 receptor seems to activate adenylate cyclase/PKA and phospholipase C/PKC signaling pathways, whereas ligand binding to VPAC2 receptor predominantly leads to stimulate adenylate cyclase/PKA. The main signal transduction pathway involved would depend on the cellular level of activation and/or differentiation, the predominant receptor subtype expressed, and

the use of cell lines. By activation of these signaling pathways, VIP shows a variety of biological functions in many tissues (Said 1991; Delgado et al. 1999b; Onoue et al. 2001a; Abad et al. 2006; Onoue et al. 2007).

Conformational analyses of VIP and related peptides

The study on the SAR of VIP is very important to characterize its interaction with the receptors and to modulate its stability and affinity. Moreover, to support SAR investigations, several efforts have been made to identify the preferred conformation of VIP and related peptides with various techniques such as nuclear magnetic resonance (NMR; Goossens et al. 1996) and circular dichroism (CD) spectral analyses (Haghjoo et al. 1996; Onoue et al. 2004d), and computational evaluation (Filizola et al. 1997). In general, after initial CD studies, a standard strategy was followed using homonuclear 2D ^{1}H NMR techniques [correlation spectroscopy (COSY), total correlation spectroscopy (TOCSY), and nuclear overhauser enhancement spectroscopy (NOESY)], under various solution conditions, to identify amino acid spin systems and give sequence-specific assignments (Wuthrich 1989). Secondary structure regions were initially deduced from the difference in Hα chemical shifts from their random coil values and qualitative nuclear overhauser effect (NOE) data. Detailed structures at the atomic level were then calculated from qualitative NOE data, which were used as distance restraints in molecular dynamics and energy minimization calculations (Wuthrich 1998). Structural information has been used to guide the design of new analogues and mimetic versions of VIP. As shown in Table 1, all glucagons–secretin family peptides are short linear peptides consisting of approximately 30 amino acid residues, and they show significant sequence homology and even a secondary structure, featuring random coil structures at the N terminus and long α-helical structures at the C terminus (Clore et al. 1988; Wray et al. 1993; Thornton and Gorenstein 1994; Blankenfeldt et al. 1996; Wray et al. 1998; Onoue et al. 2004b). Of all glucagons–secretin family peptides, PACAP27, a PAC1 receptor agonist, has the highest sequence homology, as much as 68%, with VIP, and helodermin, a VPAC2 receptor agonist, also exhibit high structural similarities with VIP. Previous structural analyses, including circular dichroism (CD), nuclear magnetic resonance (NMR) and theoretical calculations, suggest remarkable similarities in secondary structures among the glucagon/secretin peptide family (Onoue et al. 2004b). With respect to the secondary structure of VIP, numerous conformational studies, including spectral and computational analyses, have characterized that VIP possesses two

segments of secondary structures: a random coil structure in the N-terminal region containing approximately ten amino acid residues between positions 1 and 9, and a long α-helical structure in the C-terminal region stretching from position 10 to its C terminus (Bodanszky et al. 1974; Robinson et al. 1982; Bodanszky and Bodanszky 1986). As VIP acts through the interaction with two subclasses of G protein-coupled receptors, VPAC1 and VPAC2, it would be necessary to provide an artificial hydrophobic environment that mimics the vicinity of the peptide/receptor interface when performing conformational studies on VIP. The solvent system of water/alcohol, including methanol and trifluoroethanol, or micelle has often been used as a membrane-mimetic medium (Park et al. 1998; Kobayashi et al. 2000), and α-helical content of VIP dissolved in the water/alcohol system seemed to be much higher than that in water, possibly because of the enhanced intramolecular hydrogen bonding or other interactions (Bodanszky et al. 1974). Thus, α-helical contents estimated by spectroscopic analyses were variable, depending on solvent systems such as pH and polarity; however, N-terminal random coil and C-terminal helical structures were maintained under any conditions tested.

Structure–activity relationships of VIP

In past years, several chemical modification studies were carried out with different techniques, and valuable SAR information about receptor-binding pharmacophore and the stabilization of VIP have been reported (Table 3). Most SAR studies on VIP aimed to produce simplified VIP analogs having high affinity and selectivity for one VIP receptor subtype and enough metabolic stability for the following reasons: (1) VIP has high affinity for both VPAC1 and VPAC2 receptor subtypes and, therefore, does not discriminate between the two VIP receptor subtypes; (2) VIP undergoes rapid degradation in vivo with a half-life of less than 1 min (Hassan et al. 1994). In practice, outcomes from these SAR studies were not limited to a theoretical basis, as a number of VIP receptor agonists or antagonists have been identified and proposed as drug candidates (Tables 3 and 4).

