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Human Cannabinoid 1 GPCR C-Terminal Domain Interacts with Bilayer Phospholipids to Modulate the Structure of its Membrane Environment

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Abstract. G protein-coupled receptors (GPCRs) play critical physiological and therapeutic roles. The human cannabinoid 1 GPCR (hCB1) is a prime pharmacotherapeutic target for addiction and cardiometabolic disease. Our prior biophysical studies on the structural biology of a synthetic peptide representing the functionally significant hCB1 transmembrane helix 7 (TMH7) and its cytoplasmic extension, helix 8 (H8), [hCB1(TM7/H8)] demonstrated that the helices are oriented virtually perpendicular to each other in membrane-mimetic environments. We identified several hCB1(TM7/H8) structure-function determinants, including multiple electrostatic amino-acid interactions and a proline kink involving the highly conserved NPXXY motif. In phospholipid bicelles, TMH7 structure, orientation, and topology relative to H8 are dynamically modulated by the surrounding membrane phospholipid bilayer. These data provide a contextual basis for the present solid-state NMR study to investigate whether intermolecular interactions between hCB1(TM7/H8) and its phospholipid environment may affect membrane-bilayer structure. For this purpose, we measured ¹H-¹³C heteronuclear dipolar couplings for the choline, glycerol, and acyl-chain regions of dimyristoylphosphocholine in a magnetically aligned hCB1(TM7/H8) bicelle sample. The results identify discrete regional interactions between hCB1(TM7/H8) and membrane lipid molecules that increase phospholipid motion and decrease phospholipid order, indicating that the peptide's partial traversal of the bilayer alters membrane structure. These data offer new insight into hCB1(TM7/H8) properties and support the concept that the membrane bilayer itself may serve as a mechanochemical mediator of hCB1/GPCR signal transduction. Since interaction with its membrane environment has been implicated in hCB1 function and its modulation by small-molecule therapeutics, our work should help inform hCB1 pharmacology and the design of hCB1-targeted drugs.

KEY WORDS: conformational switch; integral-membrane protein topology; molecular hinge; NMR; signal transduction.

INTRODUCTION

Nuclear magnetic resonance spectroscopy (NMR) and X-ray crystallography are standard approaches for direct experimental elucidation of 3D protein structure (1,2). Application of these biophysical techniques to G protein-coupled receptors (GPCRs), a superfamily of integral-membrane proteins and prime targets of both marketed and potential drugs (1-4), faces serious technical challenges, such as purifying the required quantities of the GPCR in intact

form and maintaining protein structural stability in a membrane environment (2). Consequently, although NMR has been successfully applied to solve the structures of protein/GPCR fragments in membrane-mimetic environments (3-5), high-resolution, NMR-derived structures of functional GPCR holoreceptors have yet to be reported. Even with the impressive technical advances in X-ray crystallography, high-resolution structures of very few of the over 1,000 genetically encoded GPCRs (6) [i.e., the human β_2 adrenergic (7,8), A_{2A} adenosine (9), and opsin (10,11) receptors and the turkey β_1 adrenergic receptor (12)] have been solved since the first X-ray diffraction structure of a class-A GPCR (bovine rhodopsin) was reported a decade ago (13). Available crystallographic structures and computer-driven homology modeling data support a traditional class-A GPCR structural signature characterized by an extracellular amino terminus, seven hydrophobic transmembrane helices (TMHs) connected by intra- and extracellular loops, and an intracellular carboxyl terminus (2,14). In the C-terminal GPCR region, transmem-

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brane helix 7 (TMH7) is contiguous with a small cytoplasmic helical extension, helix 8 (H8), and contains a highly conserved NPXXY motif (2,13,14). The C-terminal domain of traditional class-A GPCRs holds particular functional significance. The NPXXY motif is a candidate structural feature of GPCR activation and conformational switching among multiple GPCR activity states, and H8 is a component of the intracellular GPCR binding site to G proteins (14–17).

