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REVIEW

Conformational switches in the $VPAC_1$ receptor

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The vasoactive intestinal peptide receptor 1 ($VPAC_1$) belongs to family B of GPCRs and is activated upon binding of vasoactive intestinal peptide (VIP) and pituitary AC-activating polypeptide neuropeptides. Widely distributed throughout body, $VPAC_1$ plays important regulatory roles in human physiology and physiopathology. Like most members of the GPCR-B family, $VPAC_1$ receptor is predicted to follow the actual paradigm of a common 'two-domain' model of natural ligand action. However the precise structural basis for ligand binding, receptor activation and signal transduction are still incompletely understood due in part to the absence of X-ray crystal structure of the whole receptor and to significant structural differences with the most extensively studied family of receptor, the GPCR-A/rhodopsin family. Here, we try to summarize the current knowledge of the molecular mechanisms involved in $VPAC_1$ receptor activation and signal transduction. This includes search for amino acids involved in the two-step process of VIP binding, in the stabilization of $VPAC_1$ inactive and active conformations, and in binding and activation of G proteins.

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Abbreviations

IC, intracellular loop; PACAP, pituitary AC-activating polypeptide; TM, transmembrane; VIP, Vasoactive Intestinal Peptide

The vasoactive intestinal peptide receptor 1 ($VPAC_1$) is a member of the family B of GPCRs, which includes $VPAC_2$, pituitary AC-activating polypeptide receptor 1 (PAC_1), secretin, glucagon, glucagon-like peptide (GLP) 1 and 2, calcitonin, gastric inhibitory polypeptide (GIP), corticotropin-releasing factor (CRF) 1 and 2, and parathyroid hormone (PTH) receptors. The endogenous ligands of $VPAC_1$ receptor are vasoactive intestinal polypeptide (VIP) and pituitary AC-activating polypeptide (PACAP), two neuropeptides that contribute to the regulation of intestinal motility and secretion, exocrine and endocrine secretions, and to homeostasis of the immune system (Dickson and Finlayson, 2009). Like all members of the GPCR-B family, $VPAC_1$ receptor is preferentially coupled to Gαs protein that stimulates AC activity and induces cyclic AMP increase, although a coupling to the PLC and the calcium/inositol trisphosphate pathway through either Gαq or Gαi is also effective (Dickson and Finlayson, 2009). $VPAC_1$ receptor was also reported to interact with receptor activity-modifying proteins (RAMP), in particular RAMP2, inducing a significant increase of agonist-induced inositol trisphosphate production without modifying cAMP stimulation (Christopoulos *et al.*, 2003). Like most GPCRs, $VPAC_1$ receptor also forms constitutive homodimers as well as hetero-oligomers with $VPAC_2$ receptors, as demonstrated using biophysical methods (Harikumar *et al.*, 2006), but the physiological consequences of those oligomerizations remain to be elucidated. Indeed, pharmacological studies performed on CHO cells co-expressing $VPAC_1$ and $VPAC_2$ receptors did not identify any differences in VIP or selective agonist affinities or potencies. Similarly, VIP receptors co-expression did not modify receptor internalization and trafficking patterns following agonist exposure (Langer *et al.*, 2006).

Major advances in structural biology of GPCRs came a few years ago from solving the first X-ray crystal structures of rhodopsin and ligand-activated GPCR-A family members bound to an antagonist and an agonist (Palczewski *et al.*, 2000; Cherezov *et al.*, 2007; Rasmussen *et al.*, 2007; Jaakola *et al.*, 2008; Park *et al.*, 2008; Scheerer *et al.*, 2008; Warne

et al., 2008; Rosenbaum *et al.*, 2011; Xu *et al.*, 2011). However, the mechanisms regulating the GPCR-B family signal transduction are less precisely understood, since no X-ray crystal structure of the whole receptor is available, and conserved motifs of the GPCR-A family (E/DRY at TM3, NPXXY at TM7) are absent in the GPCR-B family. They also differ from family A members by their larger binding site located both on N-terminal extracellular domain and transmembrane (TM) helices. Although recent studies have solved the structure of the N-terminus of several family B receptors (CRF, PTH, PAC_1, GIP, GLP-1, calcitonin receptor-like/RAMP1) and clarified their role in ligand binding (Grace *et al.*, 2007; Parthier *et al.*, 2007; Sun *et al.*, 2007; Pioszak and Xu, 2008; Runge *et al.*, 2008; ter Haar *et al.*, 2010), information on the events that follow ligand binding only came from site-directed mutagenesis and pharmacological studies. These will be developed in this review, trying to highlight the current knowledge of the molecular switches driving $VPAC_1$ from inactive to active conformation and subsequent G protein binding and activation.