Some groups attempted to identify the backbone and side-chain conformational requirements for interaction at the VIP receptor and to elicit high biological potency. These studies were accomplished by characterizing the in vitro and in vivo pharmacological effects of (1) analogues truncated sequentially at the N and C termini (O'Donnell et al. 1991; Onoue et al. 2004b), (2) analogues systematically modified with single Ala substitutions (O'Donnell et al. 1991; Nicole et al. 2000; Igarashi et al. 2002; Igarashi et al. 2005), and (3) analogues modified with D-amino acid

substitutions (Bolin et al. 1995; Fig. 1). Site-directed modification through single-point Ala substitutions is a useful way to determine the potential binding and biological pharmacophores of peptides and small proteins. Once identified, it should be possible to enhance the biological potency of a specific compound by increasing binding energy at these positions. In SAR studies using Ala scanning to identify the VIP pharmacophore for hVPACs, analogues with Ala replacements in positions Asp^3, Phe^6, Thr^7, Tyr^{10}, Thr^{11}, Tyr^{22}, and Leu^{23} exhibited a significant loss in potency in the bioassay (O'Donnell et al. 1991). These results, taken together with previous findings about the crucial role of His^1 (Bolin et al. 1989), the binding pharmacophore for VIP molecule was thought to be composed of three aromatic/hydrophobic moieties at residues 6, 10, and 22, a negatively charged site at residue 3, and a lone pair structure at residue 1. As shown in Fig. 1, functional analysis of the structure of VIP revealed that residues His^1, Asp^3, Phe^6, Arg^{12}, Arg^{14}, and Leu^{23} interact with the VPAC1 receptor (Igarashi et al. 2002). Nicole et al. (2000) identified His^1, Asp^3, Val^5, Phe^6, Thr^7, Asp^8, Tyr^{10}, Arg^{12}, Arg^{14}, Lys^{15}, Lys^{20}, Lys^{21}, Leu^{23}, and Ile^{26} as being important for binding to human VPAC1 receptor and subsequent stimulation of adenylate cyclase activity. In addition, Igarashi et al. (2005) also reported that a number of amino acids, particularly, Thr^{11}, Tyr^{22}, Asn^{24}, Leu^{27}, and Asn^{28}, were more important for VPAC2 than hVPAC1 affinity.

PACAP27 and VIP displayed similar binding activities toward VPAC1 and VPAC2, whereas the binding affinity of PACAP27 to PAC1 was much higher than that of VIP. To clarify the structural elements of VIP/PACAP for receptor selectivity, our group previously investigated the biological activities and structural/physicochemical properties of VIP/PACAP chimeric peptides (Onoue et al. 1999; Onoue et al. 2001b). Chemical modifications in the N-terminal random coil regions of both VIP and PACAP resulted in significant changes in their biological activities, including receptor-binding activity (Fig. 2) and activation of adenylate cyclase. In CHO cells stably expressing PAC1 short-form receptor, biological activities of $[G^4, I^5]$-VIP increased significantly in comparison with VIP, whereas that of $[A^4, V^5]$-PACAP27 was decreasing compared with PACAP27. Chemical modification of VIP/PACAP27 in the N-terminal region showed no significant influence on the C-terminal helical structure as determined by CD spectral analyses. In contrast, a high degree of similarity between N-terminal structures of PACAP and $[G^4, I^5]$-VIP, and between those of VIP and $[A^4, V^5]$-PACAP was observed (Fig. 2), suggesting that replacement of positions 4 and 5 between PACAP27 and VIP induced mutual conformational conversion. The results demonstrated the crucial role of the N-terminal structure of VIP/PACAP, especially residues