A prominent pharmacotherapeutic target for substance abuse, drug addiction, weight-control, and cardiometabolic disease/metabolic syndrome (18–20), the human cannabinoid receptor 1 (hCB1) is a class-A, rhodopsin-like GPCR that can be activated by intrinsic lipid mediators (endocannabinoids) and synthetic cannabimimetic agents (21). Indirect evidence from site-directed mutation studies and computational homology modeling suggests that TMH7 and H8 are critical to ligand recognition and signal transmission by hCB1 (22–26). Consequently, the importance of the hCB1 C-terminal domain to the design and targeting of drugs that modulate hCB1 activity for therapeutic benefit is well appreciated (19,20,23,26). In marked contrast to the detailed knowledge of the structural features of rhodopsin photoactivation (13–15), the interactions and dynamics between the hCB1 C-terminal region and its plasma-membrane environment across the hCB1 activity spectrum are not well characterized. The relatively limited homology between rhodopsin and hCB1—particularly with respect to their C-terminal domain—precludes direct extrapolation of the rhodopsin structure to hCB1 (25,26). Our and others' investigations on hCB1 higher-order structure indicate that H8 acquires a helical conformation in membrane-mimetic environments with a juxtamembrane orientation parallel to the membrane surface and perpendicular to the TMH7 intramembrane bundle (4,27–30). Multiple electrostatic amino-acid interactions underpin TMH7/H8 conformation (4), and interactions between these two hCB1 α -helical segments have been implicated in ligand-induced hCB1 conformational transitioning (3).

We have recently provided NMR and site-directed spin labeling/electron paramagnetic resonance spectroscopy (SDSL/EPR) evidence of hCB1 TMH7 conformational plasticity such that its intramembrane orientation and its topology relative to H8 are influenced by membrane-bilayer thickness (3,27). These results have led us to postulate that the dynamic interaction of the hCB1 C-terminal domain with its membrane phospholipid environment could be an important modulator of hCB1 activation and determinant of hCB1 ligand pharmacology. Our postulate gains strength from accumulating evidence that GPCR/hCB1 function (ligand-binding competency, signal transmission) is influenced decisively by lipid microdomains in the plasma membrane (31–33). From the perspective of hCB1-related medicines, inhibition of cancer-cell proliferation by the first-in-class hCB1 inverse-agonist drug, rimonabant, requires hCB1 interaction with lipid rafts/caveolae (34), suggesting that lipid microdomains serve as hCB1 regulatory signaling elements potentially amenable to therapeutic exploitation (35).

The present report extends our prior investigations on the structural biology of the hCB1 C-terminal domain (3,4,27). We first recall selected results from our solution and solid-state NMR analyses of a chemically synthesized peptide corresponding to an extensive hCB1 TMH7 segment and its entire contiguous H8 domain (i.e., fourth cytoplasmic loop). This

peptide is designated as hCB1(TM7/H8). The experiments allowed us to solve the NMR solution structure of hCB1(TM7/H8) in a membrane-mimetic environment, determine the precise orientation of TMH7 and H8 in model-membrane phospholipid bicelles, and detail the influence of bilayer phospholipid thickness on TMH7 structure. The results form the contextual foundation for the current study, which utilizes this hCB1(TM7/H8) peptide as reconstituted and aligned in a defined dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) model-membrane system. Aligned bicelle samples afford high spectral resolution and are well-accepted for analyzing the structure of membrane proteins and investigating membrane properties using heteronuclear dipolar couplings (36–39). With this experimental system, we now extend our previous findings by identifying and characterizing directly specific intermolecular interactions between hCB1(TM7/H8) and its membrane phospholipid environment. For this purpose, we have applied a highly sensitive, solid-state NMR approach with proton-decoupled local field (PDLF) sequence to measure potential 2D ^1H – ^{13}C -dipolar interactions between the lipid molecules in the DMPC bilayer and hCB1(TM7/H8) reconstituted therein (40). We demonstrate that specific regional interactions between hCB1(TM7/H8) and membrane phospholipid molecules in its immediate environment reduce the lipid order parameter, suggesting that the hCB1 C-terminal domain, by partly traversing the membrane bilayer, may induce localized motion and conformational disorder/deformation in the membrane phospholipids. Since membrane environment can influence hCB1 activity and its response to small-molecule drugs (31,32,34), our findings implicate discrete intermolecular crosstalk between the C-terminal domain of hCB1 and plasma-membrane phospholipids in the regulation of hCB1 signaling.

MATERIALS AND METHODS

Peptide Synthesis and Purification

The 40-mer [$^{377}\text{TVFAFCSML}\underline{\text{ALLN}}\text{STVNPIIYALRSKDLRHAFRSMFPS}\underline{\text{AE}}^{416}$] corresponding to hCB1 TMH7/H8 was synthesized by a standard 9H-fluoren-9-ylmethoxycarbonyl (Fmoc)–polyamide method at the Molecular Biology Core Facility, GenScript Corporation (Scotch Plains, NJ, USA). Two C386A and C415A substitutions were made in the native sequence (as indicated, above) in order to improve spectral resolution for the structural calculations. The peptide as utilized experimentally was isolated by reverse-phase LC to >95% purity according to LC and MALDI-TOF mass spectrometry (MS) analyses (3,4,27). This synthetic peptide is designated herein as: hCB1(TM7/H8).

