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Class-B GPCR activation: is ligand helix-capping the key?

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The class B family of G-protein-coupled receptors (GPCRs) regulates essential physiological functions such as exocrine and endocrine secretions, feeding behaviour, metabolism, growth, and neuro- and immuno-modulations. These receptors are activated by endogenous peptide hormones including secretin, glucagon, vasoactive intestinal peptide, corticotropin-releasing factor and parathyroid hormone. We have identified a common structural motif that is encoded in all class B GPCR-ligand N-terminal sequences. We propose that this local structure, a helix N-capping motif, is formed upon receptor binding and constitutes a key element underlying class B GPCR activation. The folded backbone conformation imposed by the capping structure could serve as a template for a rational design of drugs targeting class B GPCRs in several diseases.

The class B family of GPCRs

G-protein-coupled receptors (GPCRs) are a large family of proteins that contain a seven transmembrane helical structural motif [1]. They mediate responses to a wide variety of ligands by binding and activating intracellular heterotrimeric G proteins and are considered to be the most important group of drug targets. The largest family of these receptors, the class A GPCRs, or rhodopsin-like receptors, which comprises several hundred members [1] and represents >30% targets for marketed drugs [2,3], is well characterized [1].

More recently, a second family of receptors, the class B GPCRs (also referred to as class II GPCRs or secretin-like receptors), comprising 15 members, has received attention. There are several differences between class A and class B GPCRs, most notably their low overall sequence homology and the presence of a large and structured N-terminal ectodomain (N-ted) in class B receptors, which is usually small in most class A receptors (with the notable exception of glycoprotein hormone receptors) [4]. Moreover, whereas the structure of the transmembrane core of the class A receptors is known [5,6], that of the class B receptors is not.

Class A GPCRs can transduce signals as diverse as photons, organic odorants, amines, nucleotides, nucleosides, peptides, proteins or lipids. In contrast to the wide diversity in chemical structure that characterizes the class A GPCR ligands, the set of natural ligands (>20) for class B GPCRs seems to be remarkably homogeneous: they are all peptides with >27 amino acid residues synthesized and released by endocrine cells, neurons and/or immune cells. In humans, these ligands include secretin, glucagon, glucagon-like peptides (GLPs), vasoactive intestinal peptide (VIP), pituitary adenylate cyclase-activating peptide (PACAP), growth hormone-releasing factor (GRF), gastric inhibitory polypeptide (GIP), corticotropin-releasing factor (CRF), parathyroid hormone (PTH) and calcitonin [7]. These peptides strongly influence human physiopathology and have been proposed as promising candidates for the treatment of several diseases: PACAP for neurodegenerative diseases [8]; VIP for inflammatory diseases [9]; GRF for dwarfism [10]; glucagon, GLP-1 and GIP for diabetes [11–13]; GLP-2 for short bowel syndromes [14]; PTH and calcitonin for osteoporosis [15,16]; and CRF for chronic stress [17]. The main drawback of natural peptides is their very short half-life in biological fluids, including blood, owing to their rapid degradation. Therefore, the development of stable non-peptide agonists of class B GPCRs is an important goal. This is a marked contrast with class A GPCRs, for which antagonists rather than agonists represent the main marketed drugs.

Although all peptides that activate class B GPCRs lack a well-defined conformation in aqueous solution, they exhibit a marked propensity to form α-helices. This property is clearly revealed under structure-inducing conditions, that is, in the presence of organic solvents and lipid micelles [18–28], and in crystals [29,30]. The only direct structural information about the conformation of a peptide agonist bound to its receptor concern the PACAP21 peptide bound to its pituitary adenylate cyclase 1 (PAC1) receptor [23]: when bound to its receptor, PACAP21 is mainly α-helical [23]. The class B GPCR peptide ligands also share common sequence-activity relationships. Of note, truncations in their C-terminal segments result in a marked decrease in receptor affinity. Moreover, the results of truncations in their N-terminal segments indicate that the first residues of their sequences are essential for the activation process [31].