Table 3 Reported SAR information of VIP

SAR information and major outcomes	Report
Binding pharmacophore	
VIP(10–28) as a VPACs-R antagonist (IC$_{50}$: 45 μM)	(Taylor and Pert 1979)
GHRF analog as a VPACs-R antagonist (IC$_{50}$: 1.2 μM)	(Waelbroeck et al. 1985)
[(4Cl)$_D$F^6,L^{17}]-VIP as a VPACs-R antagonist	(Pandol et al. 1986)
Crucial role of His^1 in VIP for receptor binding	(Bolin et al. 1989)
Neurotensin/VIP chimeric peptide as VPACs-R antagonist (IC$_{50}$: 0.7 μM)	(Gozes et al. 1989)
Ala scanning experiment	(O'Donnell et al. 1991)
VIP/PACAP chimeric peptide as PAC1-R agonist (IC$_{50}$: 30 nM)	(Gourlet et al. 1996a)
Substituted VIP/GRF chimeric peptide as VPAC1-R agonist (IC$_{50}$: 1.0 nM)	(Gourlet et al. 1997a)
[R^{16}]-chicken secretin as a VPAC1-R agonist (IC$_{50}$: 60 nM)	(Gourlet et al. 1997b)
Lipophilic VIP analog as a VPACs-R agonist (IC$_{50}$: 1–5 nM)	(Gourlet et al. 1998)
Mapping the active site in VIP	(Gozes et al. 1999)
1) Ala scanning experiments 2) Essential amino acids for VPAC1-R binding	(Igarashi et al. 2002)
Stability improvement & binding pharmacophore	
Cyclic disulfide analog of VIP as a VPACs-R agonist (IC$_{50}$: 2.5 μM)	(Bolin et al. 1993)
1) Ala scan and D-amino acid substitution experiments 2) Metabolically stable analogs of VIP	(Bolin et al. 1995)
Polycationic analogs of VIP/PACAP (IC$_{50}$: 2.5 nM)	(Kashimoto et al. 1996b)
1) Role of Met^{17} for secondary structure and activity 2) Minimum sequence of VIP for VPAC-R binding	(Onoue et al. 2004b)
[R^{15,20,21}, Leu^{17}]-VIP(1–23) as a long-acting VIP analog (IC$_{50}$: 48 nM)	(Onoue et al. 2004d)
Multi-alaninated analogs of VIP as a VPAC1-R agonist (IC$_{50}$: 11.5 nM)	(Igarashi et al. 2005)
Chemical modification of VIP to protect Met^{17} against oxidation	(Stiuso et al. 2006)
Molecular modeling & binding pharmacophore	
Crucial role of Ala^4, Val^5 in VIP for binding selectivity	(Onoue et al. 1999) (Onoue et al. 2001b)
Ala scanning experiment	(Nicole et al. 2000)

4 and 5, for receptor selectivity. These findings were consistent with other reports about the structural biology of PACAP (Sakiyama et al. 1991; Inooka et al. 2001). When replacing the N-terminal region of VIP with that of PACAP, the chimeric peptide, PACAP-(1–15)–VIP-(16–28), showed PACAP-like activity instead of VIP-like activity (Sakiyama et al. 1991). According to Inooka's work, the presence of Gly^4 was indispensable for the formation of the stable type II′ β-turn, possibly maintaining relative orientation between the hydrophobic cluster and N-terminal residues 1–3. Thus, the local main chain conformation of Gly^4 and Ile^5 is important for PACAP-receptor binding, and the weak binding of VIP to the PACAP receptor could be partly attributed to an inability to form a stable β-turn at this position. In addition to SAR investigation of the N-terminal region of VIP/PACAP, our group demonstrated that the binding activities of C-terminally truncated VIP analogues

against lysates from rat lungs that express VPAC receptors were reduced in accordance with the decrease in α-helical contents (Onoue et al. 2004b). Gourlet et al. (1996b) also clarified that the presence of the C-terminal end of VIP is important for its interaction with the VPAC1 receptor. Hence, we conclude that N-terminal regions forming random coil structures in both VIP and PACAP may play a crucial role in the selective recognition of VPAC or PAC1 receptors and that the formation of the α-helical structure in the C terminus of VIP is critical to its binding to VPAC receptors.