Sample Preparation and NMR Experiments

NMR Sample Preparation. Sample preparation for the solid-state NMR experiments (below) was conducted as previously detailed (3,27), yielding the aligned bicelle system composed of hCB1(TM7/H8) reconstituted in a DMPC bilayer.

Solid-State NMR. Solid-state NMR experiments were conducted at 37°C on a 700-MHz Bruker AVANCE II NMR spectrometer using a 5-mm double resonance probe under static sample conditions. Solid-state ^{13}C NMR-oriented spectra were collected utilizing a standard cross-polarization

pulse sequence (1,5,36,41,42). A ramped cross-polarization sequence with a contact time of 5 ms was used to record the 1D ^{13}C experiments. 2D ^{13}C spectra were obtained at 37°C using 128 t_1 experiments, 128 scans, a 5-s recycling delay, and 20 kHz ^1H decoupling (36).

Values from the ^1H - ^{13}C dipolar coupling spectrum were converted into an order parameter (S_{CH}) profile for the DMPC phospholipid molecule by using the relationships:

$$D_{\text{ch}} = D_{\text{O}} S_{\text{CH}} (3 \cos^2 \theta - 1) / 2 \quad (1)$$

and

$$\Delta\nu = 2\kappa [D_{\text{CH}} + J_{\text{CH}}/2] \quad (2)$$

where $D_{\text{O}} = 21.5$ kHz is the dipolar coupling constant for rigid C-H bond; the angle $\theta = 90$ defines the orientation of the bicelle normal relative to the magnetic field; $\Delta\nu$ is the experimental splitting; J_{CH} is the scalar coupling constant; and κ = scaling factor of the homonuclear decoupling constant, which has a value of 0.42 (36).

RESULTS

Precedent for the Current Work: Key Structural Features of hCB1(TM7/H8) in Membrane-Mimetic Environments

Except for H8, very little direct structural information is available for the C-terminal region of GPCRs, including hCB1 (2,14,30). We previously solved the NMR solution structure of a synthetic peptide 40-mer, hCB1(TM7/H8), representing the hCB1 C-terminal domain (4) and reported the first application of solid-state NMR, along with SDSL/EPR, to study the peptide's higher-order structure in defined phospholipid bilayers (3,27). Since certain results from those studies provide a contextual rationale for the new solid-state NMR experiments to follow, select findings are briefly recalled here. As illustrated by the ribbon depiction in Fig. 1a and elaborated upon in different, previously published schema (4), the structural signature of hCB1(TM7/H8) in membrane-mimetic 30% aqueous trifluoroethanol solution evidences four structurally distinct regions: the rigid TMH7 α -helix; a loop-like region interconnecting TMH7 and H8 and containing the highly conserved NPXXY motif with a proline kink; a short H8 α -helix, and an unstructured C terminus end. Multiple short-distance electrostatic interactions appear to be critical determinants of this hCB1(TM7/H8) solution structure: cation-phenolic and cation- π interactions promote formation of an interhelical microdomain that may act as a hinge during ligand-induced hCB1 conformational transitioning, as depicted in Fig. 1b and visualized differently in other schematics published elsewhere (4). Our prior solid-state NMR study of hCB1(TM7/H8) reconstituted in defined phospholipid model membranes indicates that the two hCB1 TMH7 and H8 α -helical segments are oriented virtually orthogonally to each other, as they are in solution: TMH7 is disposed within the membrane bilayer, and H8 is parallel to the phospholipid membrane surface (3,4,27). Our molecular dynamics simulations (27) and SDSL/EPR results (3) further demonstrate that TMH7's intramembrane conformation and relative orientation with respect to H8 are

Fig. 1. **a** Representation of the ribbon structure of TMH7/H8 obtained from NOESY and TOCSY experiments. Only the backbone atoms are shown. **b** Interhelical TMH7-H8 microdomain. High degree of conformational restriction for Tyr397 results from the side-chain proximity of residues from conserved NPXXY motif and Arg405. Cation-phenolic and cation- π interactions are observed between Tyr397 and Arg405. The short-distance amino-acid interaction pattern forms an H-bonding microdomain

dynamically modulated by its membrane environment. A final characteristic of hCB1(TM7/H8) germane to the present work, as first defined by us (4,27) and substantiated by others (30), is the amphipathicity of H8: the relative disposition therein of a hydrophilic cationic cluster contralateral to a hydrophobic “face” of nonpolar residues is likely important for optimal H8 orientation/interaction with both the plasma membrane and the G-protein subunits to which hCB1 couples for information transmission and intracellular signal propagation.

