CANNABIDIOL ACTS AS AN ALLOSTERIC MODULATOR OF CANNABINOID CB2 RECEPTORS

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ABSTRACT

In a heterologous expression system consisting of mammalian preosteoblasts of cells (MC3T3-E1) expressing the CB2-human receptor, we have confirmed using a synthetic radioligand agonist, [3H]-WIN 55,212-2, that CBD does not bind with high affinity to CB2R. However, in the absence of the agonist, CBD decreases the binding of the fluorescent labeled CM-157 to CB2R, and inhibit the response of inhibition of forskolin-induced cAMP produced by the selective CB2R agonist JWH-133 (Figure 3A). Accordingly, CBD was suspected as acting as allosteric modulator of cannabinoid receptors (Rimoldi and Bow, 2016; Laprairie et al., 2015). This hypothesis was confirmed by functional experiments in which the effect of a selective CB2R agonist was modulated by CBD at physiologically relevant concentrations. Therefore, we have confirmed using radioligand binding experiments and a slightly different binding mode of radiolabeled WIN 55,212-2 or fluorescence-labelled CM-157 to CB2R. This effect is dose-dependent and time-dependent, as shown in the Figure 3B and C, respectively.

MATERIALS AND METHODS

Radioligand binding assays

Competition binding experiments were performed in 96 well plates expressing human CB2Rs (CM-157-CB2R) by incubation with 3 nM [3H]-WIN 55,212-2 and different concentrations of the non radioactive compounds with metabolites isolated from CB2R cells (112 pM protein per sample for 60 minutes at 30°C). Non-specific binding was determined in the presence of 1 μM WIN 55,212-2. Signal was detected using an EnVision microplate reader (Perkin-Elmer). The competitive binding was performed using a Perkin Elmer Cetus 96-well microtiter plate.

Non-fluorescent ligand binding assays

For cAMP determinations, serum-starved cells were detached and resuspended in growing medium containing 50 μM forskolin or vehicle. HTRF measures after 15 minutes incubation were performed in quadruplicate. HTRF ratio = 665 nm acceptor signal/620 nm donor signal. Kinetic binding assays were performed by incubating cells expressing Tb-labelled SNAP-CB2R with 24 nM or 48 nM red CB2R ligand (CM-157) in the presence of increasing concentrations of CBD. The fluorescent labeled CM-157 was excited using a Perkin Elmer Cetus 96-well microtiter plate equipped with a FRET counter (Perkin Elmer). Data results from at least 3 independent experiments. The data in the graphs are the mean ± SEM. Statistical analysis was performed in SPSS 18.0 software. Significant differences were considered when p<0.05.

RESULTS

For cAMP determinations, we have confirmed that CBD is not able to replace the binding of WIN 55,212-2 to the orthosteric sites of the CB2R. The HTRF data, however, showed that the binding of the selective CB2R agonist JWH-133 to CB2R cells expressing CBD was not statistically different from the binding of CBD alone. Therefore, we have confirmed that the effect of CBD on the binding site is not significant in the HTRF binding studies. The most plausible explanation of these data is an inability effect of CBD on the CB2R orthosteric binding site; however, we have not excluded the possibility that CBD modulates the binding of CB2R to other molecules such as proteins or nucleic acids.

CONCLUSION AND DISCUSSION

The hypotheses were confirmed that CBD does not bind with high affinity to CB2R. However, in the absence of the agonist, CBD decreases the binding of the fluorescent labeled CM-157 to CB2R, and inhibit the response of inhibition of forskolin-induced cAMP produced by the selective CB2R agonist JWH-133. According to our results, CBD acts as an allosteric modulator of CB2R.

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REFERENCES


CM-157

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