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Themed Section: Secretin Family (Class B) G Protein-Coupled Receptors – from Molecular to Clinical Perspectives

REVIEW

VPAC receptors: structure, molecular pharmacology and interaction with accessory proteins

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The vasoactive intestinal peptide (VIP) is a neuropeptide with wide distribution in both central and peripheral nervous systems, where it plays important regulatory role in many physiological processes. VIP displays a large biological functions including regulation of exocrine secretions, hormone release, fetal development, immune responses, etc. VIP appears to exert beneficial effect in neuro-degenerative and inflammatory diseases. The mechanism of action of VIP implicates two subtypes of receptors (VPAC1 and VPAC2), which are members of class B receptors belonging to the super-family of GPCR. This article reviews the current knowledge regarding the structure and molecular pharmacology of VPAC receptors. The structure–function relationship of VPAC1 receptor has been extensively studied, allowing to understand the molecular basis for receptor affinity, specificity, desensitization and coupling to adenylyl cyclase. Those studies have clearly demonstrated the crucial role of the N-terminal ectodomain (N-ted) of VPAC1 receptor in VIP recognition. By using different approaches including directed mutagenesis, photoaffinity labelling, NMR, molecular modelling and molecular dynamic simulation, it has been shown that the VIP molecule interacts with the N-ted of VPAC1 receptor, which is itself structured as a 'Sushi' domain. VPAC1 receptor also interacts with a few accessory proteins that play a role in cell signalling of receptors. Recent advances in the structural characterization of VPAC receptor and more generally of class B GPCRs will lead to the design of new molecules, which could have considerable interest for the treatment of inflammatory and neuro-degenerative diseases.

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Abbreviations
Bpa, benzophenone; KO, knock-out; N-ted, N-terminal ectodomain; TM, transmembrane domain; VPAC, vasoactive Intestinal Peptide receptor; VPAC1, vasoactive intestinal peptide receptor 1; VPAC2, vasoactive intestinal peptide receptor 2

The vasoactive intestinal peptide (VIP), a ubiquitous neuropeptide

VIP is a ubiquitous neuropeptide of 28 amino acids, discovered in porcine duodenum by Said and Mutt (1970), which is present in central and peripheral nervous systems (Vaudry and Laburthe, 2006). VIP has been more recently identified in immune system where it plays the role of a 'cytokine-like peptide' (Delgado *et al.*, 2001; Gomariz *et al.*, 2001). In agreement with this widespread distribution, VIP is involved in many physiological and pathophysiological processes related to development, growth, cancers, immune responses, circadian rhythms, control of neuronal and endocrine cells and functions of the digestive, respiratory, reproductive and cardiovascular systems (Table 1). VIP belongs to a structural family of related peptides referred to as secretin/VIP family (Figure 1). This family encompasses VIP, pituitary adenylate cyclase activating peptide (PACAP), secretin, growth hormone-releasing factor (GRF), peptide having an histidine residue in N-terminal position and an isoleucine residue in C-terminal position (PHI and its human homolog PHM),

Table 1

Major physiological and pathophysiological actions of VIP

Short-term	Exocrine secretions
	Hormone release
	Muscle relaxation (vasodilator, bronchodilator)
	Metabolism
Long-term	Circadian rhythms
	Growth regulator of whole fetuses and embryonic brain
Other effects	Neuroprotection
	Suppression of inflammation
	Immunomodulation
	Psychiatric disorders
	Effects on cell proliferation in cancer

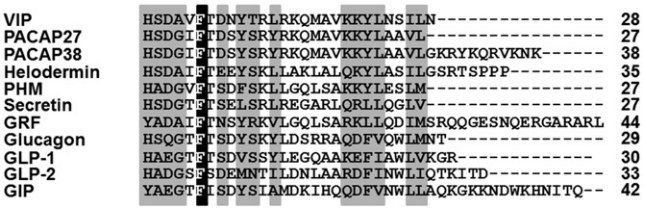

Figure 1

Sequence comparison of peptides of the VIP/secretin family. Sequence identity is represented by a black box, and sequence homologies are represented by grey boxes. Numbers indicate the length of the peptides.

helodermin, glucagon, gastric inhibitory polypeptide (GIP) and glucagon-like peptide 1 and 2 (GLP-1 and GLP-2). These natural ligands share some common properties: (1) they are all peptides with 27 to 44 amino acid residues; (2) they are synthesized and released by endocrine cells, neurons and/or immune cells; (3) they exhibit a marked propensity to form α-helices; (4) they contain a N-Cap structure in the N-terminal part (Neumann *et al.*, 2008), which consists of an hydrophobic cluster between N-terminal hydrophobic residues and an hydrogen bond between two polar residues (Figure 2). All these peptides play an important role in physiological processes and strongly impact on human physiopathology (Table 2). The purpose of this review is to provide a selection of data regarding the current knowledge of the structure and molecular pharmacology of VIP receptors and also their ability to interact with accessory proteins.