Whereas the pharmacological potency of a peptide itself may be of general importance, chemical and physical stability is also a critical factor in the drug design of peptide for overall therapeutic success. In particular, enzymatic digestion, induced by neutral endopeptidase on bronchial epithelial cells, as well as tryptase and chymase

Table 4 Sequences and binding activities of VIP, PACAP27, and VPAC receptors agonists

	Affinity (IC$_{50}$: nM)[a] VPAC1	VPAC2		Sequence (positions 5 · 10 · 15 · 20 · 25 · 30)
Naturally occurring peptides				
VIP	1.6	5.3		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
PACAP27	2.0	10		– – – G I – – – S – S – Y – – – – – – – – – – A A V –
VPAC1 agonists			*Selectivity*	
[A^{11,22,28}]-VIP Nicole et al. 2000	0.9	2,352	2,613	– – – – – – – – – – A – – – – – – – – – – A – – – – – A
[K^{15}, R^{16}, L^{27}]-VIP(1–7)/GRF(8–27) Robberecht et al. 1998	1.0	>30,000	>30,000	– – – – – – N S – R K V L – R L S A R – L – Q D – –
GRF-6 Ito et al. 2001	2.6	>30,000	>11,538	– $_D$A – – I – – A$_{ib}$ A – R K V L A A L A$_{ib}$ A R – A – A A A G$_{ab}$
[R^{16}]-chicken Secretin Robberecht et al. 1998	60	5,000	83	– – – G L – – S E – S K M – G R A Q V Q – F I Q N L M
[Y^9, Dip^{18}]-VIP Tams et al. 2000	0.1[b]	53[b]	482	– – – – – – – Y – – – – – – – Dip – – – – – – – – – –
[A^{2,8,9,11,19,22,24,25,27,28}]-VIP Igarashi et al. 2005	11.5	>30,000	>2,609	
VPAC2 agonists			*Selectivity*	
Ro 25-1392 Xia et al. 1997	>1,000[c]	9.6[c]	>104	acetyl-– – – – – – E – $_m$Y – K – – – – Nle – A – K* – – – D* L K K
Ro 25-1553 Bolin et al. 1995	800	1.0	800	acetyl-– – – – – – E – – – K – – – – Nle – A – K* – – – D* L K K G G T
BAY 55-9837 Tsutsumi et al. 2002	8,700	60	145	– – – – – – – – – – – – – – – V – A – – – – Q – – K – K R Y
BAY(Q9Q28C32)PEG43 Pan et al. 2007	>10,000	200	>50	– – – – – – – Q – – – – – – V – A – – – – Q – – K Q K R Y C-PEG(43kDa)
PG 96-277 Moreno et al. 2000	1,000	3.0	333	acetyl-– – – – – – E – – – K – – – R Nle – A – Nle – – – N L K K G G T
Nonselective agonists				
stearyl-[Nle^{17}]-VIP Gourlet et al. 1998	5	1		stearyl-– – – – – – – – – – – – – – – – Nle– – – – – – – – – – –
[R^{15,20,21}, L^{17}]-VIP-GRR Onoue et al. 2004a	1.4			– – – – – – – – – – – – – – R – L – – R R – – – – – – G R R
[R^{15,20,21}, L^{17}]-VIP(1–23) Onoue et al. 2004d	48			– – – – – – – – – – – – – – R – L – – R R – –

All peptides are amidated at the C terminus.

Aib α-Aminoisobutyric acid, *Gab* γ-aminobutyric acid, *Dip* β,β-diphenyl-L-alanine, and *mY* methoxy-tyrosine

[a] Binding activity toward human VPAC receptors, except for [Y^9, Dip^{18}]–VIP (rat VPACs), [R^{15,20,21}, L^{17}]–VIP–GRR and [R^{15,20,21}, L^{17}]–VIP (1–23) (rat lung).

[b] Ki values

[c] Kd values

[d] Side-chains of Lys^{21} and Asp^{25} in Ro25-1392 and Ro25-1553 form lactam ring

derived from mast cells, could be significant. The blood half-life of VIP was found to be less than 1 min in rats (Hassan et al. 1994) and approximately 1 min in humans (Domschke et al. 1978). Herein, the rapid degradation of VIP after systemic administration is part of the reason for the limitation of its clinical applications. Therefore, for the clinical application of VIP as a therapeutic agent, a metabolically stable analogue of VIP needs to be developed, and many SAR studies focusing on VIP stabilization

have been carried out (Table 3). According to comprehensive researches on the enzymatic cleavage and metabolism of VIP, VIP was found to be easily hydrolyzed by several enzymes, including neutral endopeptidase, mast cell chymase, and mast cell tryptase, and two primary cleavage sites on the VIP molecule were identified as amide bonds between Ser at 25 and Ile at 26, and Thr at 7 and Asp at 8 (Bolin et al. 1992; Lilly et al. 1993). After the identification of major cleavage sites, this information was

