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Membrane environment and endocannabinoid signaling

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Two main molecular targets of Δ^9-tetrahydrocannabinol (Δ^9-THC), the psychoactive principle of *Cannabis sativa*, are type-1 (CB_1) and type-2 (CB_2) cannabinoid receptors (Howlett et al., 2010). In the past few years many endogenous agonists of CB receptors have been characterized, and are collectively called "endocannabinoids" (Maccarrone et al., 2010). They are mainly amides and esters of long-chain polyunsaturated fatty acids isolated from brain and peripheral tissues and, although structurally different from plant cannabinoids, share critical pharmacophores with Δ^9-THC (Pertwee, 2010). Two arachidonate derivatives, *N*-arachidonoylethanolamine (anandamide, AEA) and 2-arachidonoylglycerol (2-AG), were shown to mimic Δ^9-THC by functionally activating CB receptors, and these are the endocannabinoids whose biological activity has been best characterized to date (Di Marzo, 2009; Maccarrone et al., 2010).

CB_1 receptor is the most abundant G protein-coupled receptor (GPCR) in the brain (Howlett et al., 2010). Together with its endogenous agonists (AEA, 2-AG, and other congeners), CB_1 belongs to an ancient neurosignaling system that plays important control functions within the central nervous system (Katona and Freund, 2008). Alterations in this so-called "endocannabinoid system" have been extensively investigated in a wide range of neurodegenerative and neuroinflammatory disorders, spanning from Alzheimer's disease, Parkinson's disease and Huntington's disease, to amyotrophic lateral sclerosis and multiple sclerosis (Bisogno and Di Marzo, 2010). For this reason, research on the therapeutic potential of drugs modulating the endocannabinoid system is very intense (Di Marzo, 2009). More recently, it has become evident the involvement of membrane lipids, especially cholesterol and glycosphingolipids, in regulating the function of GPCRs like β_2-adrenergic and serotonin$_{1A}$ receptors, as well as of several other membrane-associated proteins like caveolins (Pontier et al., 2008; Prinetti et al., 2009; Paila et al., 2010; Shrivastava et al., 2010). Also a role for membrane cholesterol in the functional regulation of CB_1 has been well-documented (for an updated review see Dainese et al., 2010). Acute cholesterol depletion by methyl-β-cyclodextrin has been shown to double CB_1-dependent signaling via adenylyl cyclase and mitogen-activated protein kinases in neuronal cells (Bari et al., 2005a,b). Conversely, it has been reported that in the same cells CB_1-dependent binding and signaling was significantly reduced by cholesterol enrichment (Bari et al., 2005a,b, 2006). Notably, the CB_2 receptor that is structurally and functionally related to CB_1 is completely insensitive to the modulation of membrane cholesterol content (Bari et al., 2006), and does not reside in cholesterol-rich microdomains like lipid rafts (Bari et al., 2006; Rimmerman et al., 2008). As yet, the molecular basis for the different response of these two receptor subtypes to cholesterol remains unclear, although its impact on the therapeutic exploitation of CB_1-dependent endocannabinoid signaling *versus* that dependent on CB_2 could be immense.

Here, I would like to comment that subtle, yet specific, differences might underpin the differential sensitivity of CB_1 and CB_2 to membrane cholesterol, possibly explaining the apparent redundancy of having two largely overlapping receptor subtypes that are activated by similar compounds (endocannabinoids) and trigger similar transduction pathways: (i) inhibition of adenylyl cyclase, (ii) regulation of ionic currents (e.g., inhibition of voltage-gated L, N, and P/Q-type Ca^{2+} channels, and activation of K^+ channels), and (iii) activation of focal adhesion kinase, mitogen-activated protein kinase, and cytosolic phospholipase A_2 (Di Marzo, 2009; Maccarrone et al., 2010).

In general, cholesterol may act on the conformation of a membrane receptor by indirectly altering the physico-chemical properties of the bilayer, or by directly interacting with the receptor itself. Although a unique conserved structural determinant for protein interaction with cholesterol has not yet been identified, a well-known motif is the cholesterol interaction/recognition amino acid sequence consensus [L/V-$X_{(1-5)}$-Y-$X_{(1-5)}$-R/K], named CRAC (Epand, 2006). This motif has been demonstrated in caveolin-1, peripheral-type benzodiazepine receptor (Li and Papadopoulos, 1998; Jamin et al., 2005), and in other proteins targeted to lipid rafts (Xie et al., 2010). Interestingly, by sequence alignment of human CB_1 and CB_2 we have recently identified the presence of CRAC in the last 11 amino acids of the transmembrane helix 7 of both CB_1 and CB_2 (Oddi et al., 2011). In particular, we found that in the highly conserved CRAC region (82% amino acid identity), CB_1 differs from CB_2 for one residue only: lysine 402 of CB_1 (**Figure 1**) corresponds to glycine 304 in CB_2 (Oddi et al., 2011). We also found that the CB_1(K402G) mutant where the CRAC sequence of CB_1 was converted into that of CB_2 had a reduced propensity to reside in cholesterol-rich membrane regions, and lost its sensitivity to membrane cholesterol enrichment (Oddi et al., 2011). Therefore, one residue in complex proteins like GPCRs can be enough to direct their interaction with membrane lipids, thus affecting signal transduction thereof.