The VPAC1 receptor, a class B GPCR prototype

VIP triggers biological responses through interaction with two subtypes of receptors, VPAC1 and VPAC2. VPAC1 and

Table 2

Potential therapeutical interest of natural ligands of class B GPCRs in diseases

Ligand	Diseases	References
VIP	Inflammation	Delgado *et al.*, 2004
	Neurodegeneration	Gozes *et al.*, 2003
PACAP	Neurodegeneration	Gozes *et al.*, 2003
	Inflammation	Abad *et al.*, 2006
GRF	Dwarfism	Campbell *et al.*, 1995
Glucagon	Diabetes	Brubaker, 2007
GLP-1	Diabetes	Brubaker, 2007
GLP-2	Short bowel syndrome	Jeppesen, 2006
GIP	Diabetes	Inzucchi and McGuire, 2008
PTH	Osteoporosis	Epstein, 2007
Calcitonin	Osteoporosis	Mulder *et al.*, 2006
CRF	Stress	Gilligan and Li, 2004

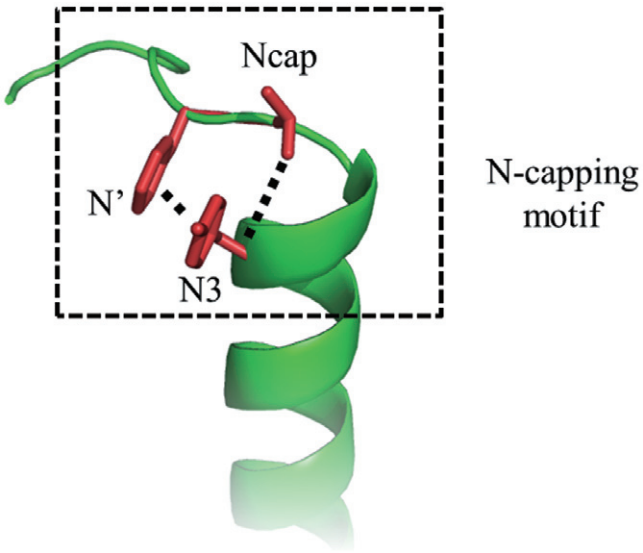

Figure 2

Generic representation of N-capping motif of class B GPCRs peptide ligands. The N-capping motif (type IA) is represented as (1) the hydrophobic interactions between side-chain groups of N′ and N3 residues (dashed lines); (2) the hydrogen bond between side chain of Ncap residue and backbone atom of N3 residue. See Neumann *et al.* (2008) for details.

VPAC2 receptors bind with the same-affinity VIP and the other neuropeptide PACAP. These two receptors subtypes are mainly coupled to the G-protein Gs and stimulate cellular adenylyl cyclase activity (Laburthe *et al.*, 2007). It should be noted that some groups have reported the ability of VIP to

increase calcium levels in different cells (Dickson and Finlayson, 2009). This may be related to the fact that the VPAC1 receptor is able to interact with RAMPs (receptor activity-modifying proteins), in particular RAMP2, inducing a significant enhancement of agonist-mediated inositol trisphosphate production and subsequent effect on cellular calcium without affecting the coupling to adenylyl cyclase (Christopoulos *et al.*, 2002). A previous report indicated that the VPAC1 receptor is able to homodimerize and heterodimerize with VPAC2 or secretin receptors (Harikumar *et al.*, 2006). However, the relation between receptor oligomerization and the ability of VPAC receptor to trigger biological responses remains conjectural.