The disparity in drug development for the A and B families could result from the different structural characteristics described here. Notably, these differences include:

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(i) the large extracellular domain involved in ligand binding for class B GPCRs in contrast to class A GPCRs (except for the glycoprotein receptor); (ii) the generally small size of class A GPCR ligands enables these ligands; (iii) the absence of solved structures for the transmembrane core of the class B receptors.

Facing the paucity of structural data on class B GPCRs, rather than relying solely on commonly used empirical approaches, we aimed to identify a structural characteristic shared by all the class B GPCR ligands to would provide a rational basis for understanding the activation process of these receptors. We therefore focused on the sequences of all class B GPCRs peptide ligands in light of their common specificities. In particular, they all exhibit a marked propensity to form helices, and truncations in their N-terminal segments demonstrate that the first residues of their sequences are essential for the activation process [31,32]. We searched for a common structural motif encoded in the sequences of these ligands, specifically in their N-terminal regions. Throughout our analysis of the many natural ligand peptide sequences for the class B GPCRs in various species [33], we identified such a motif – a helix N-capping structure that could serve as a template for rational drug design. We therefore believe that this structure will contribute to a realization of the full therapeutic potential for class B GPCRs.

A helix N-capping motif in class B GPCR peptide ligands

The α-helix is characterized by main-chain hydrogen bonds between successive amide hydrogen donors and carbonyl oxygen acceptors located four residues prior in the sequence. Therefore, the initial four amide hydrogens are not hydrogen bonded, and additional 'capping' hydrogen bonds that satisfy the initial four amide hydrogens might also be present. These capping motifs, termed helix N-capping, are well-defined structures that strongly stabilize helical N termini by inhibiting fraying and imposing a specific local fold through a pronounced change in the backbone direction [34,35] (Figure 1).

To begin to investigate the structural motifs encoded in the N-terminal sequences of class B GPCR ligands, we

