Molecular Pharmacology Fast Forward. Published on April 21, 2006 as doi:10.1124/mol.106.025932

MOL 25932

Class B GPCRs: A hidden agonist within ?

Martin Beinborn

Molecular Pharmacology Research Center, Tufts-New England Medical Center,

Boston, Massachusetts, 02111

Molecular Pharmacology Fast Forward. Published on April 21, 2006 as DOI: 10.1124/mol.106.025932
This article has not been copyedited and formatted. The final version may differ from this version.

Running title: Built-in agonist epitope in class B receptors

Corresponding author:

Martin Beinborn, Molecular Pharmacology Research Center, Molecular Cardiology Research Institute, Tufts-New England Medical Center, 15 Kneeland Street, Boston, Massachusetts 02111, Tel. 617 636-7740; Fax 617 636-8692.

E-Mail: mbeinborn@tufts-nemc.org

Number of text pages: 20

Number of tables: 0

Number of figures: 1

Number of references: 39

Number of words in abstract: 197

Number of words in introduction: -

Number of words in discussion: 2173 (main text, excluding acknowledgements, figure legend, references)

Abbreviations used:

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

Molecular Pharmacology Fast Forward. Published on April 21, 2006 as DOI: 10.1124/mol.106.025932
This article has not been copyedited and formatted. The final version may differ from this version.

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 carboxy-termini of cognate peptides bind to the amino-terminus of their GPCRs, and that the peptides' amino terminal segments then dock to the heptahelical receptor portion to induce signaling. In the current issue of Molecular Pharmacology, Dong et al. 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 lacking amino acid homology with the secretin hormone. These non-conventional 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.

Molecular Pharmacology Fast Forward. Published on April 21, 2006 as DOI: 10.1124/mol.106.025932
This article has not been copyedited and formatted. The final version may differ from this version.

Perspective

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 (George et al., 2002; Howard et al., 2001). To date, much of what is known regarding the principles and mechanisms that underly GPCR activation has been derived from studying members of a major subfamily of these proteins, class A rhodopsin-type receptors (Ballesteros et al., 2001; Gether, 2000). 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 (Harmar, 2001; Ulrich et al., 1998). 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 which either mimic or block the function of endogenous peptide ligands.

In the current issue of Molecular Pharmacology, Dong et al. propose a novel paradigm of ligand-induced class B receptor activation (Dong et al., 2006b). Their observations, primarily made 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.

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 (Al-Sabah and Donnelly, 2003; Castro et al., 2005; Chorev, 2002; Grauschopf et al., 2000; Hjorth and Schwartz, 1996; Tan et al., 2006; Unson, 2002). In this model, the carboxy-terminus of a peptide ligand initially binds to the long extracellular amino-terminus of its cognate receptor. Subsequently, 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., 2004; Dong et al., 2003).

Molecular Pharmacology Fast Forward. Published on April 21, 2006 as DOI: 10.1124/mol.106.025932
This article has not been copyedited and formatted. The final version may differ from this version.

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. Interestingly, 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. Intriguingly, 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 to that of secretin (a 27 amino acid molecule).

How may 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, may be dependent on the action of a 'built-in agonist' sequence.

Dong et al. 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). Since 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 if and how 'built-in agonist' function really relates to conventional ligand binding.

While 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. 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-

Molecular Pharmacology Fast Forward. Published on April 21, 2006 as DOI: 10.1124/mol.106.025932
This article has not been copyedited and formatted. The final version may differ from this version.

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 appears 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 than 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

Molecular Pharmacology Fast Forward. Published on April 21, 2006 as DOI: 10.1124/mol.106.025932
This article has not been copyedited and formatted. The final version may differ from this version.

eptitope, 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 of note that the apparent docking site of the receptor-derived agonist peptide (top of transmembrane domain 6) represents the same receptor segment where 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, while not directly inducing receptor activation, help to position the receptor amino-terminus as 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 of interest 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 (Montrose-Rafizadeh et al., 1997; Rosenblatt and Potts, 1981; Unson et al., 1987). 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. on future drug discovery efforts deserves particular attention. Despite major screening efforts within the pharmaceutical industry, clinically desirable small molecule non-peptide agonists

Molecular Pharmacology Fast Forward. Published on April 21, 2006 as DOI: 10.1124/mol.106.025932
This article has not been copyedited and formatted. The final version may differ from this version.

