Structural biology study of human cannabinoid receptor CB1

Marijuana from *Cannabis sativa L.* has been used for both therapeutic and recreational purposes for many centuries. The active constituent Δ^9-tetrahydrocannabinol (THC) [1], exerts its psychotropic effects mainly through cannabinoid receptor 1 (CB1) [2], which is also the primary target of the endocannabinoids, anandamide (AEA) and 2-arachidonoyl glycerol (2-AG) [3] (Fig. 1). CB1 belongs to the class A G protein-coupled receptor (GPCR) family, signal through inhibitory $G\alpha_{i/o}$ heterotrimeric G proteins, and interact with β-arrestins (Fig. 1). Moreover, CB1 is the most highly expressed GPCR in the human brain and is expressed throughout the body, with the highest levels found in the central nervous system.

CB1-selective small-molecule agonists have shown therapeutic promise in a wide range of disorders, including pain and inflammation, multiple sclerosis, and neurodegenerative disorders. Antagonists of CB1 have been explored as potential therapeutics for obesity-related metabolic disorders, mental illness, liver fibrosis and nicotine addiction. The molecular details defining the binding modes of both endogenous and exogenous ligands are still unknown. In order to address this deficit in understanding, we have determined the crystal structures of CB1 in complex with antagonist [4] and agonist [5]. The structures provide very important molecular basis of how ligands engage to modulate the cannabinoid system and reveal important insights into the activation mechanism of CB1.

Crystallization of GPCRs, especially active (active-like) –state, has been challenging due to their inherent conformational flexibility and biochemical instability. To facilitate crystallization, Flavodoxin was identified as a stabilizing fusion partner and the receptor was truncated on both the N and C termini by 98 and 58 residues, respectively. In order to further improve the expression and thermostability, four computationally and rationally designed mutations were introduced to the CB1 sequence. Fortunately, the modified CB1 construct has comparable affinity for both antagonists and agonists as the wild-type receptor. Finally, we obtained the crystals of CB1 in complex with antagonist AM6538 and agonist AM11542, respectively (Fig. 2). The diffraction datasets were collected at the SPring-8 **BL41XU**, GM/CA-CAT beamline 23ID-B of APS and beamline X06SA of Swiss Light Source.

The overall CB1 contains seven transmembrane (7TM) α-helices (I to VII) connected by three extracellular loops (ECL1-3), three intracellular loops (ICL1-3), and an amphipathic helix VIII. The antagonist AM6538 binds quite deep in the binding pocket and adopts an extended conformation (Fig. 2). The agonist AM11542 adopts an L-shape conformation in the orthosteric-binding pocket, which is much smaller than the more expanded binding domain of AM6538 (Fig. 2 and Fig. 3(c)). Both the AM6538 and AM11542 are forming mainly hydrophobic interactions with the receptor.

Comparisons between the agonist- and antagonist-bound CB1 reveal significant structural rearrangements

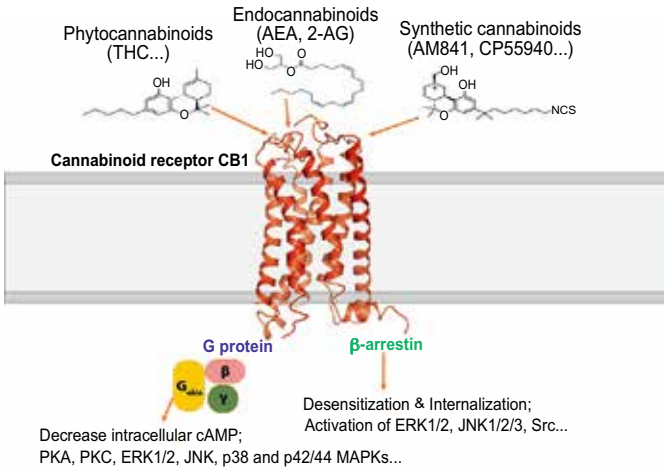

Fig. 1. Schematic representation of CB1-mediated signaling pathways. CB1 can be activated by phytocannabinoids, endocannabinoids and synthetic cannabinoids and induce the G protein or β-arrestin activation pathways.