The 'two-domain' model for ligand-receptor interaction

The commonly accepted model for agonist action of family B GPCRs suggests that the N-terminal domain of the receptor is the principal binding site for the central and the C-terminal regions of the natural ligand and ensures correct ligand positioning, whereas binding of residues 1–6 of the ligand to the extracellular loops and TM helices drives the receptor activation (Hoare, 2005). Following agonist binding, subsequent conformational changes are expected within the TM domains of the receptor causing key sequences located in the intracellular loops to be exposed and to interact with the G proteins. More recently, it has also been proposed that a helix N-capping motif, identified in the N-terminus of all GPCR-B family ligands and stabilizing their helical conformation, was probably formed upon receptor binding and could also constitute a key element in receptor activation (Neumann *et al.*, 2008).

A large number of site-directed mutagenesis studies suggests that VIP–$VPAC_1$ receptor interaction also follows this paradigm and pointed out that the N-terminus of the $VPAC_1$ receptor plays a key role in agonist binding (Laburthe *et al.*, 2007). Solano *et al.* (2001) also found, using reciprocal substitution mutants in both ligand and receptor, that D^3 of VIP forms a salt bridge with R^{188} of the $VPAC_1$ receptor and that this interaction was necessary for receptor activation. More recently, photoaffinity experiments performed by the group of Couvineau and Laburthe showed that benzophenone-residues in position 6, 22, 24 and 28 of VIP are in direct contact with D^{107}, G^{116}, C^{122} and K^{127} respectively, four residues located in the N-terminus of $VPAC_1$ receptor (Couvineau *et al.*, 2010). Interestingly, they also observed, using a VIP and a $VPAC_1$ antagonist affinity probe in position 0, that the N-terminal domain of VIP (agonist) and of the $VPAC_1$ antagonist recognizes two different microdomains in the N-terminus of the $VPAC_1$ receptor, while the central and the C-terminal regions of these ligands seem to share the same binding site (Ceraudo *et al.*, 2008a) (Figures 1 and 2).

Molecular mechanisms involved in $VPAC_1$ receptor activation

As mentioned before, the recent solving of the X-ray crystal structures of several GPCR-A family members provides clues to the TM helix rearrangements that result from agonist binding and subsequent receptor activation. These include the disruption of an ionic interaction between the cytoplasmic face of TM3 (E/DRY motif) and TM6 (E residue) maintaining the receptor preferentially in a ground inactive conformation in absence of agonist (ionic lock), a 'rotamer toggle switch' (modulation of the helix conformation around a proline-kink) in TM6 causing key sequences to be exposed to cytoplasmic binding partners and a conformational change of Y residue of the NPXXY motif located in TM7 stabilizing the active conformation (Rosenbaum *et al.*, 2009; Rosenbaum *et al.*, 2011). In the absence of X-ray crystal structure of the $VPAC_1$ receptor, only model structures have been reported, which used as template the structures of the N-terminal domain of the CRF 2β receptor (Ceraudo *et al.*, 2008b) or structures of family A GPCRs for the TM domains (Conner *et al.*, 2005; Chugunov *et al.*, 2010). However, the low sequence identity between the $VPAC_1$ receptor sequence and the templates used for homology modelling prevents direct transposition of molecular switches that drive GPCR-A members activation.

As all members of GPCR-B family, $VPAC_1$ receptor lacks the E/DRY sequence. On the basis of subtle changes observed when Y^{239} and L^{240}, located in TM3 of $VPAC_1$, were substituted with alanine it was proposed that this YL sequence was equivalent to the E/DRY motif of GPCR-A family (Tams *et al.*, 2001). Another model based on a three-dimensional analysis of the GLP-1 receptor proposed that an E/DRY motif could be formed by three non-adjacent residues consisting in R^{174} in the cytoplasmic end of TM2, E^{236} and Y^{239} in the distal part of TM3 of $VPAC_1$ (Frimurer and Bywater, 1999). But in our hands $Y^{239}A$, $L^{240}A$, $E^{236}A$, $Y^{239}A$ and $R^{174}A$ mutants were undistinguishable from the wild-type receptor (Nachtergael *et al.*, 2006). One possible explanation for the discrepancy can be the fact that Tams *et al.* (2000) studied cyclic AMP measurements in intact cells a more sensitive model than the AC assay on membrane used in our study. Nevertheless, even if the YL motif of GPCR-B family and E/DRY motif of GPCR-A family have the same location, they certainly do not have the same importance for receptor activation (Figure 1).