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. To date, the vast majority of known small molecule ligands of class B GPCRs target only two family members, the glucagon (Duffy et al., 2005; Kurukulasuriya et al., 2004; Ladouceur et al., 2002; Madsen et al., 2002) and CRF (Hartz et al., 2004; Kehne and De Lombaert, 2002) receptors. All of these compounds appear to lack functional activity and act as neutral antagonists. Given this limitation, it is all the more remarkable that three amino acids were sufficient to induce full (albeit low potency) agonism as reported by Dong et al. (Dong et al., 2006b). Will such compounds provide leads to develop higher potency agonists, and possibly antagonists as well ? 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. While 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 non-targeted 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 appear to act via the PTH receptor's heptahelical body, i.e. independent of this GPCR's amino terminus (Castro et al., 2005; Shimizu et al., 2001). These findings suggest that, at least for the PTH receptor type 1, known peptide agonists likely 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. provide an exciting fresh view on class B GPCRs. The seminal idea that a hidden 'built-in agonist', rather than 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 a molecular pharmacologist's standpoint, 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.

Molecular Pharmacology Fast Forward. Published on April 21, 2006 as DOI: 10.1124/mol.106.025932
This article has not been copyedited and formatted. The final version may differ from this version.

Acknowledgements

I thank Edward McBride for carefully reading this manuscript and for helpful suggestions.

References

Al-Sabah S and Donnelly D (2003) A model for receptor-peptide binding at the glucagon-like peptide-1 (GLP-1) receptor through the analysis of truncated ligands and receptors. *Br J Pharmacol* 140:339-46.

Ballesteros JA, Shi L and Javitch JA (2001) Structural mimicry in G protein-coupled receptors: implications of the high-resolution structure of rhodopsin for structure-function analysis of rhodopsin-like receptors. *Mol Pharmacol* 60:1-19.

Carter PH and Schipani E (2006) The roles of parathyroid hormone and calcitonin in bone remodeling: prospects for novel therapeutics. *Endocr Metab Immune Disord Drug Targets* 6:59-76.

Castro M, Nikolaev VO, Palm D, Lohse MJ and Vilardaga JP (2005) Turn-on switch in parathyroid hormone receptor by a two-step parathyroid hormone binding mechanism. *Proc Natl Acad Sci U S A* 102:16084-9.

Chorev M (2002) Parathyroid hormone 1 receptor: insights into structure and function. *Receptors Channels* 8:219-42.

Dong M, Hosohata K, Pinon DI, Muthukumaraswamy N and Miller LJ (2006a) Differential Spatial Approximation Between Secretin and Its Receptor Residues in Active and Inactive Conformations Demonstrated by Photoaffinity Labeling. *Mol Endocrinol*. Mar 2; [Epub ahead of print]

Dong M, Li Z, Pinon DI, Lybrand TP and Miller LJ (2004) Spatial approximation between the amino terminus of a peptide agonist and the top of the sixth transmembrane segment of the secretin receptor. *J Biol Chem* 279:2894-903.

Dong M, Li Z, Zang M, Pinon DI, Lybrand TP and Miller LJ (2003) Spatial approximation between two residues in the mid-region of secretin and the amino terminus of its receptor. Incorporation of seven sets of such constraints into a three-dimensional model of the agonist-bound secretin receptor. *J Biol Chem* 18:18.

Dong M, Pinon DI, Asmann YW and Miller LJ (2006b) Possible endogenous agonist mechanism for activation of secretin family G protein-coupled receptors. *Mol Pharmacol*.

Dong M, Pinon DI and Miller LJ (2005) Insights into the structure and molecular basis of ligand docking to the G protein-coupled secretin receptor using charge-modified amino-terminal agonist probes. *Mol Endocrinol* 19:1821-36.