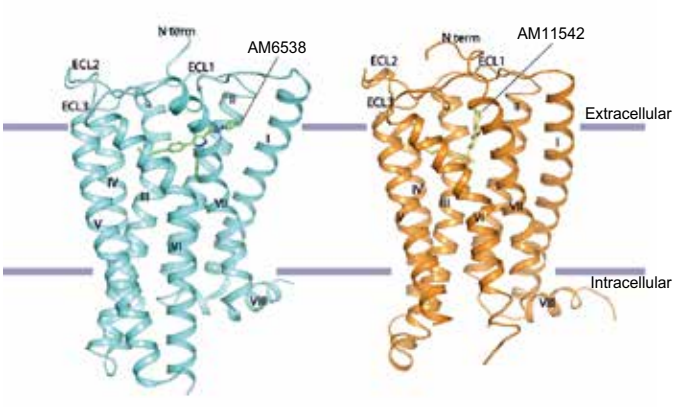

Fig. 2. The overall structures of antagonist- and agonist-bound CB1. The antagonist AM6538 (green sticks) bound CB1 structure and agonist AM11542 (yellow sticks) bound CB1 structure is shown in cyan and orange cartoon, respectively.

(C_α r.m.s.d. of the overall structure without fusion protein: 3.52 Å). The notable conformational change occurs in helices I and II. The extracellular part of helix I bends inwards by 6.6 Å and helix II rotates in by about 6.8 Å, respectively in the AM11542-bound structure (Fig. 3(a)). Similarly, conformational changes are also observed in the cytoplasmic part of the receptor, in which helix VI moves outwards by about 8 Å (Fig. 3(b)). This is the largest structural change, especially within the extracellular portion, observed in the solved agonist/antagonist-bound pairs of class A GPCRs.

The agonist-induced conformational changes probably trigger the activation and downstream signaling. CB1 seems to use an extended molecular toggle switch involving a synergistic conformational change between Phe200$^{3.36}$ and Trp356$^{6.38}$, which we refer to as the 'twin toggle switch' (Fig. 3(d)). Comparing previously proposed 'toggle switch' of Trp356$^{6.38}$, the synergistic movement of two residues during the activation of the receptor has never been observed before and we speculate that this 'twin toggle switch' is related to CB1 activation.

A notable feature of the CB1 agonist-bound structure is the large (53%) reduction in volume in the ligand-binding pocket. Such plasticity in the orthosteric binding pocket enables CB1 to respond to a diverse array of ligands with considerably different sizes, shapes and associated functions, consistent with the repertoire of CB1 to modulate such variety of physiological and psychological activities (Fig. 1).

In summary, the current study reported the first crystal structure of antagonist-bound CB1 and also the agonist-bound CB1, which reveal the inactive and active-like states of the receptor and provide important structural insights to the activation mechanism of the receptor. In most agonist- and antagonist-bound structural pairs of class A GPCRs, the extracellular half shows small changes while larger conformational changes in the intracellular half. As an exception, CB1 has the largest ligand-binding pocket volume change, contributed mainly by the movements of the extracellular half of helices I and II. The balloon-like flexibility of CB1 in the extracellular region may also occur in other GPCRs. Therefore, in designing GPCR agonists or antagonists using structure-based strategies, multiple, structurally varied receptor models should be considered.

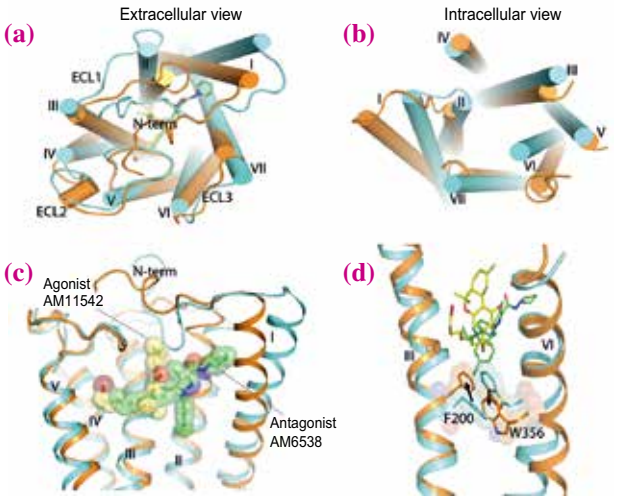

Fig. 3. Comparison of agonist- and antagonist-bound CB1 structures. The extracellular **(a)** and intracellular **(b)** views of the compared receptors. **(c)** Comparison of agonist-bound (orange cartoon) and antagonist-bound (cyan cartoon) CB1 ligand-binding pockets. AM11542 (yellow) and AM6538 (green) are shown in sticks and sphere representations. **(d)** The 'twin toggle switch', F200/W356, is shown in sticks and spheres.

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References

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