Fig. 1 Summary of binding pharmacophores of VIP. Pharmacophoric amino acid residues (*circles*) were deduced by several authors with different techniques and purposes
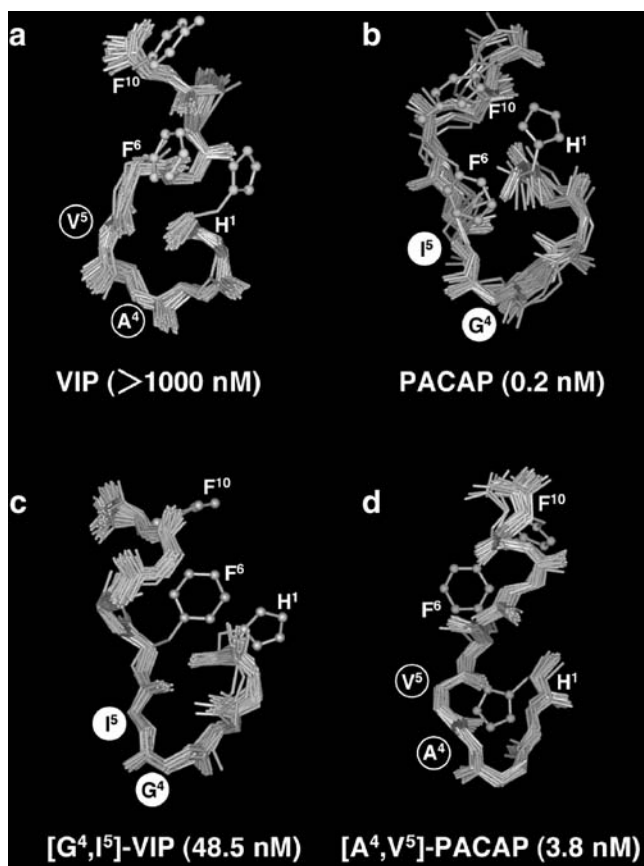
Fig. 2 Solution structures of VIP/PACAP N-terminal analogues determined by NMR spectral analysis and molecular simulation. **a** VIP, **b** PACAP, **c** [G^4, I^5]–VIP, and **d** [A^4, V^5]–PACAP. The 50 structures sampled during the last 100 ps of molecular dynamics at 313 K were superimposed so as to minimize root-mean-square deviation values of the backbone atoms N, C, C′, O and NH. IC$_{50}$ values, shown in *parentheses*, were established from inhibition curves of tracer binding of [^{125}I]–PACAP27 for PAC1 receptor by increasing concentrations of unlabelled peptides

used to synthesize cyclic peptides that incorporated disulfide and lactam ring structures (Bolin et al. 1993; Bolin et al. 1995). With respect to the stabilization of VIP against enzymatic cleavage, our group previously performed chemical modification studies by using a lot of synthetic VIP analogues. Based on the data obtained, the replacement of Lys residue at positions 15, 20, and 21 by Arg in VIP resulted in a significant improvement in metabolic stability (Kashimoto et al. 1996a; Kashimoto et al. 1996b; Onoue et al. 2004d). In addition to proteolytic degradation, Met at 17 is easily oxidized followed by conformational changes and disruption of the helical structure, and a significant decrease of biological activities was observed after oxidation of Met^{17} (Onoue et al. 2004b). Therefore, chemical modifications at Met^{17} have been proposed to improve the stability of VIP by (1) replacement of Met by Leu (Kashimoto et al. 1996b; Onoue et al. 2004b) and (2) Gln^{16}-diaminopropane derivative (Stiuso et al. 2006).

Development of potent VIP receptor agonists

VIP has been considered a pharmaceutical agent candidate for several diseases, and clinical interest in developing novel VIP receptor agonists has recently been highlighted. Because of diverse actions of VIP on various receptor subtypes, considerable effort has been made to design peptide analogues that are selective for receptor subtypes. Whereas directed approaches to substitute or alter specific amino acids have been attempted, Ala and D-amino acid scanning has resulted in the design of stable and highly selective novel analogues.