Duffy JL, Kirk BA, Konteatis Z, Campbell EL, Liang R, Brady EJ, Candelore MR, Ding VD, Jiang G, Liu F, Qureshi SA, Saperstein R, Szalkowski D, Tong S, Tota LM, Xie D, Yang X, Zafian P, Zheng S, Chapman KT, Zhang BB and Tata JR (2005) Discovery and investigation of a novel class of thiophene-derived antagonists of the human glucagon receptor. *Bioorg Med Chem Lett* 15:1401-5.

George SR, O'Dowd BF and Lee SP (2002) G-protein-coupled receptor oligomerization and its potential for drug discovery. *Nat Rev Drug Discov* 1:808-20.

Gether U (2000) Uncovering molecular mechanisms involved in activation of G protein-coupled receptors. *Endocr Rev* 21:90-113.

Grace CR, Perrin MH, DiGruccio MR, Miller CL, Rivier JE, Vale WW and Riek R (2004) NMR structure and peptide hormone binding site of the first extracellular domain of a type B1 G protein-coupled receptor. *Proc Natl Acad Sci U S A* 101:12836-41.

Grauschopf U, Lilie H, Honold K, Wozny M, Reusch D, Esswein A, Schafer W, Rucknagel KP and Rudolph R (2000) The N-terminal fragment of human parathyroid hormone receptor 1 constitutes a hormone binding domain and reveals a distinct disulfide pattern. *Biochemistry* 39:8878-87.

Harmar AJ (2001) Family-B G-protein-coupled receptors. *Genome Biol* 2:REVIEWS3013.

Hartz RA, Nanda KK, Ingalls CL, Ahuja VT, Molski TF, Zhang G, Wong H, Peng Y, Kelley M, Lodge NJ, Zaczek R, Gilligan PJ and Trainor GL (2004) Design, synthesis, and biological evaluation of 1,2,3,7-tetrahydro-6h-purin-6-one and 3,7-dihydro-1h-purine-2,6-dione derivatives as corticotropin-releasing factor(1) receptor antagonists. *J Med Chem* 47:4741-54.

Hjorth SA and Schwartz TW (1996) Glucagon and GLP-1 receptors: lessons from chimeric ligands and receptors. *Acta Physiol Scand* 157:343-5.

Hollenberg MD and Compton SJ (2002) International Union of Pharmacology. XXVIII. Proteinase-activated receptors. *Pharmacol Rev* 54:203-17.

Howard AD, McAllister G, Feighner SD, Liu Q, Nargund RP, Van der Ploeg LH and Patchett AA (2001) Orphan G-protein-coupled receptors and natural ligand discovery. *Trends Pharmacol Sci* 22:132-40.

Kehne J and De Lombaert S (2002) Non-peptidic CRF1 receptor antagonists for the treatment of anxiety, depression and stress disorders. *Curr Drug Targets CNS Neurol Disord* 1:467-93.

Knudsen LB (2004) Glucagon-like peptide-1: the basis of a new class of treatment for type 2 diabetes. *J Med Chem* 47:4128-34.

Kurukulasuriya R, Sorensen BK, Link JT, Patel JR, Jae HS, Winn MX, Rohde JR, Grihalde ND, Lin CW, Ogiela CA, Adler AL and Collins CA (2004) Biaryl amide glucagon receptor antagonists. *Bioorg Med Chem Lett* 14:2047-50.

Ladouceur GH, Cook JH, Doherty EM, Schoen WR, MacDougall ML and Livingston JN (2002) Discovery of 5-hydroxyalkyl-4-phenylpyridines as a new class of glucagon receptor antagonists. *Bioorg Med Chem Lett* 12:461-4.

Lisenbee CS, Dong M and Miller LJ (2005) Paired cysteine mutagenesis to establish the pattern of disulfide bonds in the functional intact secretin receptor. *J Biol Chem* 280:12330-8.

Lopez de Maturana R, Willshaw A, Kuntzsch A, Rudolph R and Donnelly D (2003) The isolated N-terminal domain of the glucagon-like peptide-1 (GLP-1) receptor binds exendin peptides with much higher affinity than GLP-1. *J Biol Chem* 278:10195-200.