More recently, by combining pharmacological and *in silico* approaches, we have identified a network of interactions between residues located in helices 2, 3 and 7 of the $VPAC_1$ receptor, which are involved in the stabilization of the receptor in the absence of agonist and in early steps of receptor activation. We proposed that, in the absence of ligand, interaction between R^{188}, N^{229} and Q^{380} ties helices 2, 3 and 7 together (Figure 3). Upon VIP binding, the interaction between R^{188} and N^{380} is broken, and a stronger interaction (salt bridge) is established between R^{188} and the D^3 side chain of VIP. TM2 and, probably, other helices undergo conformational changes causing key sequences located in intracellular loops to be exposed and to interact with the G proteins. In the meantime, the interaction network involving N^{229} and Q^{380} maintains TM7 in a conformation necessary for proper

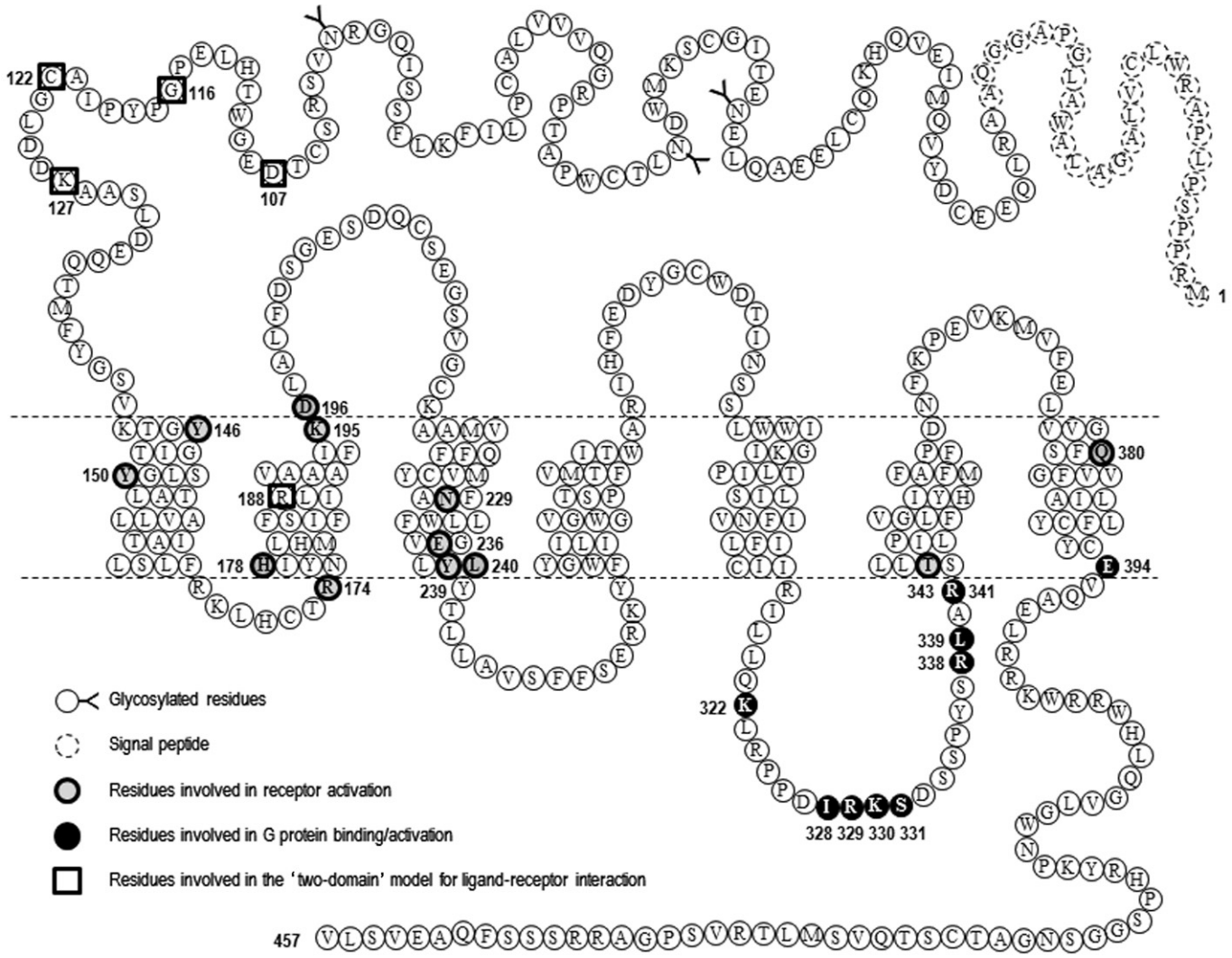

Figure 1

Snake plot representation of $VPAC_1$ receptor. Amino acid sequence of human $VPAC_1$ receptor, the position of signal peptide, glycosylated residues and amino acids important for VIP binding, receptor activation and G protein coupling are also labelled.

