Structural basis of natural ligand binding and activation of the Class II G-protein-coupled secretin receptor

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Abstract

The secretin receptor is prototypic of Class II GPCRs (G-protein-coupled receptors), based on its structural and functional characteristics and those of its natural agonist ligand. Secretin represents a linear 27-residue peptide with diffuse pharmacophoric domain. The secretin receptor includes the typical signature sequences for this receptor family within its predicted transmembrane segments and the highly conserved six cysteine residues contributing to three intradomain disulfide bonds within its long N-terminus. This domain is critical for secretin binding based on receptor mutagenesis and photoaffinity labelling studies. Full agonist analogues of secretin incorporating a photolabile moiety at various positions throughout the pharmacophore covalently label residues within this region, while only N-terminal probes have labelled the core helical bundle domain. Combining insights coming from receptor structural studies, peptide structure–activity relationship considerations, photoaffinity labelling, and application of fluorescence techniques has resulted in the development of a working model of the secretin–receptor complex. This supports the initial docking of the peptide agonist within a cleft in the receptor N-terminus, providing the opportunity for an endogenous sequence within that domain to interact with the core of the receptor. This interaction is believed to be key in the molecular basis of conformational change associated with activation of this receptor. The site of action of this endogenous agonist could also provide a possible target for small molecule agonists to act.

Introduction

The superfamily of guanine nucleotide-binding protein (G-protein)-coupled receptors represents the largest group of cell membrane receptors and the most common type of target for existing drugs. This reflects the extraordinary structural diversity in natural agonist ligands capable of binding and activating these receptors and the presence of these receptors on essentially every excitable and regulatable cell in the body. Consistent with the diversity of natural ligands for these receptors, ranging from photons and odorants to lipids, peptides, large proteins and even to viral particles, the receptors can be organized into families and subfamilies based on structural motifs that often correlate with the structures of the ligands. Although essentially all receptors in this superfamily share a heptahelical TM (transmembrane) structure and couple with heterotrimeric G proteins, a series of large groups or classes of these receptors are organized based on conserved motifs and signature sequences, while smaller subgroups or families are organized based on finer degrees of structural details [1]. Sequence characteristics of cloned orphan receptors in this superfamily have often been utilized to identify the natural ligands for those receptors [1].

Key words: G-protein-coupled receptor (GPCR), ligand binding, mutagenesis, photoaffinity labelling, receptor activation, secretin receptor.

Abbreviations used: GPCR, G-protein-coupled receptor; TM, transmembrane.

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Class II GPCRs (G-protein-coupled receptors) share the characteristics of having signature sequences within their predicted TM segments and having a relatively long N-terminal extracellular tail region in excess of 115 residues that contains six highly conserved cysteine residues that form three conserved intradomain disulfide bonds [2,3]. All of the Class II receptors bind moderately long peptides in excess of 25 residues that have diffuse pharmacophoric domains. Receptors in this family include potentially important drug targets that bind and are activated by hormones such as glucagon, parathyroid hormone, calcitonin, vasoactive intestinal polypeptide, secretin and corticotrophin-releasing factor.

