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PERSPECTIVE

Class B GPCRs: A Hidden Agonist Within?

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ABSTRACT

Class B G protein-coupled receptors (GPCRs) regulate a wide range of endocrine and neuroendocrine functions and are endogenously stimulated by moderately large peptide hormones. Current evidence suggests that the carboxyl termini of cognate peptides bind to the amino terminus of their G protein-coupled receptors (GPCRs) and that the peptides' amino terminal segments then dock to the heptahelical receptor portion to induce signaling. In this issue of *Molecular Pharmacology*, Dong et al. (p. 206) propose an alternative model of ligand-induced class B GPCR activation. Based primarily on studies with the secretin receptor, a prototype class B family member, they provide evidence that the endogenous peptide hormone does not function as an activator per se. Instead, this hormone (secretin) exposes a hidden, built-in agonist epitope that is present within the amino terminus of its target GPCR. Isolated oligopeptide fragments containing this epitope act as full agonists on the secretin receptor despite their lack of amino acid homology with the secretin hormone. These nonconventional agonists can be minimized to tripeptide molecules and still maintain biological activity. The study to be discussed introduces a novel paradigm of class B GPCR function, and may facilitate the elusive goal of finding small molecule agonist drugs for this therapeutically attractive group of receptors.

G protein-coupled receptors (GPCRs) form a large group of membrane proteins that are activated by cognate hormones and transmitters to trigger intracellular signaling cascades. These receptors play an important role in many physiological and pathophysiological processes (Pierce et al., 2002). Not surprisingly, GPCRs therefore provide therapeutic targets for a major portion of currently used drugs (Howard et al., 2001; George et al., 2002). To date, much of what is known regarding the principles and mechanisms that underlie GPCR activation has been derived from studying members of a major subfamily of these proteins, class A rhodopsin-type receptors (Gether, 2000; Ballesteros et al., 2001). Considerably less is known about respective processes that apply to class B, secretin-type GPCRs.

The class B family includes receptors for moderate-sized peptides that are involved in regulating important endocrine and neuroendocrine functions (Ulrich et al., 1998; Harmar, 2001). In addition to the prototype member, the secretin receptor, structurally related mammalian class B GPCRs include the calcitonin and calcitonin receptor-like, corticotropin-releasing factor (CRF), gastric inhibitory peptide, glucagon, glucagon-like peptide, growth hormone-releasing hormone, parathyroid hormone (PTH), pituitary adenylate cyclase-activating peptide, and vasoactive intestinal polypeptide receptors. Although these GPCRs share a similar seven transmembrane domain topology with their class A counterparts, there is virtually no amino acid conservation between the two groups of proteins. The relative paucity in current knowledge on ligand interaction and receptor activation of class B GPCRs is reflected by the difficulty in identifying synthetic, small molecule drugs that either mimic or block the function of endogenous peptide ligands.

In the current issue of *Molecular Pharmacology*, Dong et al. (2006b) propose a novel paradigm of ligand-induced class B receptor activation. Their observations, made primarily with the secretin receptor, not only extend current views on how at least some of these proteins may function but also offer the potential to open exciting new avenues in drug discovery.

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ABBREVIATIONS: GPCR, G protein-coupled receptor; CRF, corticotropin-releasing factor; PTH, parathyroid hormone.

An Unexpected Agonist Epitope within the Secretin Receptor

Earlier studies based on complementary mutagenesis, photoaffinity cross-linking, and structural modeling approaches of several receptors and cognate peptides had led to a consensus model of ligand binding and activation of class B GPCRs (Hjorth and Schwartz, 1996; Grauschopf et al., 2000; Chorev, 2002; Unson, 2002; Al-Sabah and Donnelly, 2003; Castro et al., 2005; Tan et al., 2006). In this model, the carboxyl terminus of a peptide ligand initially binds to the long extracellular amino terminus of its cognate receptor. Thereafter, the ligand's amino terminus is believed to dock to the body of the receptor (i.e., the heptahelical membrane bundle including the extracellular loops). This, in turn, induces a conformational change that enables intracellular portions of the receptor to trigger signaling events. This basic model of class B receptor activation implies that interaction of the amino-terminal portion of the ligand with the receptor body is required for receptor activation and in large part determines the degree of agonist efficacy (Luck et al., 1999; Nielsen et al., 2000; Shimizu et al., 2000).

The results of a series of photoaffinity cross-linking studies with the class B secretin receptor generally supported the current model (Dong et al., 2003, 2004). However, in a recent publication, it was found that secretin peptides with minor amino-terminal modifications no longer directly cross-linked with the receptor's body. It is noteworthy that these secretin derivatives bound only to the amino terminus of the receptor yet still acted as full agonists (Dong et al., 2005). This observation led the authors to hypothesize that secretin's role in tethering the receptor's amino terminus with its body was not as critical for receptor activation as is commonly believed. Instead, they proposed an alternative mechanism of receptor activation in which an agonist epitope hidden within the receptor's amino terminus is exposed by a ligand-induced conformational change within this domain (Fig. 1). Supporting this theory, they demonstrated that isolated peptides corresponding to segments of the secretin receptor's amino terminus activated the full-length receptor (no effect was observed in cells where the targeted GPCR was not expressed). The receptor-derived agonist polypeptides had low potency and low affinity (in the micromolar range) but full agonist efficacy. It is intriguing that they share no appreciable sequence similarity with the traditional agonist, secretin. Furthermore, these polypeptides could be minimized to as little as three amino acids and still retain full efficacy, comparable with that of secretin (a 27-amino acid molecule).