HSDAVFTDNYTRLRKQMAVKKYLNSILN

Figure 2

Amino acid sequence of VIP. Amino acids that were experimentally mapped into the $VPAC_1$ receptor binding site are in bold and those involved in the helical N-cap are underlined.

activation of G proteins. The three-dimensional model also suggested that Q^{380} could function as a floating 'ferry-boat', switching between R^{188} and N^{229} residues' side-chains, hence contributing to signal transduction propagation and activation of G proteins (Chugunov *et al.*, 2010). Likewise, other studies have pointed out the importance of TM2 and TM7 in G protein activation. Indeed, the mutation into arginine of H^{178} located at the bottom of TM2 led to a constitutively activated $VPAC_1$ receptor (Gaudin *et al.*, 1998). On the other hand, it has also been shown that E^{394} located at the junction of TM7 and the C-terminus of $VPAC_1$ was important for VIP-induced cAMP production but was not directly involved in Gαs binding (Couvineau *et al.*, 2003; Langer and Robberecht, 2005). Moreover, we found that phosphorylation levels and internalization of $N^{229}A$ and $N^{229}Q$ $VPAC_1$ receptors (mutants that failed to generate the G protein active state and, therefore, to activate AC properly and to stimulate intracellular calcium increase but with a preserved affinity for VIP and sensitivity to GTP) were comparable with that of the wild-type receptor (Nachtergael *et al.*, 2006). These later results thus suggest that receptor conformation necessary for activation and regulatory mechanisms, such as desensitization and internalization, could be different.

When considering other site-directed mutagenesis studies, it is likely that a complex and larger network of interaction between TM helices must be considered for stabilization of $VPAC_1$ inactive and active conformations (Figure 1). Indeed, mutation of T^{343}, located at the junction of

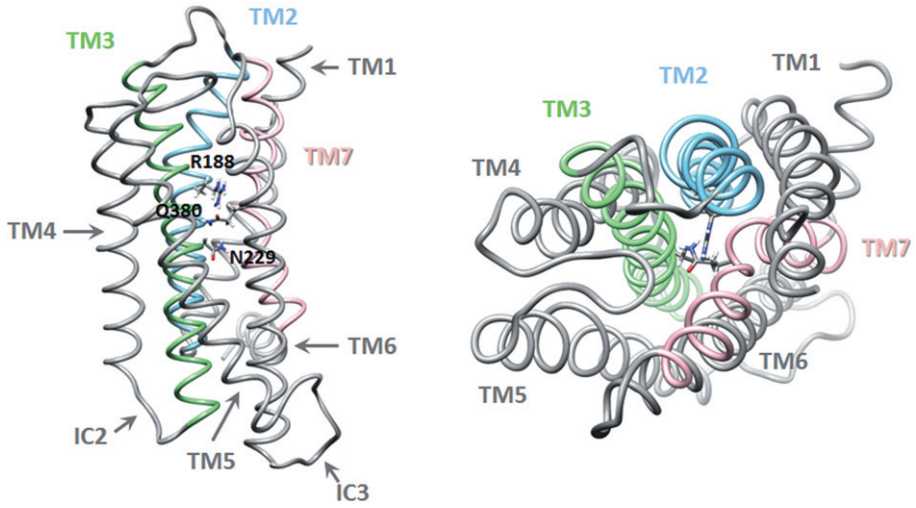

Figure 3

Three-dimensional model of the TM domains of the $VPAC_1$ receptor. Lateral (left) and top (right) view of a working model of human VPAC1 receptor, TM and residues identified as important for receptor stabilization are also labelled. Details regarding modelling procedure are described in Chugunov *et al.* (2010).

the third intracellular loop and TM6 of $VPAC_1$, into lysine, proline or alanine also led to a constitutively activated receptor (Gaudin *et al.*, 1999). Another study showed that Y^{146} and Y^{150}, located in TM1 of $VPAC_1$, do not interact directly with VIP but stabilize the correct active receptor conformation (Perret *et al.*, 2002). Similarly, we observed that K^{195} and D^{196} located at junction of TM2 and the first extracellular loop were essential for $VPAC_1$ activation but were not directly involved in VIP recognition (Langer *et al.*, 2003).