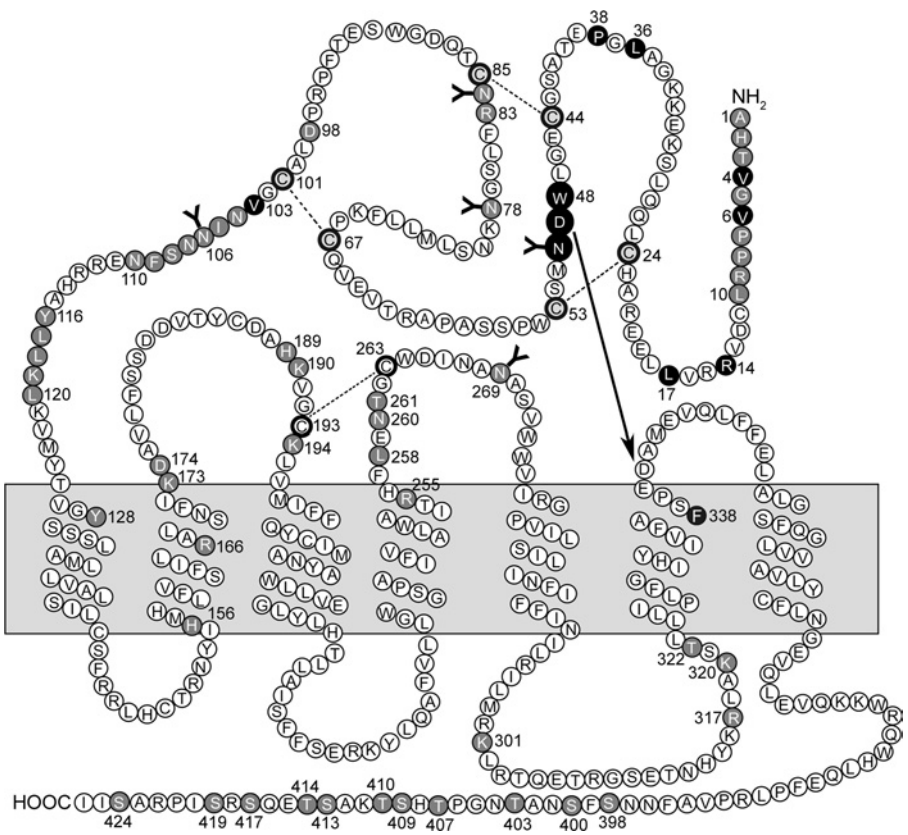
Secretin receptor

The first receptor in this family to be cloned was the secretin receptor [4]. It is prototypic of the family based on all characteristics reported to date, including its structural motifs, its structure–function relationships, its mode of signalling and its mechanism of regulation [5]. This receptor is present on ductular epithelial cells of the pancreas and hepatobiliary tree, as well as certain regions of the brain. Major physiological functions attributed to secretin include the stimulation of alkaline secretion into the upper intestine.

Much has been learned about the structure and molecular basis of activation of the secretin receptor, based on

Figure 1 | Schematic diagram of the rat secretin receptor sequence highlighting sites believed to be important for ligand binding or receptor function

Residues that have been covalently labelled in photoaffinity labelling studies are illustrated in filled black circles. Potential sites of phosphorylation and those residues demonstrated by mutagenesis to be functionally important are shown as filled grey circles. Disulfide bonds are shown using broken lines and potential sites of glycosylation are indicated by forked symbols. The endogenous agonist sequence within the N-terminus of the receptor (WDN) is highlighted in large filled black circles and an arrow points towards the region proposed to represent its site of action.



structure–activity relationships of ligand and receptor, receptor mutagenesis, photoaffinity labelling studies and use of fluorescence to probe microenvironments [6–9]. These studies, along with the key structural insight recently coming from the solution of the NMR structure of the isolated N-terminus of the closely related corticotrophin-releasing factor receptor [2], have provided a coherent working hypothesis for the mode of binding and mechanism of action of this hormone. This is also consistent with data reported for other members of this receptor family and will likely be predictive of a theme of action broadly applicable to these receptors. Figure 1 illustrates many of the features of the secretin receptor that have come from a variety of experimental approaches that are detailed further below.

Secretin peptide

Secretin is a linear 27-amino-acid peptide with important residues distributed throughout its length [5]. The C-terminal half of this peptide has been shown to be critical for high affinity binding, while the N-terminal half of this peptide is key for its biological activity [10]. This type of observation has led to a hypothesis for two domains of binding, with the C-terminus of the ligand binding to the receptor N-terminus, and the N-terminus of the ligand possibly interacting with the receptor body for activation [11]. While there have been numerous observations supporting the former and the critical importance of the N-terminus of Class II receptors for natural ligand binding [12–15], the importance of proposed direct interaction of the ligand with the receptor body is less clear. While such interactions could provide the basis for the peptide to act as a tether to exert tension on the receptor body to change its conformation to an active conformation [11], an alternative mechanism for activation was recently proposed [16]. In this possible mechanism, the peptide binds to the N-terminus of the receptor, resulting in a conformational change in that domain that exposes a previously hidden epitope (WDN) within it that can act as an endogenous agonist ligand, which interacts with the receptor body to effect the activating conformational change [16]. While the N-terminal regions

Table 1 | Spatial constraints between secretin and its receptor identified by photoaffinity labelling

Bpa, *p*-benzoyl-L-phenylalanine; (BzBz)Lys, *p*-benzoylbenzoyl-L-lysine.

Photolabile secretin probe	Labelled receptor domain	Labelled receptor residue	Reference
[Bpa^{1}]secretin	TM domain 6	Phe^{338}	[11]
[Bpa^{6}]secretin	N-terminal domain	Val^{4}	[18]
[(BzBz)Lys^{12}]secretin	N-terminal domain	Val^{6}	[6]
[Bpa^{13}]secretin	N-terminal domain	Val^{103}	[21]
[(BzBz)Lys^{14}]secretin	N-terminal domain	Pro^{38}	[6]
[(BzBz)Lys^{18}]secretin	N-terminal domain	Arg^{14}	[20]
[Bpa^{22}]secretin	N-terminal domain	Leu^{17}	[19]
[Bpa^{26}]secretin	N-terminal domain	Leu^{36}	[17]

of natural peptide ligands are probably located in spatial approximation both with the receptor N-terminus and with the receptor body, the action of the peptide as a tether and its critical or required interaction with the receptor body has not been established.