During the nineties, VPAC receptors were cloned (Lutz *et al.*, 1993; Couvineau *et al.*, 1994) as were many other receptors for peptides of the VIP/secretin family (Couvineau *et al.*, 2010). These studies revealed the existence of a new GPCR subfamily named class B GPCR or class II GPCR or also 'secretin-like' receptors family. This small GPCR subfamily shares with the other GPCR classes the same general structural scheme with the presence of seven-transmembrane helices denoted as TM I to TM VII, which are interconnected by extracellular and intracellular loops (Fredrikson and Schiöth, 2006). The class B receptor family comprises 15 members including receptors for VIP, PACAP, secretin, glucagon, glucagon-like peptide-1, glucagon-like peptide-2, GRF, GIP and also includes receptors for peptides that have no sequence homology with VIP, including parathyroid hormone, calcitonin, calcitonin gene-related peptide and corticotropin-releasing factor (CRF) (Laburthe *et al.*, 2007). The natural ligands of these class B GPCRs strongly influence human physiopathology and have been proposed as a promising candidates for the treatment of several diseases (Table 2).

Class B receptors have low sequence homologies with other members of the GPCR superfamily (Laburthe *et al.*, 2007) and share several specific properties: (i) the presence of a large (>120 residues) and structured N-terminal ectodomain (N-ted), which is usually small in most class A GPCRs, the prototypes of which are rhodopsin and adrenergic receptors. The N-teds contain six highly conserved cysteine residues connected by three disulfide bridges, representing a signature of class B GPCRs. The N-ted of each class B receptor represents the major binding site for its cognate natural peptide ligand. (ii) The presence of a signal peptide probably involved in insertion of receptor in plasma membrane. (iii) The absence of archetypical class A GPCR motifs such as E/D-R-Y or NP-xx(x)-Y. (iv) A complex gene organization with many introns (Laburthe *et al.*, 2007).

Cloning (Couvineau *et al.*, 1994) of the human VPAC1 receptor allowed its extensive studies by site-directed mutagenesis and molecular chimerism (Laburthe *et al.*, 2007), providing new insights into the molecular basis of interaction of VIP with its receptor in terms of (1) affinity (Couvineau *et al.*, 1995); (2) specificity (Couvineau *et al.*, 1996a,b; Du *et al.*, 2002); (3) cellular addressing of the receptor to the plasma membrane (Couvineau *et al.*, 1996a,b); (4) desensitization of the receptor (Marie *et al.*, 2003); (5) association of the VPAC1 receptor with RAMP proteins (Christopoulos *et al.*, 2002); (6) coupling to adenylyl cyclase (Couvineau *et al.*, 2003). These studies revealed that the receptor N-ted plays a

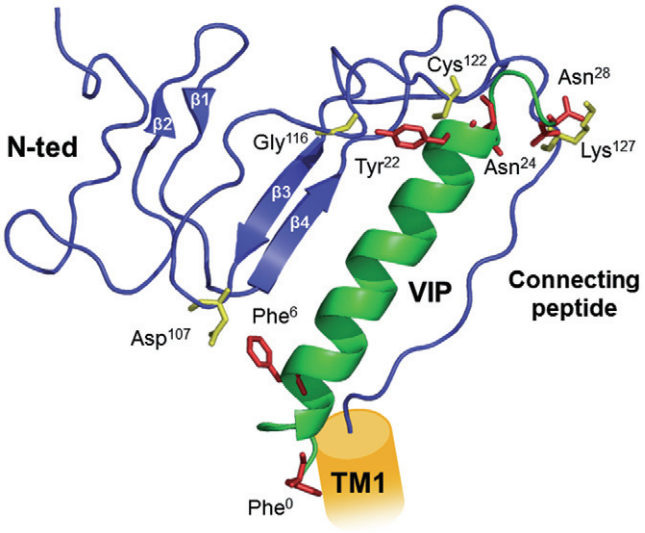

Figure 3

Structural model of VPAC1 receptor N-ted and docking of VIP. The figure shows a ribbon representation of receptor N-terminal ectodomain (N-ted; sequence 44–137) and docking of VIP. VPAC1 receptor N-ted: *blue*, main chain; VIP is shown in *green*. Photoaffinity experiments showed that Asp^{107}, Gly^{116}, Cys^{122}, Lys^{127} (*yellow*) in the N-ted are in contact with the side chains of Phe^{6}, Tyr^{22}, Asn^{24}, Asn^{28} and Phe° (see Laburthe *et al.*, 2007 for details) in VIP (*red*) respectively. The connecting peptide is an 8 amino acid sequence connecting the receptor N-ted to the first transmembrane domain (TM1) of the receptor. The structure of the connecting peptide is currently unknown.

crucial role in peptide agonist binding (Laburthe *et al.*, 2007). The structure–function relationship of VIP has been analysed in details by a complete alanine scan (Nicole *et al.*, 2000), showing that the peptide has a diffuse pharmacophoric domain. The N-terminal 1–5 segment plays a crucial role in activation of adenylyl cyclase. Recently, we have identified a common structural motif that is encoded in all class B GPCR ligand N-terminal sequences (Neumann *et al.*, 2008). This structural motif named N-cap was suggested to be involved in receptor activation and could serve as a template for rational design of drugs targeting VPAC receptors and more generally class B GPCRs (Neumann *et al.*, 2008).