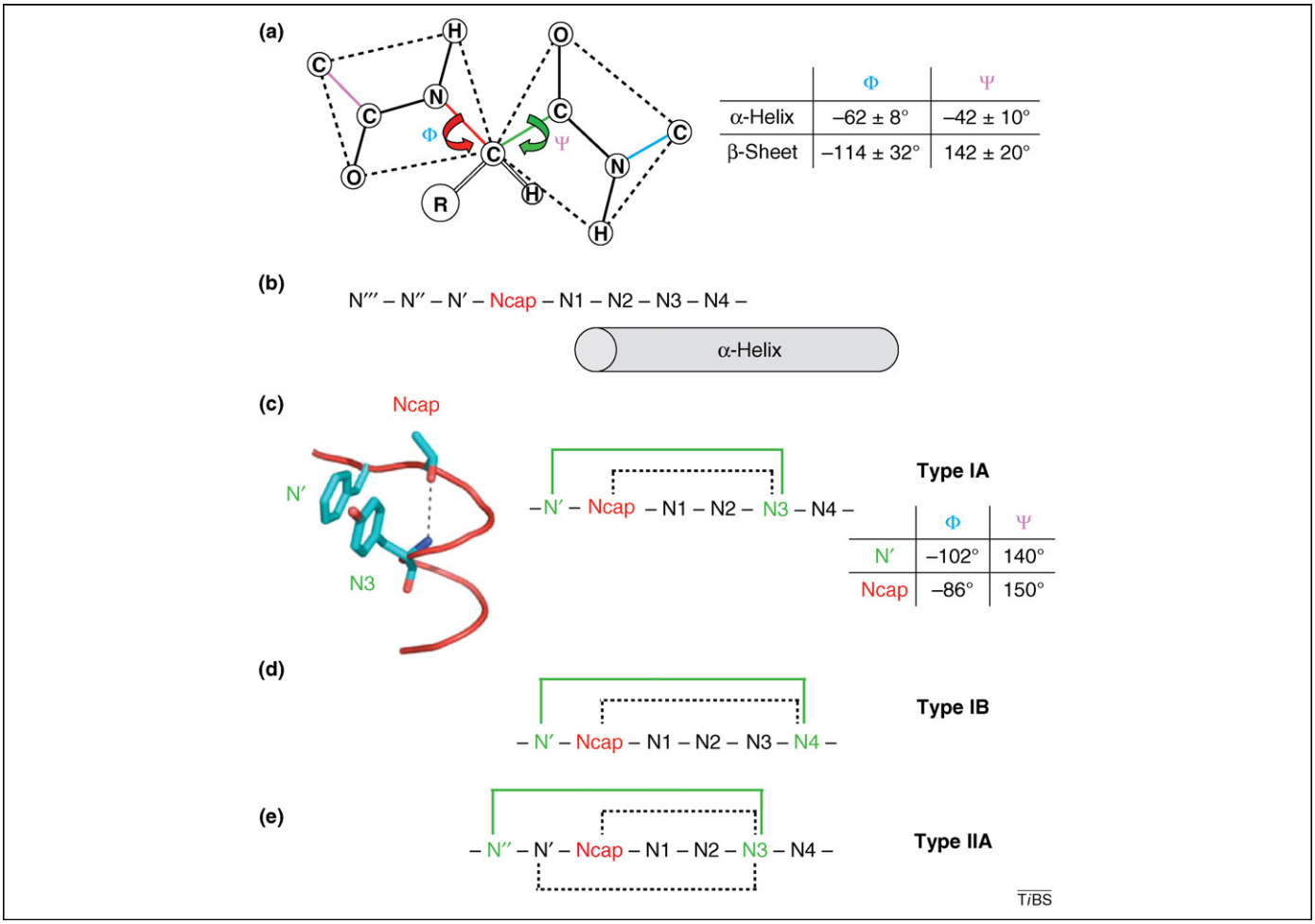

Figure 1. General scheme for the peptide bond and N-capping motif of class B GPCR peptide ligands. Polypeptide chain secondary structure is characterized by torsion angles between planes linking the backbone of consecutives amino acids. **(a)** Schematic representation of a peptide backbone (spheres are labelled as C, H, O, N atoms and the R side chain of an amino acid). Broken lines represent peptide planes that rotate relative to the other along the N–C bond phi (Φ; blue) angle and the C–C bond psi (Ψ; green) angle. Typical values for these angles found in the secondary structures are presented in the table [48]. **(b)** General nomenclature for the N-terminal region of an α-helix (cylinder). N‴, N″ and N′ are residues located before Ncap, which forms the boundary residue of the α-helix. N1, N2, N3 and N4 are the first residues of the α-helix with helical backbone dihedral angles. **(c)** The N-capping IA motif. The 3D structure is shown on the left. The intramolecular interaction pattern is shown in the middle. The hydrophobic interactions between side-chain groups are indicated by the green line, the hydrogen bonds between side chain and backbone atoms are depicted as a black broken line. Typical values of Φ and Ψ dihedral angles found for the N′ and Ncap residues are presented in the table. **(d)** Intramolecular interaction pattern involved in the N-capping IB motif. **(e)** Intramolecular interaction pattern involved in the N-capping IIA motif.

