Mechanisms of peptide and nonpeptide ligand binding to Class B G-protein-coupled receptors

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Class B G-protein-coupled receptors are a small family of 15 peptide-binding receptors. This family includes at least six biologically attractive therapeutic targets for both peptide ligands (osteoporosis and Type II diabetes) and nonpeptide ligands (anxiety, depression and migraine). A general mechanism of peptide binding has emerged for this receptor family, termed the two-domain model. In this mechanism, the C-terminal ligand region binds the extracellular N-terminal domain of the receptor. This interaction acts as an affinity trap, promoting interaction of the N-terminal ligand region with the juxtamembrane domain of the receptor. Peptide binding to the juxtamembrane domain activates the receptor and stimulates intracellular signaling. Nonpeptide ligands bind the juxtamembrane or N-terminal domain and, in most cases, allosterically modulate peptide-ligand binding. Here, these mechanisms of peptide and nonpeptide ligand binding are reviewed, then applied in a discussion of the future strategies of drug development for Class B G-protein-coupled receptors.

The Class B family of G-protein-coupled receptors (GPCRs) (also referred to as the secretin receptor family) comprises 15 peptide-binding receptors in humans, with no apparent orphan receptors [1,2] (Table 1). These receptors comprise an extracellular N-terminal domain (of ~100–160 residues) and a juxtamembrane domain of seven membrane-spanning α-helices. Class B GPCRs share little apparent sequence homology with Class A receptors (rhodopsin-like) or Class C receptors (e.g. $GABA_B$) [1,2]. Class B receptors are activated by endogenous peptide ligands of intermediate size (typically ~30–40 amino acid residues). These peptides include hormones, neuropeptides and autocrine factors that mediate diverse physiological functions (Table 1). The peptide-binding Class B receptors share low sequence homology with two other groups of receptors that have been suggested to belong to the Class B family: (i) the frizzled/smoothened receptors, which bind proteins of 350–360 amino acids (Wnts); and (ii) large N-terminal receptors, which bear single or repeating extracellular consensus sequences within their N-termini [2]. These two receptor groups are not considered in this review.

Several Class B GPCR–ligand systems are biologically attractive for treatment of disease (see Table 2 for a detailed list). For example, calcitonin and parathyroid hormone (PTH) regulate bone turnover and calcium homeostasis [3,4], and both of these peptides are effective treatments for osteoporosis [3,5] (Table 2). Two peptides that modulate insulin release and glucose homeostasis are potential novel therapies for Type II diabetes [glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP) [6]; see Table 2]. Corticotropin-releasing factor (CRF) is a principle mediator of the body's response to stress [7], and antagonism of central CRF_1 receptors has been rationalized as a potential next-generation treatment for anxiety and depression

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TABLE 1

Classification of human Class B G-protein-coupled receptors and their peptide ligands[a]

Receptor	Peptide ligand(s)	Principal biological actions	Refs
CRF_1	CRF	ACTH release, central stress responses	[7]
	UCN1	Central stress responses	[7]
CRF_2	UCN1	Central stress responses	[7]
	UCN2	Cardiac contractility	[7]
	UCN3	Hearing	[7]
GHRH	GHRH	Release of growth hormone	[22,78]
GIP	GIP	Insulin secretion	[22,78]
Glucagon	Glucagon	Regulation of blood glucose	[22,78]
GLP-1	GLP-1	Insulin and glucagon secretion	[22,78]
GLP-2	GLP-2	Gut mucosal growth	[22,78]
PTH1	PTH	Ca^{2+} homeostasis	[4]
	PTHrP	Developmental regulator	[79]
PTH2	TIP39	Hypothalamic secretion, nociception	[80]
Secretin	Secretin	Pancreatic secretion	[22,78]
$VPAC_1$	VIP	Vasodilation, neuroendocrine functions	[22,81]
	PACAP	Neurotransmission, neuroendocrine functions	[22,81]
$VPAC_2$	VIP	Vasodilation, neuroendocrine functions	[22,81]
	PACAP	Neurotransmission, neuroendocrine functions	[22,81]
PAC_1	PACAP	Neurotransmission, neuroendocrine functions	[22,81]
Calcitonin	Calcitonin	Ca^{2+} homeostasis	[3]
Calcitonin; RAMP1	CGRP	Vasodilation	[20,23]
	Amylin	Reduces feeding	[20,23]
Calcitonin; RAMP3	Amylin	Reduces feeding	[20,23]
CL; RAMP1	CGRP	Vasodilation	[20,23]
CL; RAMP2	Adrenomedullin	Vasodilation	[20,23]
CL; RAMP3	Adrenomedullin	Vasodilation	[20,23]
	CGRP	Vasodilation	[20,23]