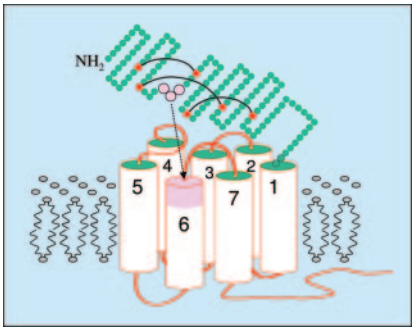

Fig. 1. Schematic diagram of a class B GPCR with an amino-terminal agonist epitope. Shown is the receptor's heptahelical transmembrane domain bundle (numbered cylinders) with connecting loops (red lines) as well as the long extracellular amino terminus (individual amino acids symbolized by circles). A defining structural feature of the amino terminus in secretin-type class B GPCRs is the presence of six highly conserved cysteine residues (red), which form three disulfide bridges (black lines) (Grauschopf et al., 2000; Lisenbee et al., 2005). The hidden built-in agonist epitope, comprising three core amino acids that are centered on a highly conserved aspartate in class B GPCRs (residue 49 in the secretin receptor) is symbolized by pink circles. It is proposed that binding of a cognate peptide hormone (not shown) induces a conformational change in the receptor's amino terminus that enables the built-in agonist epitope to dock near the top of transmembrane domain 6 (arrow). This in turn triggers a conformational change in the heptahelical bundle, thereby initiating downstream signaling.

How Might It Work?

In follow-up experiments, the authors altered the predicted three amino acid agonist epitope within the amino terminus of the secretin receptor. Substitution of tryptophan in position 48 with leucine (W48L) greatly reduced the efficacy of the conventional agonist (secretin) in stimulating this mutant GPCR. However, the W48L variant remained fully responsive to an isolated oligopeptide containing wild-type biologically active tripeptide sequence. The oligopeptide ligand thus showed markedly higher efficacy than secretin at the mutant receptor. These results provided initial evidence that the observed function of the conventional hormone, secretin, might be dependent on the action of a "built-in agonist" sequence.

Dong et al. (2006b) postulated that secretin may stimulate its GPCR by inducing a conformational change in the receptor's amino terminus, thereby exposing a hidden built-in agonist epitope. Earlier work by others lends credence to this theory. Recent structural studies with the isolated amino terminus of the CRF receptor type 1 have shown ligand-induced conformational changes that were most pronounced in the region that corresponds to the putative "hidden agonist" sequence in the secretin receptor (Grace et al., 2004). Because these studies with the CRF receptor were performed only with an antagonist ligand and used a receptor amino terminus that was isolated from the adjacent heptahelical domain, extrapolations that can be made to the current secretin receptor study are limited. Structural analyses of secretin receptor-ligand complexes will be needed to experimentally evaluate whether and how built-in agonist function really relates to conventional ligand binding.

Although the exact interplay between secretin and the receptor's agonist epitope remains speculative at this point, there is initial evidence suggesting a possible mode of action by the built-in agonist. Through an elegant series of photoaffinity cross-linking and mapping experiments using site-specific enzymatic digests, Dong et al. (2006b) were able to demonstrate that an oligopeptide comprised of built-in agonist sequence docked near the top of transmembrane domain 6 in the secretin receptor, at the transition with extracellular loop 3. This observation suggests that the secretin receptor's amino terminus folds down to make contact with its body, thereby enabling the built-in agonist sequence to interact with transmembrane domain 6, a region well known to play a key role in the activation of many GPCRs (Gether, 2000).

Questions and Puzzles

The concept of a built-in agonist, although new and unexpected for a class B GPCR, shows certain similarities with

the activation mechanism of the class A, proteinase activated receptors (Hollenberg and Compton, 2002). Unlike the secretin receptor, the built-in agonist of the proteinase activating receptors is irreversibly exposed upon cleavage of amino-terminal receptor sequence by activating enzymes. The amino terminus of the class A glycoprotein hormone receptors also seems to play a role in receptor activation. However, the amino termini of the former GPCRs are believed to act as inverse agonists in the resting state. Binding of a peptide ligand activates the receptor by switching this domain to an agonist (Vassart et al., 2004). The existence of these precedents strengthens the plausibility of the new activation mechanism now proposed for the secretin receptor.