Secretin–receptor docking

Photoaffinity labelling is a powerful experimental approach to explore spatial approximations between residues within a ligand and within its receptor, as normally docked. When affinity labelling of a receptor residue is accomplished using a fully efficacious analogue of the natural hormone that incorporates a photolabile site of covalent attachment within the ligand pharmacophore, the spatial approximation between the two residues can be safely inferred. We have successfully applied this technique to the secretin receptor using photolabile secretin analogue probes with sites of covalent attachment distributed throughout the peptide, in positions 1, 6, 12, 13, 14, 18, 22 and 26 [6,11,17–21]. These probes labelled the secretin receptor in positions noted in Table 1. It is remarkable that all of these probes, except for those at the N-terminus [11], covalently labelled residues within the receptor N-terminal domain. In fact, most of them labelled the distal N-terminus within the first 36 residues.

This is particularly interesting, since the NMR structure of the corticotrophin-releasing factor receptor N-terminus was poorly refined in the regions analogous to those in which the secretin receptor has been affinity labelled. The NMR structure was best established for only a relatively stable core of the receptor N-terminus [2]. Of note, the structure of that region did change upon ligand binding (the antagonist astressin was utilized). However, it was not clear if this was also a site of direct ligand interaction with the receptor. The photoaffinity labelling data for the secretin receptor is actually most consistent with ligand interaction with less conserved regions of the N-terminus, subsequently yielding a conformational change in the conserved core of this domain. Indeed, we recently proposed that ligand binding and conformational change in the receptor N-terminus lead to exposure of a previously hidden conserved epitope that can act as an endogenous agonist ligand [16].

Mechanism of ligand-induced receptor activation

The N-terminal domain of Class II GPCRs has its structure constrained by three conserved disulfide bonds that yield three broad loops [2]. Another smaller loop region seems to be established by antiparallel β-sheets shown in the NMR structure of the corticotropin-releasing factor receptor within the larger region between the second and third conserved cysteine residues [2]. Indeed, it was this more focused region that was shown to have agonist activity at the secretin receptor [16]. This could even be localized and minimized to a cyclic tripeptide structure containing the sequence WDN. While being only weakly potent ($EC_{50} = 5.9\ \mu M$), its affinity for binding (approximated using an IC_{50} value) was shown to be $0.9\ \mu M$. This was still consistent with the proposed mechanism of action, since this is a 'tethered' ligand held in place near its proposed site of action by the approximation of receptor N-terminus with receptor body [16]. The region of action within the receptor body was suggested by demonstrating that it continued to have full agonist activity with an N-terminally-truncated receptor construct that had no response to natural secretin. Further refinement was effected by building two photolabile analogues of the cyclic WDN peptide that had a benzoyl-phenylalanine attached to either its N- or its C-terminus. Both covalently labelled the same region of the secretin receptor predicted to reside above TM segment six [16].

This key concept of endogenous agonist within the receptor N-terminus was extended to the calcitonin receptor and to the VPAC1 (vasoactive intestinal peptide/pituitary adenylate cyclase-activating peptide receptor 1) receptor [16], consistent with a conserved mechanism of action throughout this receptor family. This should be useful for the correct alignment of the N-terminus of Class II GPCRs with their TM helical confluence in molecular models. It is noteworthy that the helical confluence of receptors in this family may be quite distinct from the better defined helical confluence of Class I receptors such as rhodopsin, based on the chemical nature of conserved residues within the aligned sequences of Class II receptor TM segments [22,23]. Signature sequences within the TM segments of Class I and Class II receptors are quite distinct [23].

Fluorescence studies

Fluorescence techniques can provide complementary insights into the environment of indicators that can be situated at distinct sites throughout a ligand bound to its receptor. We have characterized the fluorescence of secretin agonist ligands incorporating Alexa Fluor® into positions at the N-terminus, C-terminus and into positions 13 and 22 within the peptide [7]. Collisional quenching with potassium iodide demonstrated that the N-terminal probe was most accessible of all of these probes to the aqueous milieu, consistent

with the evolving molecular model. Of interest, quenching constants for each probe were higher when bound in the active state than in the G-protein-uncoupled, inactive, low-affinity state. The most marked changes in fluorescence properties associated with change in receptor affinity state occurred for the two mid-region probes. Anisotropy and fluorescence lifetime data were consistent with this set of observations, with higher anisotropy and longer lifetimes when the receptor was in its inactive state [7]. These data suggest that the mid-region of secretin is more buried and protected by the receptor in its inactive state and is more exposed to the aqueous milieu with the shift to the active conformation. This, too, supports a substantial conformational change in the N-terminal secretin-binding domain upon receptor activation.

Summary

The secretin receptor is prototypic of Class II GPCRs, binding a long peptide ligand having a diffuse pharmacophoric region to the receptor N-terminal domain. Although the details of this are not yet fully established, there seems to be a peptide-binding cleft above the conserved core of this receptor domain. The peptide is likely to reside within this cleft, oriented so that its N-terminus is directed towards the receptor body. A conserved portion of the receptor N-terminus can be exposed and oriented towards the receptor body upon agonist docking, and can independently act as an endogenous agonist ligand to elicit a conformational change, to induce G-protein coupling and to initiate intracellular signalling. It will be critical to refine our insights into this sequence of events and to establish how well this proposed molecular mechanism might be conserved throughout this receptor family.

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