[a]Abbreviations: ACTH, adrenocorticotropin hormone; CGRP, calcitonin gene-related peptide; CL, calcitonin receptor-like receptor; CRF, corticotropin-releasing factor; GHRH, growth hormone-releasing hormone; GIP, glucose-dependent insulinotropic peptide; GLP, glucagon-like peptide; PTH, parathyroid hormone; PTHrP, parathyroid hormone-related protein; TIP39, tuberoinfundibular peptide of 39 residues; PAC, pituitary adenylate cyclase; PACAP, pituitary adenylate cyclase-activating polypeptide; RAMP, receptor activity-modifying proteins; VIP, vasoactive intestinal peptide; UCN, urocortin.

[8]. Nonpeptide antagonists of the CRF_1 receptor have reached early stages of clinical development [9] (Table 2). These results and physiological findings indicate that Class B GPCRs are highly attractive therapeutic targets from a biological perspective. However, the receptors are frequently considered difficult targets for drug development. The ligands that are in clinical use or at a late stage of development are all peptides, and drug-like nonpeptide ligands have only been identified for a small number of receptors (Table 2).

The mechanisms of ligand interaction with Class B GPCRs have been studied in detail. A general mechanism of peptide binding has emerged, and the mechanisms by which nonpeptide ligands bind and function are beginning to be elucidated. The purpose of this review is to present an overview of these mechanisms, and to consider how these mechanisms can be used to aid the future development of therapeutic ligands targeting Class B GPCRs.

Structure of Class B GPCRs and their peptide ligands

Class B GPCRs comprise a moderately sized, extracellular N-terminal domain of ~100–160 amino acid residues (here termed the N-domain), connected to a juxtamembrane domain (J-domain) of seven membrane-spanning α-helices with intervening loops and a C-terminal tail (exemplified by the PTH1 receptor in Figure 1). The receptors are glycosylated, with consensus sequences for asparagine-linked glycosylation located within the N-domain and, in some instances, extracellular loops. Intracellular loops interact with G-proteins to stimulate intracellular signaling, predominantly through G_s-coupled pathways and generally, to a lesser extent, through G_q and G_i. However, the relative strength of these signaling pathways can be regulated by accessory proteins [10].

The structure of the N-domain has been determined by nuclear magnetic resonance (NMR) spectroscopy for the $CRF_{2(b)}$ receptor [11] (Figure 2a). A central core contains a salt bridge sandwiched between aromatic side chains, surrounded by conserved residues. Two anti-parallel β-sheets are interconnected by the core [11]. The tertiary structure is stabilized by three disulfide bonds between six conserved cysteine residues, in an arrangement that has also been demonstrated for PTH1 (Figure 1, [12]), CRF_1 [13], and GLP-1 [14] receptors. The structure of the J-domain is largely unknown. This region displays no significant overall sequence homology with rhodopsin, for which the crystal structure has been determined [1]. Limited mutagenesis [15] and zinc-bridging studies [16] suggest that certain inter-helix interactions are similar to rhodopsin, for example, a putative interaction between transmembrane (TM) helix two and seven of the PTH1 receptor [15] (Figure 1). The arrangement of the TM regions has been investigated using computer modeling [1], and homology models based on the rhodopsin crystal structure have been developed (e.g. Figure 2b for the PTH1 receptor [17,18]), but the predictive utility of these models remains to be determined.

Some Class B GPCRs are highly unusual in that their ligand-binding properties are profoundly affected by accessory proteins [19]. The calcitonin receptor and calcitonin receptor-like receptor (CL receptor) interact non-covalently with receptor activity-modifying proteins (RAMPs), changing the responsiveness of these receptors to peptides of the calcitonin family (Table 1). The mechanism of the RAMP effect has been reviewed elsewhere [20]. The effect on ligand binding involves close contact between the RAMP and the GPCR, suggesting a direct participation