Findings with the secretin receptor raise even more questions that are being answered. As has been discussed, structural evidence is needed to establish that secretin binding causes a conformational change in the amino terminus of the receptor consistent with exposure of the putative hidden built-in agonist. It also remains to be investigated whether the effects of changes to functionally relevant amino acids in the receptor's putative agonist epitope are mimicked when the corresponding changes are made to a homologous oligopeptide ligand. For example, will a tryptophan-to-leucine change in the isolated agonist tripeptide (corresponding to the W48L substitution in the receptor epitope, leading to loss of secretin function) compromise efficacy of the modified ligand? This is predicted based on the proposed mechanism of action, and, if experimentally verified, would further support the underlying theory.

Furthermore, it is noteworthy that the apparent docking site of the receptor-derived agonist peptide (top of transmembrane domain 6) represents the same receptor segment to which the amino terminus of the conventional agonist, secretin, has been previously shown to bind (Dong et al., 2004). What is the significance of these very similar topologies? Does binding of secretin to this region, although not directly inducing receptor activation, help to position the receptor amino terminus to enable built-in agonist function? Considering the as-yet-unresolved relationship between secretin binding and function of the built-in agonist, it would also be interesting to know how the latter is affected by conventional peptide antagonists. For class B GPCRs, peptide antagonists are typically modified agonists which are truncated or altered at the amino terminus, where agonist function is believed to reside (Rosenblatt and Potts, 1981; Unson et al., 1987; Montrose-Rafizadeh et al., 1997). Current evidence suggests that such antagonists primarily act by competing with conventional agonists for binding to the receptor's amino terminus (Lopez de Maturana et al., 2003). If so, reported antagonists at the secretin receptor (Dong et al., 2006a) should be ineffective in blocking stimulation induced by oligopeptides derived from built-in agonist sequence.

Perspectives for Drug Discovery

The potential impact of findings reported by Dong et al. (2006b) on future drug discovery efforts deserves particular attention. Despite major screening efforts within the pharmaceutical industry, clinically desirable small-molecule nonpeptide agonists directed at verified class B GPCR targets [e.g., the glucagon-like peptide 1 receptor for the treatment of diabetes (Knudsen, 2004) or the PTH receptor type 1 for the treatment of osteoporosis (Carter and Schipani, 2006)] have yet to be reported. So far, the vast majority of known small-molecule ligands of class B GPCRs target only two family members, the glucagon (Ladouceur et al., 2002; Madsen et al., 2002; Kurukulasuriya et al., 2004; Duffy et al., 2005) and CRF (Kehne and De Lombaert, 2002; Hartz et al., 2004) receptors. All of these compounds seem to lack functional activity and act as neutral antagonists. Given this limitation, it is even more remarkable that three amino acids were sufficient to induce full (albeit low potency) agonism as reported by Dong et al. (2006b). Will such compounds provide leads to develop higher potency agonists and possibly antagonists? Can the proposed concept of a hidden built-in agonist be applied to other members of the class B family?

To begin to address these questions, the authors demonstrated that the potency of a tripeptide secretin receptor agonist (based on built-in agonist sequence) can be increased, to a limited extent, by further modification of this compound (cyclization and myristoylation). Furthermore, they found that in addition to the secretin receptor, analogous small-molecule peptide agonists can be constructed based on putative built-in agonist sequence within two other class B GPCRs, the calcitonin and vasoactive intestinal peptide type 1 receptors. Although these observations suggest that the underlying concept is more widely applicable, the initial findings also revealed that none of the new agonist peptides was fully selective for its targeted GPCR. Each tested compound cross-reacted with one of the two nontargeted control class B receptors. The extent to which these early stage compounds can be optimized in terms of receptor affinity/potency as well as selectivity through chemical engineering and/or high throughput screening remains to be determined.

Although preliminary evidence suggests that the built-in agonist concept is not limited to the secretin receptor, one should be aware that this idea can not necessarily be generalized to all secretin-type class B GPCRs. For the PTH receptor type 1, there is published evidence that even with removal of this GPCR's amino terminus, a conventional peptide ligand still functions as a full (albeit low-potency) agonist (Luck et al., 1999). Furthermore, it has been demonstrated that with this receptor, small amino-terminal peptide fragments of the conventional agonist can act as low-potency yet fully efficacious agonists. These oligopeptides and their derivatives seem to act via the PTH receptor's heptahelical body (i.e., independent of this GPCR's amino terminus) (Shimizu et al., 2001; Castro et al., 2005). These findings suggest that, at least for the PTH receptor type 1, known peptide agonists probably act in a direct and conventional manner that does not require a built-in agonist.

Despite questions that remain and caveats that should be kept in mind, the observations by Dong et al. (2006b) provide an exciting fresh view on class B GPCRs. The seminal idea that a hidden built-in agonist, rather than the cognate peptide hormones that have been traditionally studied, may be the actual trigger of receptor activation will inspire many further studies on this topic. From the standpoint of a molecular pharmacologist, these ideas promise to spur future investigation that is attractive both in furthering the understanding of the mechanisms of receptor activation as well as in potentially leading to new drugs.

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