Solid-State NMR Using Proton-Decoupled Local Field Techniques

Select data summarized above from our previous studies on the structural biology of hCB1(TM7/H8) in membrane-

mimetic environments (3,4,27) raise the following question: Does hCB1(TM7/H8) interact dynamically with membrane phospholipid in such a way as to affect bilayer molecular topology and act thereby as a determinant of membrane structure? This question prompted us to conduct additional, high-resolution solid-state NMR studies with the hCB1(TM7/H8)-DMPC bicelle system used previously and focus specifically on the phospholipid bilayer component therein. If indeed membrane phospholipid environment plays a role in defining hCB1(TM7/H8) structure and orientation, as background data summarized above indicate, then hCB1(TM7/H8) might invite structural responses by the membrane phospholipid bilayer, either along the phospholipid acyl chains and/or in the head groups. To probe for such intermolecular interactions between hCB1(TM7/H8) and DMPC membranes in magnetically aligned bicelles, we conducted 2D PDLF NMR experiments on this model system. In this approach, correlation is made between the ^{13}C chemical shift spectrum (horizontal dimension) of the DMPC bicelle and the ^1H - ^{13}C dipolar couplings (vertical dimension) of the bicelle (Fig. 2). Figure 2a shows the 1D ^{13}C chemical shift spectrum of the DMPC bicelles, and Fig. 2b displays the 2D PDLF spectrum of the bicelles. The 2D spectrum evidences highly resolved doublets in the indirect frequency dimension due to the combined dipolar and scalar interactions between directly bonded ^1H - ^{13}C spin pairs on DMPC. Most of the overlapping peaks in the chemical shift dimensions are also resolved. Notably, in the PDLF spectrum, all the resonances are resolved, which demonstrates the enhanced power of this 2D technique over 1D solid-state NMR experiments.

We next converted the dipolar coupling value for each carbon along the DMPC lipid (Fig. 3a) into an order parameter (S_{CH}) to generate the profile shown in Fig. 3b. As compared to a DMPC bicelle itself, bicelles into which hCB1(TM7/H8) had been reconstituted evidenced a decrease of S_{CH} (i.e., increased phospholipid-bilayer disorder) at the choline methylene (α - and β -sites) groups as well as at the glycerol protons and the DMPC acyl-chain region, but not at the terminal trimethylammonium (γ -site; Fig. 3b). The overall change in the S_{CH} in the glycerol head-group region induced by hCB1(TM7/H8) is indicative of increased motion (43,44). No change was observed at the terminal methyl group of the fatty-acyl chains, suggesting that the peptide had no significant effect on the more fluid, core region of the membrane bilayer.

DISCUSSION

Definition of the structural features associated with GPCR activation and pharmacological modulation by “drugable” small-molecule ligands is a major focus of translational biomedicine (2,14). However, the heptahelical, integral-membrane character of traditional, therapeutically interesting GPCRs such as hCB1 constitutes a formidable impediment to their purification as intact, functional holoreceptors for structure determination by traditional techniques such as X-ray crystallography. As an alternative, a segmental approach has been adopted by us and others whereby peptides representing the hCB1 C-terminal region have been studied by biophysical methods such as NMR and SDSL/EPR for

Fig. 2. 1D chemical shift and 2D PDLF NMR spectra of oriented bicelles. **a** The 1D ^{13}C chemical shift spectrum of DMPC, above which the labeling of the carbons in the head-group choline, glycerol and acyl chain of the lipid is indicated. **b** The 2D PDLF spectrum that correlates the ^{13}C chemical shifts (horizontal dimension) and ^1H - ^{13}C dipolar couplings (vertical dimension) of DMPC bicelle. The X-axis labeling in **b** corresponds to the designated regions of the DMPC head group (γ , β , α), glycerol carbons (g_3 , g_2 , g_1), and fatty-acyl-chain carbons (2–14) as indicated in **a**

direct experimental analysis of their structural features and dynamics, since the C-terminal component of class-A GPCRs is considered to have great functional relevance (3,4,14,27–30). With respect to the hCB1 C-terminal region, particular attention has been focused on TMH7 and its short cytoplasmic extension (H8), for these helices appear to play functional roles in selective ligand recognition and intracellular signal transmission, respectively (24,45).