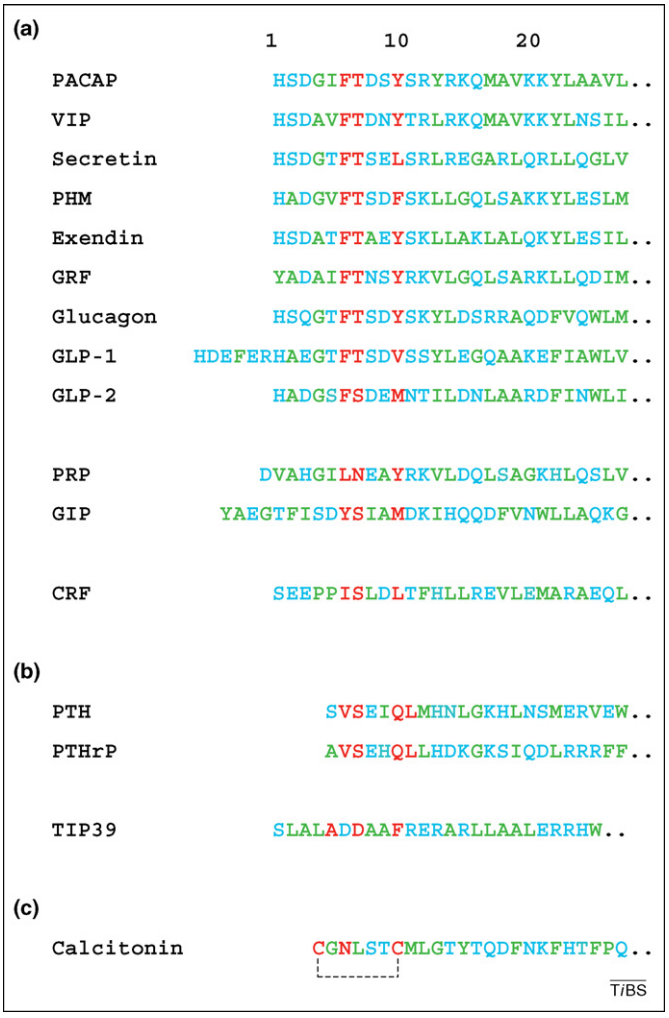

Figure 2. Human sequences of class B GPCR ligands. The first 27 amino acids of each peptide were aligned with respect to the N-capping motif. **(a)** The simplest capping structure (IA motif) involving N′, Ncap and N3 residues (red) is observed for most of the peptides. **(b)** Other capping structures involving N′, Ncap, N3 and N4 residues (IB motif) in the case of PTH and PTHrP sequences and also N′, Ncap and N3 residues (IIA motif) in the case of TIP39. **(c)** Constitutive capping observed in the case of calcitonin. The Cys–Cys bond is highlighted by a broken line. The third residue (red) is aligned with respect to the N′ residue of the other capping. Polar amino acids are blue; glycine, tyrosine, tryptophan and hydrophobic amino acids are green.

examined the 134 distinct sequences for peptides of the secretin and glucagon families that have been determined in species from tunicates to humans (listed in Ref. [33]). These ligands include PACAP, PHM (peptide histidine methionineamide) PRP (PHM-related peptide), GRF, VIP, exendin, glucagon, GLP-1, GLP-2, exendin, secretin and GIP (Figure 2, human sequences). A striking result is the presence of an N-capping IA motif in positions 6–10 of the N-terminal segment for 124 of the 134 sequences listed by Sherwood *et al.* [33], that is, those comprising hydrophobic amino acids at positions 6 and 10 (N′ and N3) and a short-chain polar amino acid at position 7 (Ncap). The N-capping in class-B GPCR ligand is highly conserved among several species and is also present in ancestral species such as tunicates. Its presence in some human peptides has been noted previously [33]. A model of the N-capping structure has been built from the human VIP sequence (Figure 3). Among the 124 N-capping motifs, 106 have a phenylalanine at position 6 (a tyrosine or a leucine for the others), 120 have a threonine or a serine at position 7 (aspartic acid or asparagine for the others) and 93 have a tyrosine at position 10 (phenylalanine, leucine, isoleucine, methionine or valine for the others). An analysis of the N-capping motif present in 3D protein structures (observed in 1316 protein helices from 274 polypeptide chains [34]) indicates that the N1 residue is most frequently a polar amino acid, as seen in the motif encoded in the 124 class B GPCR ligand sequences mentioned.