The physical sites of interaction between VIP and its VPAC1 receptor remained elusive until the development of photoaffinity experiments showing that the side chains of VIP in position 6, 22, 24 and 28 are in direct contact with different amino acids of the receptor N-ted, for example Asp^{107}, Gly^{116}, Cys^{122} and Lys^{127} (Figure 3) respectively (Tan *et al.*, 2003; 2004; 2006; Ceraudo *et al.*, 2008). Elucidation of VIP structure by NMR revealed that most of the 28 amino acid sequence has an α-helical structure (sequence 7–28) with the exception of the N-terminal 1–5 sequence, which has no defined structure in solution when unbound to the receptor (Figure 3). The development of a structural model of the VPAC1 receptor N-ted has made it possible to localize the binding site of VIP. The N-ted structure contains two antiparallel β sheets and is stabilized by three disulphide bonds between residues Cys^{50} and Cys^{72}, Cys^{63} and Cys^{105} and Cys^{86}

and Cys[122], and by a putative salt bridge involving Asp[68]-Arg[103], sandwiched between the aromatic rings of Trp[73] and Trp[110] (Figure 3). The NMR structure of VIP has been docked in the VPAC1 receptor N-ted, giving rise to a valid model in which the N-ted nicely accommodates the VIP molecule, at least for the 6–28 sequence (Figure 3). This model has been submitted to molecular dynamic simulations over 14 ns in a box of water and appears to be highly stable (Ceraudo *et al.*, 2008). As discussed above, the structure of the VIP N-terminal segment (1–5) is disordered in solution (Tan *et al.*, 2004), and the VPAC1 receptor domain involved in the recognition of VIP N-terminus is still unknown. To address this issue, we have recently developed a new VIP photoaffinity probe in position 0 (Bpa°-VIP). Photoaffinity labelling experiment and peptide mapping analysis using this probe reveal that the VIP N-terminus physically interacts with N-ted corresponding to the receptor domain connecting the N-ted and the first transmembrane helix (Figure 3). This finding is in good agreement with a speculative, but largely accepted, mechanism for peptide–ligand interaction with class B GPCRs, which is referred to a 'two-domain' model (Hoare, 2005). In this model, the central and C-terminal parts of the peptide are trapped by the N-ted, which exposes the N-terminus of the peptide ligand in an appropriate orientation for interaction with the transmembrane region of the receptor (Hoare, 2005). It is clear that the N-capping signature in the VIP N-terminus may contribute to activation of adenylyl cyclase by peptide His[1] into the receptor (Neumann *et al.*, 2008).

Structures of different recombinant N-teds of class B GPCRs such as gastric inhibitory polypeptide receptor (GIPR), parathyroid hormone receptor (PTHR), corticotropin-releasing hormone receptor 1 and 2 (CRF1R and CRF2R, respectively), glucagon-like peptide-1 receptor (GLP-1R) and pituitary adenylate cyclase-activating peptide receptor (PAC1R) have been obtained recently by X-ray crystallography or NMR spectroscopy (Parthier *et al.*, 2009). These studies seem to indicate the existence of two different binding sites for ligands in N-teds of class B receptor (Couvineau *et al.*, 2010). Analysis of these structure and/or molecular models revealed that N-teds of GIPR, PTHR, CRF1R, CRF2R and GLP-1R interact with ligands in regions encompassing the loop located between β1 and β2 sheets and the loop located between β3 and β4 sheets (Parthier *et al.*, 2009). In contrast, the N-teds of PAC1R and VPAC1R bind peptides along β3 and β4 sheets of the sushi domain (Couvineau *et al.*, 2010). However, a recent report based on the X-ray crystallography analysis of PAC1 receptor N-ted and the docking of PACAP indicates that PACAP could interact with its receptor as GIPR, PTHR, CRF1R, CRF2R and GLP-1R (Kumar *et al.*, 2011). The real significance of these differences were unclear but may be tentatively related to the following interpretations: (1) Some structural determinations were carried-out in presence of ligands that have a low affinity (micromolar range) for the recombinant N-ted, whereas in other studies, ligand affinity is higher. It could be hypothesized that low and high affinity binding occur at different sites in the N-ted structure. (2) The determination of interaction between N-teds and ligands was mainly obtained in the presence of antagonist, but it some cases in the presence of an agonist. It could be hypothesized that agonists and antagonists bind to different domains in the N-teds. (3) Finally, we cannot exclude the possibility that ligands can bind by two different ways to N-ted of class B GPCR.

