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Visions & Reflections (Minireview)

Lipid-mediated dimerization of β_2-adrenergic receptor reveals important clues for cannabinoid receptors

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Abstract. The high-resolution crystal structure of an engineered human β_2-adrenergic receptor has recently been resolved, suggesting a molecular mechanism by which cholesterol may mediate receptor dimerization. Here, we present a critical examination of new structural and functional insights derived from unprecedented preliminary homology modeling of cannabinoid receptors, obtained using the crystal structure of β_2-adrenergic receptor as a template. The structural comparison between the two cannabinoid receptor subtypes and the β_2-adrenergic receptor may be of particular interest, by providing important clues for the elucidation of the structural determinants involved in cholesterol binding. In addition, the implications of G protein coupled receptor dimerization, as well as the role of cholesterol in this process, are briefly discussed.

Keywords. β_2-adrenergic receptor, cannabinoid receptors, cholesterol, lipid binding domain, receptor dimerization.

A recent issue of *Science* reported interesting information on the high-resolution crystal structure and function of an engineered human β_2-adrenergic receptor (β_2-AR) [1, 2]. In particular, a lipid-mediated receptor dimerization has been revealed, with two cholesterol molecules and two palmitic acid molecules forming the majority of the interactions in the helix I – helix VIII interface between two receptor monomers embedded in a lipid membrane. Consistently, cholesterol modulates the physiologic function of β_2-AR, so that receptor signaling is enhanced upon cholesterol depletion from plasma membranes [3].

The β_2-AR belongs to the G protein coupled receptor (GPCR) family, which is the largest group among eukaryotic signal transduction proteins that communicate across the membrane. Similar to the β_2-AR, the activity of the type-1 cannabinoid (CB_1) receptor, which is the most abundant GPCR within our brains [4], is enhanced upon cholesterol depletion [5]. By contrast, the type-2 cannabinoid (CB_2) receptor, which is activated by the same endogenous ligands that bind to CB_1 (i.e., the "endocannabinoids") and triggers essentially the same signaling pathways [4], is independent of membrane cholesterol content [5]. CB_1 and CB_2 receptors are encoded by different genes, and exhibit 44 % amino acid identity throughout the whole protein [4]. Although their crystal structures are not available, homology models built by using bovine rhodopsin as a template, along with high resolution NMR and computer modeling [6], have revealed that the most important differences between

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CB_1 and CB_2 receptors with respect to their interaction with the surrounding lipid environment are located in the juxtamembrane domain (or helix VIII). In particular, the Arg401 residue within the CB_1 helix VIII is fully accessible, whereas the corresponding Arg302 residue in CB_2 is constrained by a salt bridge, and is thus not available for interactions with G-proteins or with cholesterol molecules [6, 7]. Another interesting feature of helix VIII that might modulate the interaction of CB receptors with the surrounding membrane lipids is the presence of a conserved cysteine residue (Cys415 in CB_1 receptor, and Cys313 in CB_2 receptor) that has been suggested to be constitutively palmitoylated in the CB_1 receptor only [8]. Notably, a cysteine residue is also constitutively palmitoylated in rhodopsin (Cys323) [9], and in β_2-AR (Cys341) [1]. Here, we discuss new structural insights derived from an unprecedented homology modeling of CB receptors, obtained by using the crystal structure of β_2-AR as a template [1, 2]. The modeled structures of the CB receptors confirm that the above mentioned cysteine residue is located at the end of helix VIII for both CB_1 and CB_2 receptors, in an appropriate position for palmitoylation (Figs. 1B–C, left panels). Other interesting insights arise from the analysis of the crevice between helices I and VIII, which are involved in the binding of ordered lipids [1, 2]. In particular, this region interacts with cholesterol and palmitic acid in β_2-AR, thus controlling receptor dimerization within the lipid bilayer [1, 2]. As expected from a region interacting with lipids, the surface electrostatic charge distribution calculated for β_2-AR shows the presence of a very hydrophobic area (Fig. 1A, right panel). Interestingly, a similar analysis of the modeled structures of CB receptors revealed that, like β_2-AR, CB_1 presents a hydrophobic surface at the helix I – helix VIII interface that is suitable for a specific interaction with cholesterol and palmitic acid (Fig. 1B, right panel). By contrast, CB_2 displays a negatively charged region within helix VIII, which is rather unfavorable for any interaction with lipids (Fig. 1C, right panel).

Overall, it can be suggested that subtle differences in the domains that interact with the surrounding lipids, such as those described for CB_1 *versus* CB_2, might be sufficient to abrogate the membrane-dependence of structure and function of GPCRs. In this context, we have recently proposed two different mechanisms by which cholesterol may control CB_1 activity within cholesterol-rich membrane subdomains such as lipid rafts: segregation within these microdomains or caveolar endocytosis [7, 10]. The above comparison with the structural features of β_2-AR suggests a further mechanism through which modulation of CB_1 by cholesterol (and palmitic acid) may depend on receptor dimerization. The latter putative regulation of CB1 is schematically depicted in Figure 2.