It is also possible to identify N-capping motifs in the ten remaining sequences that did not contain an obvious N-capping IA motif. By shifting upstream by one residue, N-capping motifs are identifiable for bowfin GLP-1 and for the four PRP sequences: Ile7-Ser-Asp-Val-Tyr11 and Leu7-[Asn/Asp]-Xaa-Xaa-Tyr11, respectively (Figure 2). In addition, in the five GIP sequences, an upstream shift of four residues reveals an N-capping motif: Tyr10-Ser-Ile-Ala-Met14 (Figure 2). In the latter case, N1 is a hydrophobic amino acid that does not constitute a limiting factor for the formation of N-capping structures in short peptides. CRF is another important member of class B GPCR ligands. Once again, we found an N-capping motif in positions 6–10 of the N-terminal segment within 13 available CRF sequences from various species (from Ref. [36]), that is, [Ile/Leu/Met]6-Ser-[Ile/Leu]-Asp-Leu10 (see the human sequence in Figure 2).

The PTH family

We next analysed 11 sequences, from various species, corresponding to another subset of class B GPCR ligands: PTH and PTHrP (PTH-related peptide) (taken from Refs [30,37]). In their N-terminal segments, we identified a more sophisticated capping motif, termed the N-capping box, which corresponds to the IB motif (Figure 1), according to the Aurora and Rose classification system [34]. In this motif, N′ and N4 residues are hydrophobic amino acids, Ncap is a short-chain polar amino acid (aspartic acid, asparagine, threonine or serine) and N3 is glutamic acid or glutamine. This structure is characterized by a reciprocal side chain (sc)–backbone hydrogen-bonding interaction between Ncap and N3, sc(Ncap)–NH(N3), and NH(Ncap)–sc(N3) and an N′–N4 hydrophobic cluster. The N′ and Ncap residues within the N-capping box exhibit dihedral angles characteristics of β-structures similar to those found for the IA motif (see earlier). The corresponding human sequences are Val2-Ser-Glu-[Ile/Met]-Gln-[Leu/Phe]7 and Val2-Ser-Glu-His-Gln-Leu7 for PTH and PTHrP, respectively (Figure 2). A model of the N-capping box structure has been built from the human PTH sequence (Figure 3). It should be mentioned that the NMR conformational study of the human PTH (1–31) fragment in aqueous solution (PDB code: 1FVY) identifies experimental constraints consistent with an N-capping motif [37]. However, the potential hydrogen bond involving the backbone amide proton of Ser3 and the side-chain oxygen atoms of Gln6 is not discernible in the structure models because it is not defined by the NMR distance constraints (PDB code: 1FVY), probably owing to conformational flexibility within this N-terminal region.

Finally, we considered the tuberoinfundibular peptide (TIP39), which has a very high affinity for PTH receptor 2;