VPAC1 receptors and accessory proteins

VPAC receptors are able to increase intracellular concentration of cAMP by coupling to adenylyl cyclase through a Gs-protein, whereas some groups have reported the ability of VIP to increase calcium levels in different cells (Dickson and Finlayson, 2009). Besides their coupling with G-proteins, GPCRs can also interact with many non-G-proteins, which have been named 'accessory proteins', 'receptor-interacting proteins' or 'GPCR interacting proteins (GIPs)' (Bockaert *et al.*, 2004). During the nineties, it became clear that GPCRs are also able to interact with non-G-proteins and to transduce signals independently of the G-proteins. A new concept of GPCR signalling was born, and various accessory proteins were found. A large new family of accessory proteins has been discovered displaying many biological functions such as modulation of receptor signalling, receptor targeting, receptor trafficking or compartmentalization, and even modulation of the pharmacological profile of receptors. Recruitment of accessory proteins by GPCRs is mainly ensured by specific sequence motifs in GPCRs, which cause recognition by accessory proteins (Bockaert *et al.*, 2004). However, some accessory proteins interact with GPCRs through highly degenerate sequences or as yet unidentified motifs. The nature of accessory proteins is diverse. Indeed, the 'accessory protein' group encompasses various families of proteins (Brady and Limbird, 2002) such as receptor-activity-modifying proteins (RAMPs), PDZ (acronym of 'PSD-95, Disc-large and ZO-1') domain-containing proteins, cytoskeletal proteins, chaperone molecules or kinases (in particular the 'kinase anchoring proteins (AKAPs)').

As mentioned above, VPAC1 but not VPAC2 is able to interact with RAMPs. RAMPs are a family of three sub-types (RAMP 1, 2 and 3) of single transmembrane proteins that heterodimerize with GPCRs (Sexton *et al.*, 2009). RAMPs are involved in regulation of glycosylation and trafficking of receptors and may drive the pharmacological profile of some GPCRs. The first observation indicating that RAMPs are able to modulate the cellular localization and the function of GPCRs was obtained from pharmacological studies of calcitonin-like receptor (CLR) where RAMPs are able to modify the pharmacological profile of CLR (Sexton *et al.*, 2009). Overexpression of VPAC1 receptor and RAMPs in CHO cells induces the translocation of RAMP1, 2 and 3 to the plasma membrane, suggesting that VPAC1 receptor physically interact with RAMPs (Christopoulos *et al.*, 2002). VPAC1 receptors are mainly coupled to the activation of adenylyl cyclase (Laburthe *et al.*, 2007). However, in the presence of RAMP2, VPAC1 is able to induce a selective increase of phosphoinositide hydrolysis without altering cAMP production (Christopoulos *et al.*, 2002). It should be noted that the functional role, if any, of VPAC1/RAMP1 and VPAC1/RAMP3 complexes remains unclear.

Sequence analysis of VPAC1 and VPAC2 receptors indicates the presence of a class I PDZ domain consensus