So far, mainly three types of VIP receptor agonists have been proposed, including VPAC1 agonists, VPAC2 agonist, and nonselective agonists (Table 4). First, as VPAC1 selective agonists, [A$^{11, 22, 28}$]-VIP (Nicole et al. 2000), [K^{15}, R^{16}, L^{27}]-VIP(1–7)/GRF(8–27) (Robberecht et al. 1998), [$_D$A^2, Aib^{8,18}, A$^{9,15,16,22,24–26}$, Gab^{27}]-hGRF(1–27) (GRF-6) (Ito et al. 2001), [R^{16}]-chicken Secretin (Robberecht et al. 1998), [Y^9, Dip^{18}]-VIP (Tams et al. 2000), and [A^{2,8,9,11,19,22,24,25,27,28}]-VIP (Igarashi et al. 2005) were designed on the basis of many SAR studies, and they, especially [K^{15}, R^{16}, L^{27}]-VIP(1–7)/GRF(8–27), exhibited specific and high affinity for human or rat VPAC1 receptor. Second, two classes of VPAC2 receptor selective agonists have been reported: (1) cyclic derivatives of VIP with several mutations and extended basic C terminus, such as acetyl-[E^8, K^{12,27,28}, Nle^{17}, A^{19}, D^{25}, L^{26}, G^{29,30}, T^{31}]-VIP(cyclo 21–25) (Ro 25–1553) (Bolin et al. 1995) and acetyl-[E^8,Ome-Y^{10}, K^{12,27,28}, Nle^{17}, A^{19}, D^{25}, L^{26}]-VIP(cyclo 21–25) (Ro 25–1392) (Xia et al. 1997), and (2) linear analogues including [V^{17}, A^{19}, Q^{24}, K^{27,29}, R^{30}, Y^{31}]-VIP (BAY 55–9837) (Tsutsumi et al. 2002), acetyl-[E^8, K^{12,27,28}, R^{16}, Nle^{17,21}, A^{19}, N^{25}, L^{26}, G^{29,30}, T^{31}]-VIP (PG 96–277) (Moreno et al. 2000), and long-acting PEGylated derivative, [Q^{9,24,28}, V^{17}, A^{19}, K^{27,29}, R^{30}, Y^{31}]-VIP-C-PEG(43 kDa) (Pan et al. 2007). They displayed high selectivity for VPAC2 over VPAC1 and PAC1 in receptor-binding and functional assays; in particular, Ro 25–1553 was found to be a potent VPAC2 receptor agonist because of its highest selectivity (IC$_{50}$ $_{VPAC1}$/IC$_{50}$ $_{VPAC2}$ ratio, 800) and affinity (IC$_{50}$, 1.0 nM) toward VPAC2 receptor. Third, nonselective VIP receptor agonists, stearyl-[Nle^{17}]-VIP (Gourlet et al. 1998), [R$^{15, 20, 21}$, L^{17}]-VIP-GRR (IK312532) (Onoue et al. 2004a), and [R$^{15, 20, 21}$, L^{17}]-VIP (1–23) (Onoue et al. 2004d) have been proposed, and they displayed attractive biological functions.

For VIP-based clinical treatments, several early studies, focusing on the improvement of stability, have been attempted to create potent drug candidates (Bolin et al. 1995; Xia et al. 1997). Chemical modification studies clarify the best multiple-substitution sites with potent cyclic peptide. Cyclic compounds, Ro25-1553 and Ro25-1392, consisting of 31 and 28 amino acids, respectively, are

highly selective for VPAC2 receptor, and they exhibited exceptionally high potency, metabolic stability, and a long duration of action (Bolin et al. 1995; Xia et al. 1997). Our group previously reported that polycationic VIP derivative, [R[15, 20, 21], L[17]]-VIP, displayed a significant improvement in metabolic stability as compared to VIP (Kashimoto et al. 1996a). [R[15, 20, 21], L[17]]-VIP showed a more sustained relaxation of rat isolated bronchial smooth muscles in vitro compared with VIP. In addition, Ito and Tachibana (1991) reported that C-terminally extended VIP derivative exhibited higher biological activity than natural VIP in vitro and in vivo. These findings led us to the successful development of a VIP analogue, IK312532; [R[15, 20, 21], L[17]]-VIP-GRR, as a long-acting bronchodilator (Onoue et al. 2004a). Interestingly, IK312532 showed much higher stability against enzymatic digestion and even higher biological activities (Onoue et al. 2004a; Ohmori et al. 2006). In addition to IK312532, further investigation, together with our previous findings on the minimal sequence of VIP for receptor binding, also resulted in the successful development of a novel shortened and stable VIP analogue, [R[15, 20, 21], L[17]]-VIP(1–23) (Onoue et al. 2004d).