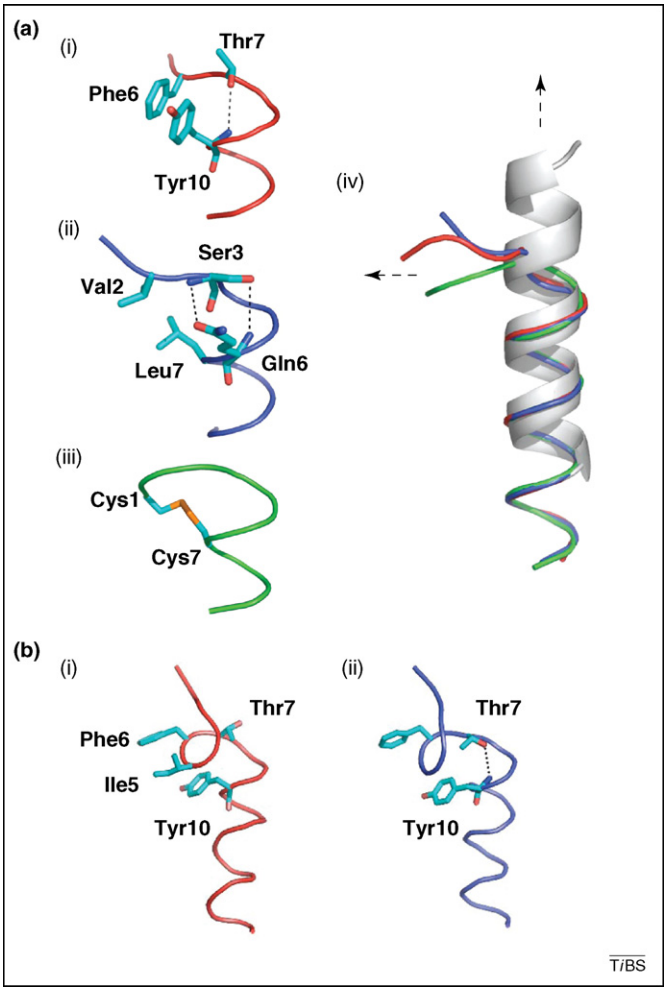

Figure 3. Structure of natural peptide ligands for class B GPCRs. Comparison of imposed N-capping motifs and natural motif in various peptides. **(a)** Models built for VIP, PTH and salmon calcitonin structures. (i) Model built from the human VIP sequence showing the N-capping structure (IA motif) imposed. It involves a hydrophobic cluster between Phe6 and Tyr10, and a hydrogen bond (broken line) between Thr7 and Tyr10. (ii) Model built from the human PTH sequence showing the N-capping structure (IB motif) imposed. It involves a hydrophobic cluster between Val2 and Leu7, and hydrogen bonds (broken lines) between Ser3 and Gln6. Only the segment at position 1–21 has been modelled for VIP and PTH peptides. The N-capping structures (IA and IB motifs for the VIP and PTH peptides, respectively) were imposed via the dihedral angles (ϕ, ψ) characterizing the N′ and Ncap residues (as described in Figure 1) and the reciprocal Ncap–N_3 hydrogen bonds. Residues from N1 to the end of the peptide were set in a helix conformation. Models were built and energy minimized using the latest version of the Sybyl software (Tripos, www.tripos.com). (iii) N-capping-like fold imposed by the disulfide bond between C1 and C7 residues in the structure of salmon calcitonin (residues 1–21) (PDB code: 2GLH). (iv) Superposition of the three structures corresponding to the VIP (red), PTH (blue) and calcitonin (green) peptides and a canonical helix segment without N-capping structure (grey ribbon). The vertical broken arrow represents the corresponding helix-axis; the horizontal arrow indicates the change in the backbone direction imposed by either the capping structure (VIP or PTH) or the S–S bond (calcitonin). **(b)** Conformation of PACAP21. (i) NMR structure of PACAP21 bound (red) to PAC1 receptor (PDB code: 1GEA). Residues Ile5, Phe6, Thr7 and Tyr10 are highlighted. (ii) A model of PACAP21 peptide (blue) with a hydrogen bond (broken line) between residues Thr7 and Tyr10 exhibits a structure similar to that observed when bound to the PAC1 receptor. The model was built and energy minimized using the latest version of the Sybyl software. Side chains, oxygen and nitrogen are shown in cyan, red and blue, respectively.

however, it does not belong to the PTH peptide family because it shares no sequence homology with PTH or PTHrP (Figure 2). TIP39 not only has a α-helical structure [38] but it is also equipped with a helix N-capping motif. This motif, different from the IA and IB patterns described, is termed II A (Figure 1) by Aurora and Rose [34]. The IIA capping motif consists of a staggered hydrogen-bonded cycle comprising sc(Ncap)–NH(N3) and NH(N′)–sc(N3). The hydrophobic cluster involves N″ and N3. The corresponding sequence N″–N′–Ncap–N1–N2–N3 in TIP39 is Ala5-Asp-Asp-Ala-Ala-Phe10 (Figure 2). Except for N1, each amino acid within the TIP39 capping motif corresponds to residues most frequently found at that position in protein helices [34].