Different non-mutually exclusive mechanisms could be proposed to explain the differential sensitivity of CB_1 and CB_2 to membrane cholesterol: (*i*) compartmentalization in cholesterol-rich microdomains; (*ii*) caveolar endocytosis; (*iii*) cholesterol-dependent receptor dimerization; (*iv*) hydrophobic mismatch; (*v*) modulation of the rate of endocannabinoid movement within the membrane (Dainese et al., 2010). Additionally, it is possible that the different effect of membrane cholesterol on CB_1 and CB_2 is due to subtle differences in the domain(s) that interact(s) with the surrounding (non-annular) lipids, by analogy

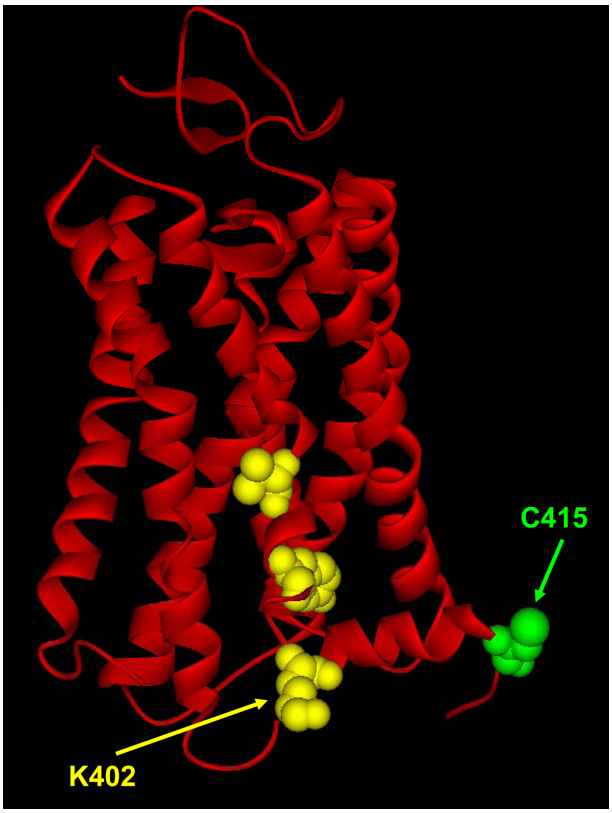

FIGURE 1 | Three-dimensional model of CB_1, based on sequence alignment with visual rhodopsin in the inactivated state (PDB code: 1F88). The model was obtained using the protein structure homology-modeling server SWISS-MODEL, integrated in the Deep-View program (Dainese et al., 2010). The three residues (V392, Y397, K402) that form the CRAC sequence are represented as yellow spheres, sized to the Van der Waals radii; these residues belong to the transmembrane helix 7 of CB_1. Recently, we have generated a mutant where a lysine residue (K402) was substituted by glycine, thus converting the CRAC sequence of CB_1 into that of CB_2 (Oddi et al., 2011). Additionally, the C-terminal component of CB_1, i.e., the intracellular juxtamembrane α-helix 8, contains a cysteine residue (C415, in green) that could be constitutively palmitoylated. See text for details. The model was kindly provided by Dr. Enrico Dainese (University of Teramo, Italy).

with other GPCRs (Paila et al., 2010). Additionally, other lipid-interacting residues might direct the interaction of CB_1 with the surrounding membrane lipids, e.g., cysteine 415 in its C-terminal (**Figure 1**), that could be the target of palmitoylation (Dainese et al., 2010). The latter reversible post-translational modification can be used by cells to regulate CB_1 targeting to cholesterol-rich subdomains of the membrane, thus influencing its interaction with coupled G proteins.

I believe that the comparison between CB_1 and CB_2 might represent an interesting paradigm that goes well-beyond endocannabinoid signaling. In fact, the modulation of CB_1 by cholesterol might disclose a novel ligand–receptor interaction, where a third player comes into the game: membrane lipids. As a consequence, the membrane environment might play a role in receptor-dependent signaling, with a potential impact on several neurotransmission pathways, as well as several neurodegenerative/neuroinflammatory diseases where CB_1 is known to play a role. More in general, it should be recalled that CB_1-dependent signaling impacts fundamental processes as different as immune response, energy homeostasis, reproduction, and skin differentiation (Di Marzo, 2009; Maccarrone et al., 2010), thus it can be anticipated that cholesterol-dependent regulation of CB_1 can have a physiological relevance well-beyond the central nervous system.

In conclusion, membrane environment seems to be critical for the regulation of signal transduction pathways triggered by G protein-coupled receptors like CB_1. Despite the three-dimensional complexity of these proteins, we learn from the comparison of CB_1 with CB_2 that just one amino acid residue can direct receptor functioning, calling for attention on the plasma membrane as a key-player in ligand recognition on the cell surface.

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