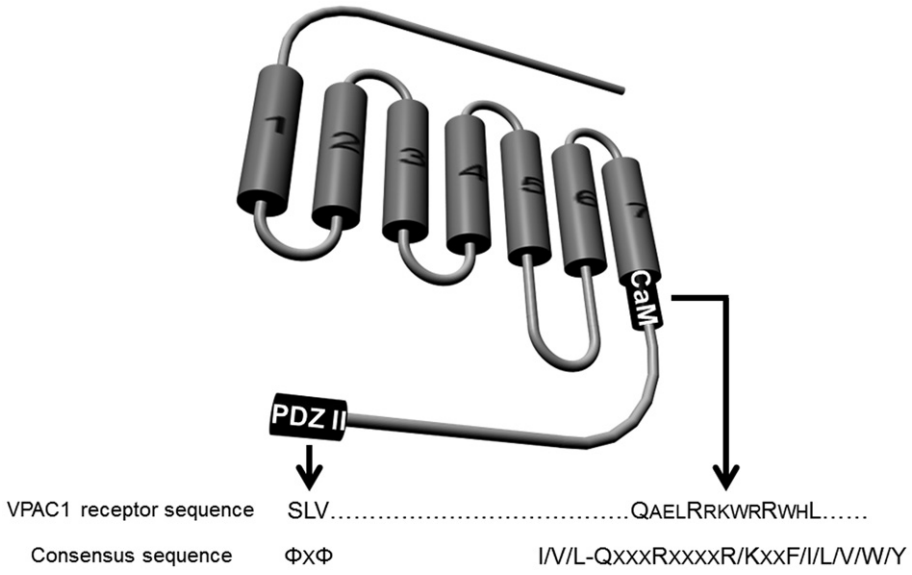

Figure 4

Schematic representation of the localization of interacting sites of accessory proteins in C-terminal tail of the VPAC1 receptor. *PDZ II*, class II PDZ (acronym of 'PSD-95, Disc-large and ZO-1') site; *CaM*, calmodulin site; *Φ*, represents a hydrophobic residue.

sequence in the last residues (Figure 4) of the receptor C-tail (SLV for VPAC1 and SVI for VPAC2 receptor). PDZ domains are repeated sequences and represent ubiquitous protein–protein interaction domains comprising about 70 to 90 residues (Kurakin *et al.*, 2007). These PDZ domains are present in multiple copies within proteins. Interaction of PDZ domains with receptors involves the three to four last residues of the C-terminal tails. Recognition consensus sequences are classed in two groups, that is class I PDZ (E/D-S/T-x-L/V/I) and class II PDZ (Φ-x- Φ), where Φ is any hydrophobic residue. About 400 different PDZ domains could be present in humans or mice (Beuming *et al.*, 2005). Recently, a yeast two-hybrid assay using the carboxy terminus of VPAC1 has revealed that the PDZ domain of S-SCAM (synaptic scaffolding molecule), also named membrane-associated guanylate kinase inverted-2 (MAGI-2), is able to bind to the VPAC1 receptor C-tail (Gee *et al.*, 2009). S-SCAM/MAGI-2 protein belongs to the membrane-associated guanylate kinase (MAGUKs) family of proteins present in junctional area where they are involved in attachment of adhesion molecules, receptors and intracellular signalling enzymes (Sheng and Sala, 2001). The guanylate kinase domain of mammalian MAGUKs is catalytically inactive but presents a Scr homology-3 (SH3) domain and a PDZ domain involved in protein–-protein interactions (Sheng and Sala, 2001). S-CAM recruits VPAC1 receptor to the junctional area near the apical end of lateral membranes of the colonic cancer cell line T84 (Gee *et al.*, 2009). This recruitment results in inhibition of cAMP production induced by VIP, an inhibition of agonist-induced internalization of VPAC1 and a decrease of the VPAC1-mediated current through the cystic fibrosis transmembrane conductance regulator (CFTR) in *Xenopus* oocytes (Gee *et al.*, 2009).

Besides the role of RAMPs and/or S-SCAM interaction with VPAC1 receptors, one report describes the possible interaction between calmodulin and this receptor (Mahon and Shimada, 2005). Calmodulin (CaM) is a ubiquitous calcium-sensing protein that regulates many intracellular proteins such as cytoskeletal elements, ion channels, kinases or phosphatases and various enzymes involved in GPCR signalling such as adenylyl cyclase, phosphodiesterase and phospholipases. CaM interacts with receptors through a degenerate motif (I/V/L-QxxxRxxxxR/Kxx-F/I/L/V/W/Y) in which hydrophobic and basic residues are crucial. An approach based on GST pull-down assay using GST-CaM, and recombinant C-tails of VPAC1 receptors (Figure 4) demonstrated a robust interaction between CaM and VPAC1 receptors (Mahon and Shimada, 2005). The functional role of this interaction still remains to be elucidated.

VIP, a promising therapeutic agent

A few years ago, VIP was identified as a potential therapeutic agent for various diseases including asthma (Groneberg *et al.*, 2001), sexual impotence (Fahrenkrug *et al.*, 1989), brain strokes (Dogrukol-Ak *et al.*, 2004), chronic inflammation (Delgado *et al.*, 2004), neuro-inflammation (Dejda *et al.*, 2005), septic shock (Delgado *et al.*, 2004) and cancers (Moody and Gozes, 2007). From these important physiopathological processes, anti-inflammatory and neuroprotective actions of VIP represent two major promising therapeutic uses of the peptide.