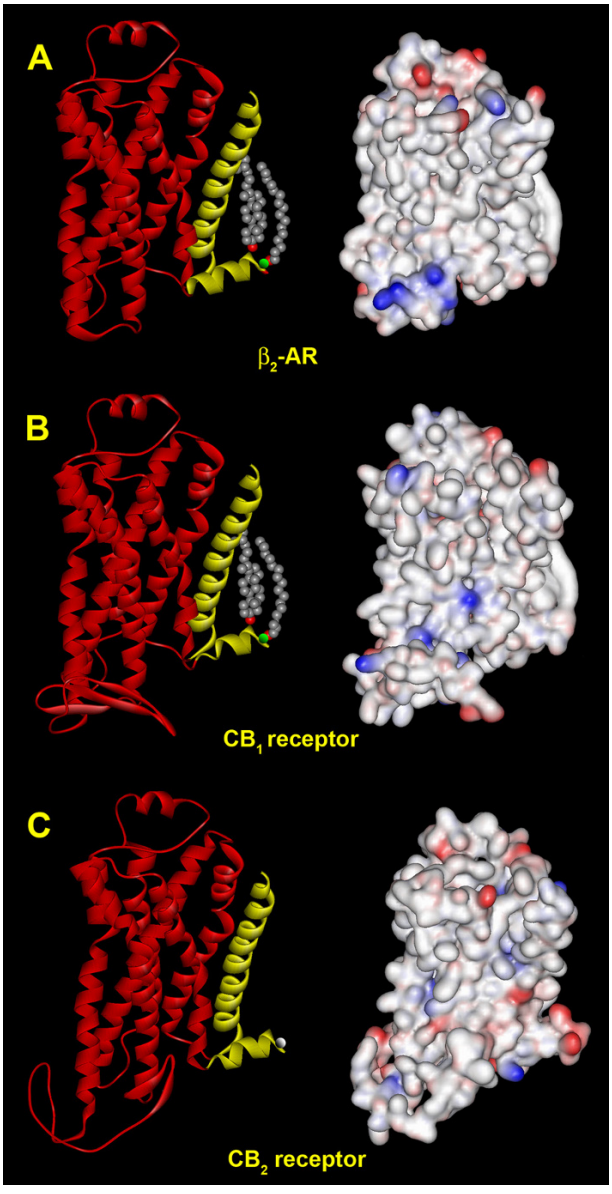

Figure 1. Comparison between the crystallographic structure of β_2-AR (*A*, left panel) and the homology models of CB_1 (*B*, left panel) and CB_2 (*C*, left panel), based on sequence alignment with β_2-AR (PDB code: 2RH1). Models were obtained using the protein structure homology-modeling server SWISS-MODEL, integrated in the Deep-View program [12]. The modeling procedure took into account the consensus sequences within each transmembrane helix. Palmitic acid and cholesterol are shown bound to the helix I-helix VIII interfaces, within the structures of β_2-AR (*A*, left panel) and of CB_1 (*B*, left panel). On the right panels, surface representations of the three receptors are reported with colors from red (−10) to blue (+10) using calculated charges and a dielectric constant of 70. Color codes for the ribbon structures (left panels) are: yellow, helices I and VIII; green spheres, palmitoylated Cys341 (within the helix VIII of β_2-AR), Cys415 (within the helix VIII of CB_1); white sphere, Cys313 within the helix VIII of CB_2 receptor.

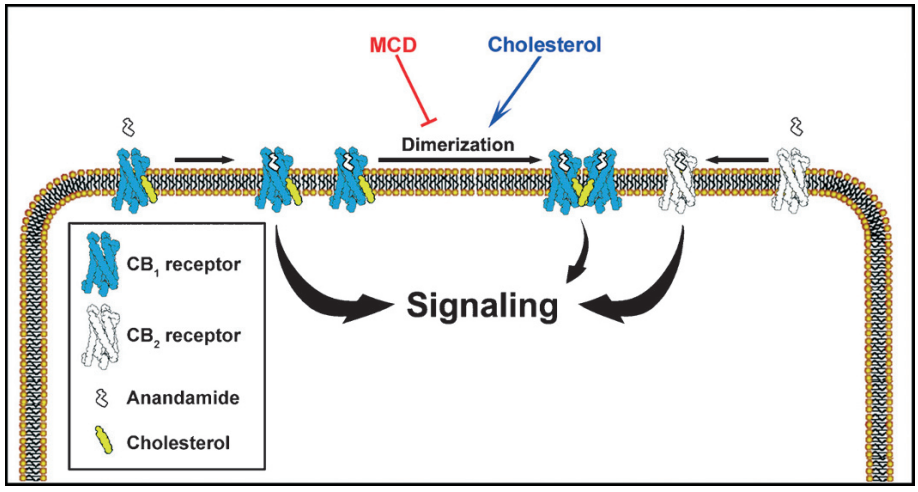

Figure 2. Putative role of cholesterol in controlling the signaling activity of CB_1 receptor. Like β_2-AR, the CB_1 receptor might be coupled to its signaling machinery more efficiently as a monomer, and less efficiently as a dimer. Thus, drugs that deplete cholesterol from the plasma membrane, such as methyl-β-cyclodextrin (MCD), are expected to interfere with CB_1 receptor dimerization, and thereby to enhance its signaling activity. Conversely, membrane cholesterol enrichment, favoring the dimerization process, could reduce CB_1-dependent signal transduction. Unlike CB_1, CB_2 does not interact with cholesterol, and therefore it is not at all influenced by membrane cholesterol perturbation.

At any rate, the structural insights into β_2-AR and the data on CB_1 receptors suggest a novel paradigm of ligand/receptor interactions, whereby a third player comes into the game: the plasma membrane [10]. This concept may be exploited for therapeutic applications, because GPCRs, including CB_1 [4, 7], might be drug targets for a wide spectrum of human diseases [11].

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