Potential clinical application of VIP and analogues

Recent progress in our knowledge of the biological functions and mechanisms of action of VIP opened a new field of therapeutic possibilities for the treatment of multiple pathologies (Gozes and Furman 2003). In practice, the use of VIP and its derivatives as therapeutic agents has been suggested for erectile and female sexual dysfunction (Dinsmore et al. 1999), skin pathologies (Granoth et al. 2000), primary pulmonary hypertension (Petkov et al. 2003), heart failure (Saetrum Opgaard et al. 2001), acute lung injury (Said and Dickman 2000), type 2 diabetes (Tsutsumi et al. 2002), gastrointestinal motility disorders (Biancani et al. 1988), and nervous system pathologies of various nature (Abad et al. 2006). In addition, VIP and analogues have been believed to be effective in various inflammatory diseases because VIP acts as an endogenous anti-inflammatory mediator by altering the balance of Th1/Th2 cells and pro-/anti-inflammatory cytokines (Pozo 2003). In this context, VIP and analogues have recently arisen as promising candidates for the treatment of inflammatory pathologies such as septic shock (Delgado et al. 1999a), rheumatoid arthritis (Delgado et al. 2001), Crohn's disease (Abad et al. 2003), and multiple sclerosis (Kato et al. 2004).

PAC1 and VPAC2 are expressed in pancreatic islets, and both PACAP and VIP have been shown to increase insulin secretion from the pancreas (Filipsson et al. 1998). Although the effect of PACAP on insulin secretion is much

higher than VIP in vitro, glucagon secretion in vivo is also stimulated in mice, dogs, and humans (Filipsson et al. 2001). Furthermore, the administration of PACAP to either mice or humans has been reported to increase plasma insulin levels without affecting plasma glucose levels. The functional role of PACAP in the control of glucose homeostasis is complex because it also plays a role in increasing glucagon and catecholamine secretion, leading to the increase of glucose output from the liver. The expression pattern of VPAC1 suggests that activation of this receptor contributes to an increase in hepatic glucose production. Therefore, VPAC2-selective agonist has been believed to be a potential candidate for diabetic drugs and would enhance pancreatic β cell insulin release without causing increased glucose production by the liver. In practice, BAY 55-9837 and its PEGylated derivatives, BAY(Q9Q28C32)PEG22 and 43, were engineered as VPAC2 selective agonists through several rounds of site-directed mutagenesis (Tsutsumi et al. 2002; Pan et al. 2007), and they could stimulate glucose-dependent insulin secretion in vivo and in vitro. In particular, PEGylated BAY 55-9837 would have higher potential and developability as diabetic drugs because they retain significant VAPC2 activity and selectivity, as well as prolonged in vivo activity as compared to BAY 55-9837 (Pan et al. 2007). In addition to the VPAC2-selective agonist, a number of GLP-1 receptor agonists also promote glucose-dependent insulin secretion and have demonstrated glucose-lowering activity in clinical studies (Riddle and Drucker 2006). However, nausea and vomiting are common side effects associated with the inhibition of gastrointestinal motility by GLP-1. It is possible that VPAC2-selective agonists induce similar undesirable side effects because of VPAC2 localization in the gastrointestinal tract. This aspect of VPAC2-selective agonists has not yet been examined and, therefore, needs further study.

VIP is abundantly present in the normal human lung (Paul and Said 1987) and VIP-immunoreactive nerves are present in the smooth muscle layer and glands of the airway, and within the walls of pulmonary and bronchial vessels. Interestingly, VIP deficiency in the respiratory system is considered to be a pathogenetic factor in pulmonary disease (Said 1989). Because of its bronchodilating and anti-inflammatory effects, VIP may be a promising agent to treat pulmonary disease, including asthma (Said 1991) and chronic obstructive pulmonary disease (COPD; Onoue et al. 2004c; Groneberg et al. 2006). Interestingly, PACAP and its derivatives also exhibited potent bronchodilation and anti-inflammatory effects via interaction with VPAC receptors (Sergejeva et al. 2004; Yoshihara et al. 2004). However, the clinical application of VIP might be limited, as VIP is susceptible to rapid chemical and biochemical digestion after systemic admin-