Most of the work related to the structural biology of the C-terminal domain of hCB1 has focused on peptides representing hCB1 TMH7 and/or H8 either in solution or, more commonly, as reconstituted into membrane mimetics. These studies have identified key structural attributes of the helices within the hCB1 C-terminal domain. For example, NMR studies from this laboratory on a synthetic peptide corresponding to an extended segment of hCB1 TMH7 and its entire contiguous H8 domain, hCB1(TM7/H8), demonstrated that hCB1 TMH7 is orientated virtually perpendicular to H8 whether hCB1(TM7/H8) is in membrane-mimetic solution or reconstituted into a phospholipid bilayer, the amphipathic H8 assuming an α -helical structure and juxtamembrane position quasi-parallel to the membrane surface, and TMH7 spanning the bilayer (3,27). The observed structures and dispositions of hCB1 TMH7 and H8 are consonant with predictions made from rhodopsin-based hCB1 homology models and are apparently

Fig. 3. **a** The structure of DMPC with labeling of the carbons in the head-group choline, glycerol and acyl chain of the lipid to indicate where the dipolar coupling values were extracted. **b** The order parameter profile determined in DMPC using dipolar coupling values. The graph is separated into three regions representing (from left to right) the DMPC choline, glycerol, and fatty-acyl chains

shared by other class-A GPCRs as well (2,7,14,22–26). Likewise, our experimentally defined structure for hCB1 H8 is reminiscent of that described for a synthetic peptide representing rat CB1 H8 in phospholipid micelles (28,29). For that peptide and a similar rat CB1 H8 peptide, an absence of secondary structure in an aqueous environment and a high degree of α -helicity in dodecylphosphocholine and sodium dodecyl sulfate micelles have been observed (28,29,46). Collectively, these data help validate our segmental experimental approach to hCB1 structure and our use of NMR to analyze hCB1(TM7/H8) reconstituted in membrane-mimetic environments.

We then sought to extend these structural descriptions of hCB1 TM7 and H8 and probed for interactions both within hCB1(TM7/H8) and between this peptide and its phospholipid bilayer environment that could impact hCB1(TM7/H8) structure. Application of biophysical techniques including NMR and SDSL/EPR allowed us to demonstrate multiple close-range electrostatic interactions between amino acids as candidate structural determinants of hCB1(TM7/H8) and implicate a flexible TMH7 region containing the conserved NPXXY domain with its proline kink as a molecular hinge that may help link TMH7 and H8 functionally (4). The flexible hCB1 TMH7 domain appears more extensive than the canonical, proline-kinked region in other class-A GPCRs (7,13,47). Nonetheless, TMH7 in both bovine rhodopsin and the human β_2 adrenergic receptor is bent due to a proline-based kink and likewise evidences a noncanonical distortion

within the NPXXY motif (2,4,7,14). By inducing local helix distortions that modulate this region's structure, the flexible NPXXY motif may play a crucial role in hCB1 signal transduction for effective coordination of ligand-binding and signal-transmission events between TMH7 and H8 (3,4,27,48,49). We also showed that TMH7 orientation can be altered by the thickness of the hydrophobic membrane bilayer (27), suggesting that hCB1 function may be influenced by its phospholipid bilayer environment.

We have now examined the structural biology of hCB1(TM7/H8) from the standpoint of its lipid environment, specifically, with respect to the peptide's potential influence on membrane phospholipid bilayer motion and order. Two-dimensional ^1H - ^{13}C heteronuclear dipolar couplings for the DMPC choline, glycerol, and acyl-chain regions were measured in a magnetically aligned hCB1(TM7/H8) bicelle system as compared to a bicelle lacking the peptide. The overall decrease in S_{CH} observed even at 0.5 mol% peptide indicates that hCB1(TM7/H8) induces disorder in the membrane-lipid acyl chains and conformational changes at the phospholipids. Early 1D NMR experiments suggested that hCB1(TM7/H8) alters the dynamic properties of the lipid component of multilamellar vesicles (43). The current 2D experiments allowed us to demonstrate directly that membrane phospholipid conformation and bilayer order are influenced by hCB1(TM7/H8) and define discrete interaction loci responsible for the peptide's impact on membrane structure. In particular, the change in S_{CH} we

observed when hCB1(TM7/H8) was incorporated into DMPC bicelles likely reflects specific intermolecular interactions between the phospholipid glycerol moiety and the peptide due to phospholipid head-group reorientation.