VIP appears to be a very potent anti-inflammatory peptide in animal models of various chronic inflammatory diseases (Table 3). This effect is mediated by modulation of T-helper balance by suppressing Th1 immune responses (Delgado *et al.*, 2004). VIP inhibits leucocyte activation and migration, decreases NF-κB activation and expression of pro-inflammatory cytokines and chemokines (Gomariz *et al.*, 2001). Although anti-inflammatory properties of VIP have

Table 3

Anti-inflammatory effects of short-term administration of VIP in animal models

Disease	Organ	Animal model	Inductor
Lung Inflammation	Lung	Rat	carrageenan
Crohn disease	Intestine	Rat	TNBS, DSS
Rheumatoid arthritis	Joints	Rat	Collagen
Septic shock	Blood	Rat	LPS
Encephalomyelitis	Brain	Rat	MOG
Hepatitis	Liver	Mouse	Con-A

Con-A, concanavalin A; DSS, dextran sulphate sodium; MOG, myelin oligodendrocyte glycoprotein; TNBS, 2,4,6-trinitrobenzene sulphonic acid.

been extensively reported in the literature (Delgado *et al.*, 2004), new data using VIP-KO or VPAC1-KO indicate that VIP can also exert pro-inflammatory actions (Abad *et al.*, 2010; Yadav *et al.*, 2011). A very recent report reveals that VIP-deficient mice are resistant to the development of encephalomyelitis (EAE), indicating that in these conditions VIP plays unexpected permissive and/or pro-inflammatory actions (Abad *et al.*, 2010). In the same way, VPAC1-KO mice are partially protected from DSS-induced colitis (Yadav *et al.*, 2011). Clearly, a short-term administration of VIP ameliorates the clinical symptoms of chronic inflammation in animal models (Delgado *et al.*, 2004), but conversely, it seems that genetic loss of VIP or VPAC1 receptor in mice result in a pro-inflammatory response. These recent results show that targeting specific VPAC receptors with agonist and/or antagonist could be considered in human therapy (Abad *et al.*, 2010; Yadav *et al.*, 2011). In spite of these recent findings, it is usually proposed that short-term administration of VIP and other VPAC receptor agonists may be beneficial in inflammatory disorders characterized by macrophage activation and Th1/Th2 misbalanced response.