Luck MD, Carter PH and Gardella TJ (1999) The (1-14) fragment of parathyroid hormone (PTH) activates intact and amino-terminally truncated PTH-1 receptors. *Mol Endocrinol* 13:670-80.

Madsen P, Ling A, Plewe M, Sams CK, Knudsen LB, Sidelmann UG, Ynddal L, Brand CL, Andersen B, Murphy D, Teng M, Truesdale L, Kiel D, May J, Kuki A, Shi S, Johnson MD, Teston KA, Feng J, Lakis J, Anderes K, Gregor V and Lau J (2002) Optimization of alkylidene hydrazide based human glucagon receptor antagonists. Discovery of the highly potent and orally available 3-cyano-4-hydroxybenzoic acid [1-(2,3,5,6-tetramethylbenzyl)-1H-indol-4-ylmethylene]hydrazide. *J Med Chem* 45:5755-75.

Montrose-Rafizadeh C, Yang H, Rodgers BD, Beday A, Pritchette LA and Eng J (1997) High potency antagonists of the pancreatic glucagon-like peptide-1 receptor. *J Biol Chem* 272:21201-6.

Nielsen SM, Nielsen LZ, Hjorth SA, Perrin MH and Vale WW (2000) Constitutive activation of tethered-peptide/corticotropin-releasing factor receptor chimeras. *Proc Natl Acad Sci U S A* 97:10277-81.

Pierce KL, Premont RT and Lefkowitz RJ (2002) Seven-transmembrane receptors. *Nat Rev Mol Cell Biol* 3:639-50.

Rosenblatt M and Potts JT, Jr. (1981) Analogues of an in vitro parathyroid hormone inhibitor: modifications at the amino terminus. *Calcif Tissue Int* 33:153-7.

Shimizu M, Carter PH and Gardella TJ (2000) Autoactivation of type-1 parathyroid hormone receptors containing a tethered ligand. *J Biol Chem* 275:19456-60.

Shimizu N, Guo J and Gardella TJ (2001) Parathyroid hormone (PTH)-(1-14) and -(1-11) analogs conformationally constrained by alpha-aminoisobutyric acid mediate full agonist responses via the juxtamembrane region of the PTH-1 receptor. *J Biol Chem* 276:49003-12.

Tan YV, Couvineau A, Murail S, Ceraudo E, Neumann JM, Lacapere JJ and Laburthe M (2006) Peptide agonist docking in the N-terminal ectodomain of a class II G protein-coupled receptor, the VPAC1 receptor: Photoaffinity, NMR and molecular modeling. *J Biol Chem*.

Ulrich CD, 2nd, Holtmann M and Miller LJ (1998) Secretin and vasoactive intestinal peptide receptors: members of a unique family of G protein-coupled receptors. *Gastroenterology* 114:382-97.

Unson CG (2002) Molecular determinants of glucagon receptor signaling. *Biopolymers* 66:218-35.

Unson CG, Andreu D, Gurzenda EM and Merrifield RB (1987) Synthetic peptide antagonists of glucagon. *Proc Natl Acad Sci U S A* 84:4083-7.

Vassart G, Pardo L and Costagliola S (2004) A molecular dissection of the glycoprotein hormone receptors. *Trends Biochem Sci* 29:119-26.

Footnotes:

Address correspondence to:

Martin Beinborn, M.D.

Molecular Pharmacology Research Center, Molecular Cardiology Research Institute

Tufts-New England Medical Center, Mailbox 7703

15 Kneeland Street

Boston, Massachusetts 02111

E-mail: MBeinborn@Tufts-NEMC.org

Molecular Pharmacology Fast Forward. Published on April 21, 2006 as DOI: 10.1124/mol.106.025932
This article has not been copyedited and formatted. The final version may differ from this version.

Legends for figures

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 around 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.

Molecular Pharmacology Fast Forward. Published on April 21, 2006 as DOI: 10.1124/mol.106.025932
This article has not been copyedited and formatted. The final version may differ from this version.