Data supporting our conclusion that hCB1(TM7/H8) can induce dynamic changes in membrane phospholipid structure have literature precedents which further suggest that such structural changes have functional significance. The interaction of cytotoxic peptides with plasma-membrane phospholipids elicits counter-directional changes at the α and β phospholipid sites and leads to the formation of pores or voltage-gated ion channels that adversely alter cell physiology (5). Stimulation of endothelial cells with fluid shear stress, hypotonic stress, or a fluidizing agent increases cellular bradykinin B2 GPCR activity (50). New data presented herein unequivocally demonstrate the occurrence of dynamic intermolecular interactions between a functionally important hCB1 signaling domain and its membrane phospholipid environment that affect the structure of the membrane bilayer at discrete loci. Collectively, these data suggest that the phospholipid bilayer itself may play a significant (mechanochemical) role in mediating GPCR/hCB1 transmission. Alterations in membrane phospholipid conformation may represent a physiological component of GPCR signal transduction. The potential for mutual structural accommodation between GPCRs (including hCB1) and their biomembrane environment to affect GPCR transmission holds significant implications for GPCR-targeted drug discovery.

Note added in proof: After submission of this work, the crystal structure of an antagonist-liganded human dopamine D3 receptor was reported (Chien EYT, Liu W, Zhao Q, Katritch V, Han GW, Hanson MA, *et al.* Structure of the human dopamine D3 receptor in complex with a D2/D3 selective antagonist. *Science*. 2010;330:1091–1095). That publication extends the findings in references [7–14] regarding the current repertoire of GPCRs whose high-resolution X-ray structures have been solved.