How all these residues cooperate to propagate signal transduction after VIP binding remains to be elucidated and would require a model of the activated receptor in complex with VIP. Particularly the two N-terminal residues of VIP, H^1 and S^2, are likely to affect, directly or indirectly, the interaction network surrounding N^{229} and Q^{380}. Of interest, so far as all residues that were identified as important for $VPAC_1$ receptor activation are highly conserved among GPCR-B family members, they may, therefore, be involved in a binding and activation mechanism that is common to the whole family.

Molecular mechanisms involved in $VPAC_1$/G protein binding and activation

The α subunit of heterotrimeric G proteins has a central role in interaction with both the receptor and the effectors. Several studies have shown that the C-terminal part of Gα subunit can directly bind to the receptor and is involved in the coupling specificity (Conklin *et al.*, 1996). The current model of GPCR activation, based on the study of family A GPCRs, proposes that when the receptor switches to its active conformation, TM movements are accompanied by intracellular loops switches leading to exposure of the G protein-binding pocket to cytosol and efficient binding to G protein. However, the diversity of sequences and loop sizes, as well as their flexibility, has made difficult the identification of a specific set of residues defining the coupling profile.

For the $VPAC_1$ receptor, Gα binding domains are mainly located in the third intracellular loop (IC3), which contains subdomains dedicated to the recognition of the different Gα subunits (Figure 1). K^{322} located in proximal part of IC3 and E^{394} located at the junction of TM7 and the C-terminal tail are required for AC activation but not for the coupling to the inositol trisphosphate/calcium pathway. The former being involved in direct interaction with Gαs (G protein binding), as demonstrated by a reduced sensitivity to GTP, while E^{394} triggering switch of Gαs from inactive to active state (G protein activation) (Couvineau *et al.*, 2003; Langer and Robberecht, 2005). Similarly, two other sequences located in IC3 have been identified as important for VIP-induced intracellular calcium increase but not cAMP production. A small sequence, I^{328}-R^{329}- K^{330}-S^{331}, located in the central part of IC3 is involved in efficient binding of $VPAC_1$ to Gαi/o and Gαq (Langer *et al.*, 2002), while R^{338} and L^{339}, located at the distal part of IC3, mediate interaction of $VPAC_1$ with Gαi/o (Langer and Robberecht, 2005). Combining mutations in the proximal and distal part of IC3 together with mutation of E^{394} gave rise to a completely inactive $VPAC_1$ receptor with respect to AC activation and intracellular calcium increase.

Among the different members of the GPCR-B family, proximal and distal domains of IC3 share conserved sequences that could therefore represent common G protein binding motifs. In line with this hypothesis, studies performed on other members of the GPCR-B family identified the proximal domain of IC3 as essential for AC activation but the amino acids involved may differ and additional conserved sequences located in other intracellular regions of the receptor may also be necessary as seen for glucagon (IC2) (Cypess *et al.*, 1999) and calcitonin gene-related peptide receptors (R^{151} located in IC1) (Conner *et al.*, 2006). The junc-

tions of IC3 loop are predicted to be α-helical and it is assumed that the correct positioning of charged amino acids plays an important role in G protein interaction. However, other data suggest that lipophilic and aromatic residues are also important for G protein interaction. It is possible that IC3 loop junctions activate G protein directly or that they may serve as regions that control the loop conformation. As mutations may change both direct interaction site and secondary structure, it is difficult to define more precisely the mechanisms involved in IC3 loop/G protein interaction. Again a structure or a model of the activated $VPAC_1$ receptor in complex with VIP could help to answer this question.

Conclusion

Identification of the precise molecular mechanisms that drive GPCRs from inactive to active state represents a major focus in functional genomics and drug development research with the ultimate aim of designing molecules able to stabilize one of these states. VIP and PACAP receptors have been identified as potential therapeutic targets for metabolic, inflammatory and neuronal diseases (Dickson and Finlayson, 2009). But the use of their natural ligands is limited by their lack of specificity (PACAP binds with high affinity $VPAC_1$, $VPAC_2$ and PAC_1 receptors while VIP recognizes both $VPAC_1$ and $VPAC_2$ receptors), their poor oral bioavailability (VIP and PACAP are 27- to 38-amino acid peptides) and their short half-life. Therefore, the development of non-peptide small molecules or specific stabilized peptidic ligands is of high interest. Up to now, only two small molecules antagonists of $VPAC_2$ receptor have been identified by high-throughput screening (Chu *et al.*, 2010), further investigation and new insight toward elucidation of VIP receptors activation mechanism would allow the rational design of potential new drugs.

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Conflict of interest

The author has no conflicts of interest and no financial links with manufacturers of reagents relevant to this work.

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