istration, resulting in low potency and short duration of action in clinical application (Takubo et al. 1991). Hence, many chemical modification studies have been attempted to develop potent VIP derivatives with sufficient chemical and biochemical stability. So far, various stabilized VIP analogues, including Ro 25-1392, Ro 25-1553, and IK312532, have the potential to ameliorate asthmatic symptoms (Bolin et al. 1995; Xia et al. 1997; Onoue et al. 2004a). Ro 25-1553 is 24 to 89 times more potent than VIP as a relaxant of the guinea pig trachea, and pulmonary responses evoked by histamine, leukotriene D$_4$, platelet-activating factor, and acetylcholine are inhibited dose-dependently by intratracheally instilled Ro 25-1553 (O'Donnell et al. 1994). IK312532 exhibits longer-lasting bronchodilation and suppression of the antigen-evoked infiltration of granulocytes in the bronchiolar mucosa as compared to VIP, possibly because of higher stability against enzymatic digestion (Ohmori et al. 2004). IK312532 also acts as protective agent against cigarette smoke-induced apoptosis of rat alveolar cells through the activation of PKA and MAP kinase signaling pathways and deactivation of caspase-3, an apoptotic enzyme (Onoue et al. 2004a). Therefore, these analogues have been proposed as candidates of asthma and/or COPD drugs, being in preclinical and clinical stages.

In addition to the stability issue, there is a possible drawback that the systemic administration of VIP may cause moderate hypotension and/or other adverse effects, depending on the rate of infusion (Morice et al. 1983). It is, thus, also required to use an effective drug delivery system for specific delivery of VIP to the target tissue. The development of effective methods to use polypeptides to treat diseases is a significant challenge to the pharmaceutical industry. Serious problems associated with systemic treatment include: poor oral availability because of gastrointestinal peptidases, high intestinal and hepatic first pass metabolism, low stability in the blood, and poor penetration to the desired site of action. Against these challenging problems, the development of efficacious drug delivery systems for VIP has been attempted. Liposomal and PEG-phospholipid micellar formulation for intravenous use have been investigated and proposed to protect VIP from degradation (Suzuki et al. 1995; Ashok et al. 2004). The association of VIP with liposomes is believed to curtail enzyme-induced inactivation of VIP, and the association of VIP with sterically stabilized biocompatible liposome also circumvents peptide inactivation by catalytic VIP antibodies, leading to enhanced vasodilation (Rubinstein et al. 2001). To cross the BBB, several strategies, such as drug lipidization or conjugation to VIP in BBB-targeting molecules, have been proposed (Bickel et al. 1993; Dufes et al. 2004). Dufes and coworkers demonstrated that the administration of systemic glucose-bearing vesicles encapsulating VIP could deliver intact VIP to specific brain areas.

Bickel and coworkers reported successful application of the drug transport vector avidin-OX26 for brain delivery of VIP, and systemic administration of a small dose of VIP resulted in an in vivo central nervous systemic pharmacologic effect. The intranasal route also offers a much simpler approach for brain drug delivery, as the olfactory receptor cells are in contact with both the nasal cavity and the CNS, the connection of which constitutes a direct pathway to the brain (Dufes et al. 2003). In practice, intact VIP could be delivered successfully to the brain using the intranasal route for administration, whereas no intact VIP was found either in the brain or blood by intravenous administration. For the treatment of asthma/COPD, our group previously suggested a dry powder inhalation system of IK312532, the use of which resulted in higher concentrations of the drug in the target organ, the lung, and minimized systemic adverse events (Ohmori et al. 2004; Onoue et al. 2007). Although VIP-based drugs have not yet been used for clinical purposes, these reported DDS, taken together with potent VIP derivatives, might provide efficacious medication in the clinical treatment of cardiovascular, CNS, and pulmonary diseases.

Concluding remarks

VIP receptors have been identified as promising drug targets for several diseases, and some VIP derivatives designed for clinical treatment of type II diabetes, asthma, and COPD are now in preclinical and clinical stages. So far, many SAR studies have been carried out to clarify the structural biology of VIP with several approaches, resulting in the production of many attractive agonists or antagonists of VIP receptors. Although therapy using developed VIP derivatives would offer potential benefits to patients, they are not yet in clinical use. For the clinical application of VIP, studies aimed at developing an appropriate delivery system of VIP will be a key direction of future research, and the combination use of potent VIP analogue and targeted DDS might provide novel insight into the therapeutic potential of VIP and its derivatives.

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