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REFERENCES

- Marassi FM, Ramamoorthy A, Opella SJ. Complete resolution of the solid-state NMR spectrum of a uniformly ^{15}N -labeled membrane protein in phospholipid bilayers. *Proc Natl Acad Sci USA*. 1997;94:8551–6.
- Topiol S, Sabio M. X-ray structure breakthroughs in the GPCR transmembrane region. *Biochem Pharmacol*. 2009;78:11–20.
- Tiburu EK, Gulla SV, Tiburu M, Janero DR, Budil DE, Makriyannis A. Dynamic conformational responses of a human cannabinoid receptor-1 helix domain to its membrane environment. *Biochemistry*. 2009;48:4895–904.
- Tyukhtenko S, Tiburu EK, Deshmukh L, Vinogradova O, Janero DR, Makriyannis A. NMR solution structure of human cannabinoid receptor-1 helix 7/8 peptide: candidate electrostatic interactions and microdomain formation. *Biochem Biophys Res Commun*. 2009;390:441–6.
- Porcelli F, Buck B, Lee D-K, Hallock KJ, Ramamoorthy A, Veglia G. Structure and orientation of pardaxin determined by NMR experiments in model membranes. *J Biol Chem*. 2004;279:45815–23.
- Fredriksson F, Schiöth HB. The repertoire of G-protein-coupled receptors in fully sequenced genomes. *Mol Pharmacol*. 2005;67:1414–25.
- Cherezov V, Rosenbaum DM, Hanson MA, Rasmussen SG, Thian FS, Kobilka TS, *et al.* High-resolution crystal structure of an engineered human β_2 -adrenergic G protein-coupled receptor. *Science*. 2007;318:1258–65.
- Rasmussen SG, Choi HJ, Rosenbaum DM, Kobilka TS, Thian FS, Edwards PC, *et al.* Crystal structure of the human β_2 adrenergic G-protein-coupled receptor. *Nature*. 2007;450:383–7.
- Jaakola VP, Griffith MT, Hanson MA, Cherezov V, Chien EY, Lane JR, *et al.* The 2.6 angstrom crystal structure of a human A2A adenosine receptor bound to an antagonist. *Science*. 2008;322:1211–7.
- Park JH, Scheerer P, Hofmann KP, Choe HW, Ernst OP. Crystal structure of the ligand-free G-protein-coupled receptor opsin. *Nature*. 2008;454:183–7.
- Scheerer P, Park JH, Hildebrand PW, Kim YJ, Krauss N, Choe HW, *et al.* Crystal structure of opsin in its G-protein-interacting conformation. *Nature*. 2008;455:497–502.
- Serrano-Vega MJ, Magnani F, Shibata Y, Tate CG. Conformational thermostabilization of the β_1 -adrenergic receptor in a detergent-resistant form. *Proc Natl Acad Sci USA*. 2008;105:877–82.
- Palczewski KT, Kumasaka T, Hori T, Behnke CA, Motoshima H, Fox BA, *et al.* Crystal structure of rhodopsin: a G protein-coupled receptor. *Science*. 2000;289:739–45.
- Rosenbaum DM, Rasmussen SG, Kobilka BK. The structure and function of G-protein-coupled receptors. *Nature*. 2009;459:356–63.
- Fritze O, Filipek S, Kuksa V, Palczewski K, Hofmann KP, Ernst OP. Role of the conserved NPxxY(x)5, 6 F motif in the rhodopsin ground state and during activation. *Proc Natl Acad Sci USA*. 2003;100:2290–5.
- Prioleau C, Visiers I, Ebersole BJ, Weinstein H, Sealfon SC. Conserved helix 7 tyrosine acts as a multistate conformational switch in the 5HT_{2C} receptor. Identification of a novel “locked-on” phenotype and double revertant mutations. *J Biol Chem*. 2002;277:36577–84.
- Hsu D, Knudson PE, Zapf A, Rolband GC, Olefsky JM. NPXY motif in the insulin-like growth factor-I receptor is required for efficient ligand-mediated receptor internalization and biological signaling. *Endocrinology*. 1994;134:744–50.
- Janero DR, Makriyannis A. Targeted modulators of the endogenous cannabinoid system: future medications to treat addiction disorders and obesity. *Curr Psychiatry Rep*. 2007;9:365–73.
- Janero DR, Vadivel SK, Makriyannis A. Pharmacotherapeutic modulation of the endocannabinoid signalling system in psychiatric disorders: drug-discovery strategies. *Int Rev Psychiatry*. 2009;21:122–33.
- Janero DR, Makriyannis A. Cannabinoid receptor antagonists: pharmacological opportunities, clinical experience, and translational prognosis. *Expert Opin Emerg Drugs*. 2009;14:43–65.
- Di Marzo V. The endocannabinoid system: its general strategy of action, tools for its pharmacological manipulation and potential therapeutic exploitation. *Pharmacol Res*. 2009;60:77–84.
- Bramblett RD, Panu AM, Ballasteros JA, Reggio PH. Construction of a 3D model of the cannabinoid CB1 receptor: determination of helix ends and helix orientation. *Life Sci*. 1995;56:1971–82.
- Barnett-Norris J, Hurst DP, Lynch DL, Guarnieri F, Makriyannis A, Reggio PH. Conformational memories and the endocannabinoid binding site at the cannabinoid CB1 receptor. *J Med Chem*. 2002;45:3649–58.
- Kapur A, Hurst DP, Fleischer D, Whitnell R, Thakur GA, Makriyannis A, *et al.* Mutation studies of Ser7.39 and Ser2.60 in the human CB1 cannabinoid receptor: evidence for a serine-induced bend in CB1 transmembrane helix 7. *Mol Pharmacol*. 2007;71:1512–24.
- Montero C, Campillo NE, Goya P, Páez JA. Homology models of the cannabinoid CB1 and CB2 receptors. A docking analysis study. *Eur J Med Chem*. 2005;40:75–83.