In summary, a helix N-capping motif is present in the 124 analysed sequences of class B GPCR ligands, apart from those of the calcitonin subset (see later). Surprisingly, despite the many NMR studies [18–28], stable N-capping conformations are not observed for the isolated peptides of the class B GPCR ligand family. In fact, whereas N-capping motifs have been identified in numerous protein 3D structures [34], they are scarcely detected in small peptides. In the case of isolated peptides, in the absence of long range intra-molecular or inter-molecular interactions the N-capping motifs might only be transiently formed [39,40] and their presence is, therefore, difficult to detect from NMR data corresponding to average values over multiple exchanging conformations. In other words, tertiary interactions are required for stabilizing N-capping structures. Taking into account the exceptional conservation of the N-capping motif highlighted by our sequence analysis of the class B GPCR ligands, we propose that these motifs are only formed or stabilized within the receptor environment. Two features strongly support the formation of a folded capping structure for the N-terminal part of a ligand interacting with its receptor: the conformational properties of the calcitonin subset and the only conformational data available for a bound peptide, that is, for PACAP21 bound to its PAC1 receptor.

S–S bond-imposed N-capping-like fold in the calcitonin structure

Calcitonin constitutes a singular subset of the class B GPCR ligand family because its sequence exhibits two cysteine residues at positions 1 and 7 (Figure 2, human sequence). The resulting S–S bond imposes a characteristic backbone conformation in the N-terminal segment of the calcitonin peptide and this locked conformation is necessarily conserved in the ligand-bound state. We compared the N-terminal backbone conformation of the salmon calcitonin structure [41] with those of our structural models built from the VIP and PTH sequences (Figure 3) by superimposing the characteristic change in the backbone direction induced by the N-capping motifs to that dictated by the S–S bond (Figure 3). The calcitonin Asn3 residue occupies a position close to that of the VIP Phe6 residue and that of the PTH Val2 residue. The three residues exhibit dihedral angles characteristics of β-structures. Therefore, the backbone conformation imposed by the S–S bond, that is, of a peptide agonist bound to a class B receptor, is similar to the backbone conformation induced by an N-capping motif.

The conformation of PACAP21 bound to its PAC1 receptor

The N-terminal backbone of the receptor-bound PACAP21 peptide drastically bends from the Thr7 residue [23], resulting in a backbone direction change similar to that