A large body of study has associated VIP with neuroprotection. In the mid-eighties, a first report demonstrated that this peptide was able to prevent neuronal death associated with electrical blockade induced by the addition of tetrodotoxin (TTX) to primary spinal cord cultures (Brenneman and Eiden, 1986). Further studies have demonstrated that VIP plays a neuroprotective effects in various neurodegenerative diseases developed in animal models including Alzheimer's disease (Gozes *et al.*, 1996), Parkinson's disease or encephalomyelitis (Gonzalez-Rey *et al.*, 2005; 2006). These neuroprotective actions of VIP were associated with glial cells possessing VPAC receptors. Clearly, VIP induced from glial cells the secretion of various trophic molecules having neuroprotective properties (Dejda *et al.*, 2005) such as IL-1, IL-6, protease nexin-1, the chemokine RANTES (Regulated upon Activation, Normal T-cell Expressed, and Secreted) and MIP (Macrophage Inflammatory Proteins). Moreover, VIP inhibits the production of pro-inflammatory cytokines such as TNF-α and/or IL-1β secreted by activated microglia, which is involved in neuro-inflammation observed in Parkinson's disease (Delgado and Ganea, 2003). VIP also induces neuroprotective effects by increasing the secretion of ADNF (activity-dependent neurotrophic factor) and/or by increasing the concentration of ADNP mRNA (activity-dependent neurotrophic protein) (Brenneman and Gozes, 1996; Gozes *et al.*, 2000). These two protective proteins that belong to the heat shock protein family are able to prevent the neuronal death (Brenneman and Gozes, 1996) and represent some of most potent neuroprotective agents secreted by astroglia in response to VIP. Although the major neuroprotective effects of VIP can be explained by activation of adenylyl cyclase through VPAC receptors (Brenneman, 2007), some reports indicate that VIP-mediated effects on protection did not involve cAMP but rather a mobilization of intracellular calcium in astrocytes (Brenneman, 2007). Recently, it has been suggested that the VPAC2 receptor could be a potential target for the development of antipsychotic drugs related to duplications of VPAC2 receptor gene in schizophrenia (Vacic *et al.*, 2011). However, a major drawback with the use of VIP in therapy is its high sensitivity to protease degradation. Indeed, removing of the first His^{1}-Ser^{2} residues by peptidases, such as DPPIV (dipeptidyl peptidase IV), induces a drastic loss of affinity (Gourlet *et al.*, 1997a,b). To circumvent this problem, VIP could be modified to increase its resistance to degradation by N-acylation of the peptide N-terminal end or by substitution of residues involved in proteolytic consensus sequences (dibasic doublets). Recent data indicated that N-terminal modifications of PACAP confer resistance to DPPIV (Bourgault *et al.*, 2008). In the same way, acetylation of N-terminal end of VIP increases its stability in the presence of human serum (personal data). Other strategies have been developed to protect peptide against degradation by insertion of VIP into micelles or nanoparticles (Fernandez-Montesinos *et al.*, 2009; Onyüksel and Mohanty, 2009). A second major obstacle that reduces the therapeutic use of VIP in humans is its ability to interact at high affinity with different receptors such as VPAC1 and VPAC2 subtypes but also, with lower affinity, with other class B GPCRs such as PACAP receptor, secretin receptor and/or GRF receptor (Laburthe *et al.*, 2007). These cross-interactions may be responsible for the existence of strong side effects induced by VIP in humans including hypotension and diarrhoea (Laburthe *et al.*, 2007). In this context, the development of specific ligands for VPAC1 and VPAC2 receptors with no affinity with other class B GPCRs is clearly crucial. It should be noted that only one non-peptide antagonist having low affinity for VPAC2 receptor is yet available (Chu *et al.*, 2010). There is an abundant literature regarding the pharmacology of these receptors (Robberecht and Waelbroeck, 1998); moreover, drastic differences between species of VPAC receptor pharmacology have been described (Laburthe *et al.*, 2002). Analysis of VIP structure–function relationships by a complete alanine scan (Nicole *et al.*, 2000) allowed us to rationally design the most potent and specific peptide agonist for VPAC1 receptor currently available, that is [$Ala^{11,22,28}$]-VIP (Nicole *et al.*, 2000). This VIP derivative has 1000 times higher affinity for the VPAC1 receptor, which is mainly involved in anti-inflammatory action of VIP (Delgado *et al.*, 2004), than for the VPAC2 receptor (Nicole *et al.*, 2000). A selective high-affinity antagonist of the VPAC1 receptor, that is [Ac-His^{1},D-Phe^{2},K^{15}, R^{16}, L^{27}]VIP(3–7)/GRF(8–27) named PG 97–269, was characterized (Gourlet *et al.*, 1997a,b).

The amino acid sequence of this antagonist has many similarities and identities with the sequence of VIP. However, one of the major differences is the presence of a D-phenylalanine in position 2 instead of a serine. This substitution probably plays an important role in antagonist properties of PG 97–269. Indeed, the presence of D-Phe, a hydrophobic residue, results in a perturbation in the formation of N-cap motif (Neumann *et al.*, 2008) involving residues 2–6. Regarding the VPAC2 receptor, the cyclic peptide analogue of VIP [Ac-Glu^{8},OCH3-Tyr^{10},Lys^{12},Nle^{17},Ala^{19},Asp^{25},Leu^{26},Lys^{27,28}-VIP(cyclo 21–25)] or Ro 25–1392 is a potent and selective agonist (Xia *et al.*, 1997). In our opinion, there is still no satisfactory VPAC2 receptor antagonist since PG 99–465, a VIP analogue that antagonizes VIP action on VPAC2 receptor, has significant agonist activity on human VPAC1 receptor (Moreno *et al.*, 2000).

Conclusions

The VPAC receptors, in particular VPAC1, are very promising targets for the development of therapeutic molecules. While new peptide derivatives specifically targeting VPAC receptor subtypes are now available, however, their very short half-life and the inconvenience related to their administration routes make them difficult to use in human therapy. It is to be hoped that recent advances of our knowledge of the structure of VPAC receptor site and more generally of class B GPCR binding sites will lead shortly to the design of non-peptide receptor agonists and/or antagonists. Such molecules would be of considerable interest in the therapy of many human diseases in particular inflammatory and neurodegenerative diseases.

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Conflicts of interest

None.

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