26. Shim JY, Welsh WJ, Howlett AC. Homology model of the CB1 cannabinoid receptor: sites critical for nonclassical cannabinoid agonist interaction. *Biopolymers*. 2003;71:169–89.
27. Tiburu EK, Bowman AL, Struppe JO, Janero DR, Avraham HK, Makriyannis A. Solid-state NMR and molecular dynamics characterization of cannabinoid receptor-1 (CB1) helix 7 conformational plasticity in model membranes. *Biochim Biophys Acta*. 2009;1788:1159–67.
28. Choi G, Guo J, Makriyannis A. The conformation of the cytoplasmic helix 8 of the CB1 cannabinoid receptor using NMR and circular dichroism. *Biochim Biophys Acta*. 2005;1668:1–9.
29. Xie XQ, Chen JZ. NMR structural comparison of the cytoplasmic juxtamembrane domains of G-protein-coupled CB1 and CB2 receptors in membrane mimetic dodecylphosphocholine micelles. *J Biol Chem*. 2005;280:3605–12.
30. Ahn KH, Pellegrini M, Tsomain N, Yatawara AK, Kendall DA, Mierke DF. Structural analysis of the human cannabinoid receptor one carboxyl-terminus identifies two amphipathic helices. *Biopolymers*. 2009;91:565–73.
31. Maccarrone M, De Chiara V, Gasperi V, Viscomi MT, Rossi S, Oddi S, *et al.* Lipid rafts regulate 2-arachidonoylglycerol metabolism and physiological activity in the striatum. *J Neurochem*. 2009;109:371–81.
32. Yang Q, Liu HY, Zhang YW, Wu WJ, Tang WX. Anandamide induces cell death through lipid rafts in hepatic stellate cells. *J Gastroenterol Hepatol*. 2010;25:991–1001.
33. Lajoie P, Goetz JG, Dennis JW, Nabi IR. Lattices, rafts, and scaffolds: domain regulation of receptor signaling at the plasma membrane. *J Cell Biol*. 2009;185:381–5.
34. Sarnataro D, Pisanti S, Santoro A, Gazzerò P, Malfitano AM, Laezza C, *et al.* The cannabinoid CB1 receptor antagonist rimonabant (SR141716) inhibits human breast cancer cell proliferation through a lipid raft-mediated mechanism. *Mol Pharmacol*. 2006;70:1298–306.
35. Dainese E, Oddi S, Bari M, Maccarrone M. Modulation of the endocannabinoid system by lipid rafts. *Curr Med Chem*. 2007;14:2702–15.
36. Dvinskikh S, Durr U, Yamamoto K, Ramamoorthy A. A high-resolution solid-state NMR approach for the structural studies of bicelles. *J Am Chem Soc*. 2006;128:6326–7.
37. Lu JX, Damodaran K, Lorigan GA. Probing membrane topology by high-resolution ^1H - ^{13}C heteronuclear dipolar solid-state NMR spectroscopy. *J Magn Reson*. 2006;178:283–7.
38. Ottiger M, Bax A. Bicelle-based liquid crystals for NMR measurement of dipolar couplings at acidic and basic pH values. *J Biomol NMR*. 1999;13:187–91.
39. Park SH, Prytulla S, De Angelis AA, Brown JM, Kiefer H, Opella SJ. High-resolution NMR spectroscopy of a GPCR in aligned bicelles. *J Am Chem Soc*. 2006;128:7402–3.
40. Schmidt-Rohr KD, Nanz D, Emsley L, Pines A. NMR measurement of resolved hetero. dipole couplings in LCs, and lipids. *J Phys Chem*. 1994;98:6668–70.
41. Cross TA. Solid state nuclear magnetic resonance characterization of gramicidin channel structure. *Meth Enzymol*. 1997;289:672–96.
42. Cross TA, Opella SJ. Solid-state NMR structural studies of peptides and proteins in membranes. *Curr Opin Struct Biol*. 1994;4:574–81.
43. Tiburu EK, Karp ES, Birrane G, Struppe JO, Chu S, Lorigan GA, *et al.* ^{31}P and ^2H relaxation studies of helix VII and the cytoplasmic helix of the human cannabinoid receptors utilizing solid-state NMR techniques. *Biochemistry*. 2006;45:7356–65.
44. Tiburu EK, Dave PC, Lorigan GA. Solid-state ^2H NMR studies of the effects of cholesterol on the acyl chain dynamics of magnetically aligned phospholipid bilayers. *Magn Reson Chem*. 2004;42:132–8.
45. Anavi-Goffer S, Fleischer D, Hurst DP, Lynch DL, Barnett-Norris J, Shi S, *et al.* Helix 8 Leu in the CB1 cannabinoid receptor contributes to selective signal transduction mechanisms. *J Biol Chem*. 2007;282:25100–13.
46. Grace CRR, Cowsik SM, Shim J-Y, Welsh WJ, Howlett AC. Unique helical conformation of the fourth cytoplasmic loop of the CB1 cannabinoid receptor in a negatively charged environment. *J Struct Biol*. 2007;159:359–68.
47. Henderson R, Baldwin JM, Ceska TA, Zemlin F, Beckmann E, Downing KH. Model for the structure of bacteriorhodopsin based on high-resolution electron cryo-microscopy. *J Mol Biol*. 1990;213:899–929.
48. Hall SE, Roberts K, Vaidehi N. Position of helical kinks in membrane protein crystal structures and the accuracy of computational prediction. *J Mol Graph Model*. 2009;27:944–50.
49. Cordes FS, Bright JN, Sansom MS. Proline-induced distortions of transmembrane helices. *J Mol Biol*. 2002;323:951–60.
50. Chachisvilis M, Zhang YL, Frangos JA. G protein-coupled receptors sense fluid shear stress in endothelial cells. *Proc Natl Acad Sci USA*. 2006;103:15463–8.