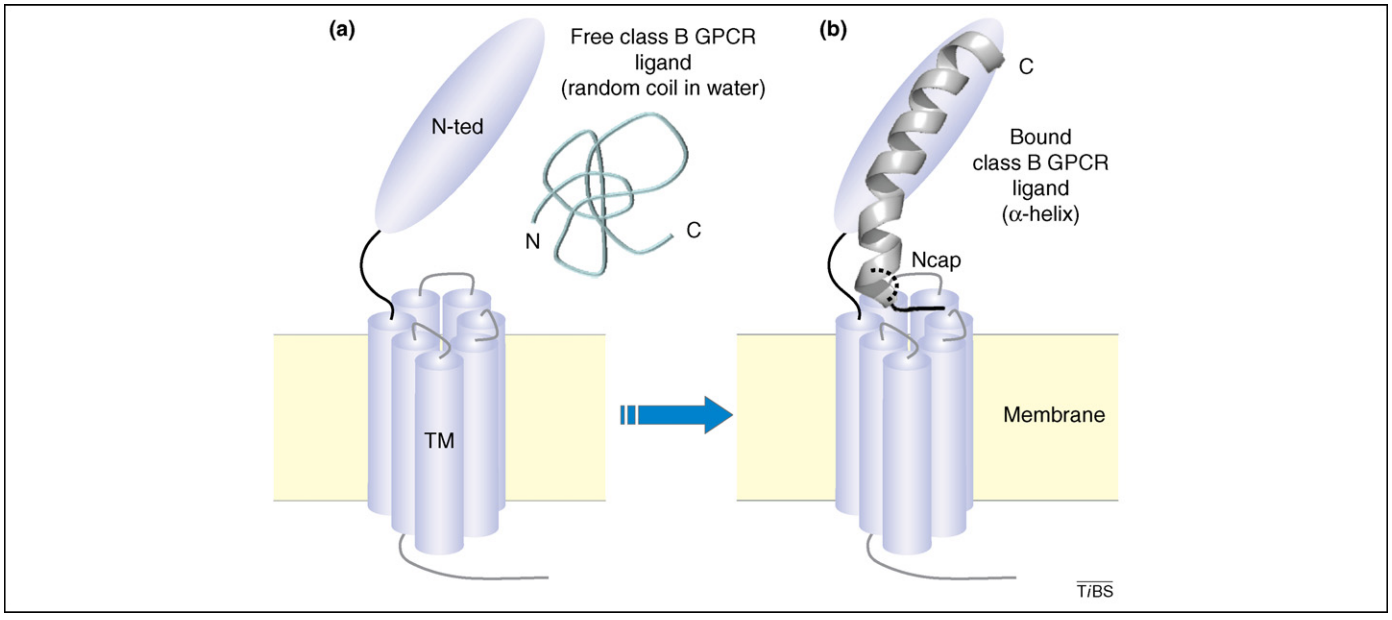

Figure 4. Cartoon representation of class B GPCR–ligand interaction. In aqueous solution, peptide-ligands do not adopt well-defined conformations (random coil), whereas they adopt a well-defined conformation (α-helix) upon binding to GPCRs. In this model, the two-domain of class B GPCR interacts with peptide ligand. **(a)** The central core of ligand peptide up to its C-terminal region (C) binds to the receptor N-terminal extracellular ectodomain (N-ted). **(b)** The N-terminal (N) portion of peptide ligand adopts the appropriate orientation (Ncap) to bind to the transmembrane (TM) core of the receptor.

characteristic of an N-capping motif (Figure 3). Moreover, the PACAP21 Phe6 residue exhibits dihedral angles characteristics of a β-structure similar to those adopted by the same residue in an N-capping structure. The hydrophobic clustering of the side chains of Tyr10, Phe6 and Ile5 observed for the bound PACAP21 peptide is also consistent with a folded capping structure. However, Thr7 does not adopt β-dihedral angles characteristic of an N-capping structure but, instead, disallows dihedral angle values, which could result from either experimental bias or environmental constrains. A model of PACAP21 bound to its PAC1 receptor [23] was built using a representative conformer with a hydrogen bond imposed between Thr7 and Tyr10 (Figure 3). In this model, Thr7 dihedral angle values now reach canonical values for N-capping and similar relative positioning of Tyr10, Phe6 and Ile5 is observed. Nevertheless, as with calcitonin, the PACAP N-terminal segment bound to its receptor exhibits a folded conformation very similar to that induced by an N-capping structure.

Concluding remarks and future perspectives

A speculative, but largely accepted, mechanism for peptide–ligand interaction with class B GPCRs has emerged and is termed the 'two-domain' model [31,32]. In this model (Figure 4), the central and C-terminal segment of a peptide ligand binds to the N-ted of the receptor, which positions the N terminus of the peptide ligand in the appropriate orientation for interacting with the transmembrane region of the receptor [42]. The recent 3D structures of several class B GPCRs N-teds in complex with a peptide ligand [43–47] indicate that the N terminus of the peptide ligand is not involved in the interaction with the extracellular domain. In the 'two-domain' model, the interaction of the peptide ligand with the receptor transmembrane region triggers the activation process and is thought to involve a specific network of ligand–receptor intermolecular interactions that probably requires well-defined spatial positioning of key N-terminal ligand side-chains. In searching for N-terminal structural motif of peptide ligands, we found that the N-capping motif is strikingly highly conserved throughout the family of class B GPCR peptide ligands [33]. Such a structural motif with a specific folded-backbone conformation imposes a specific spatial positioning of the amino acid side-chains. This positioning is likely to govern the pharmacological specificity of each class B GPCR ligand. Moreover, a natural ligand, calcitonin, contains a disulfide bond that imposes a similar backbone conformation to that of an N-capping structure. Therefore, we propose that the N-capping motif constitutes an essential structural element in the receptor-activation process. This motif could serve as a basis for the rational design of drugs that target class B GPCRs. For example, chemical structures could be synthesized that mimic capping conformations using non-natural amino acids or non-peptidic elements and position pharmacophore mimicking side-chains of amino acids specific to each class B GPCR.

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