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Coupling of the human recombinant CB_2 cannabinoid receptor to ERK and calcium elevation

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Background: Both CB_1 and CB_2 cannabinoid receptors are described to inhibit adenylyl cyclase and activate extracellular signal-regulated kinase (ERK), although only CB_1 receptors are described to regulate ion channel function[1]. In this study, we have investigated the coupling of human recombinant CB_2 cannabinoid receptors expressed in Chinese hamster ovary cells.

Methods: ERK activation of CHO-hCB_2 cells was assessed using immunocytochemistry quantified with a Li-Cor Odyssey and foetal calf serum as a positive control, while intracellular calcium levels were assessed using fluo-4 with a FlexStation and ATP as a positive control. Experiments were conducted on at least five different cell passages.

Results: HU210-evoked ERK activation reached a peak between three and five minutes. The following agents evoked maximal responses similar to the positive control (78-90 %) with the rank order: WIN55212-2 (pEC_{50} 7.7 ± 0.1), HU210 (7.4 ± 0.2), fenofibrate (7.2 ± 0.1), JWH015 (7.2 ± 0.1) > 2-AG (6.8 ± 0.1), JWH133 (6.5 ± 0.1). AEA evoked a reduced maximal response (51%, 5.7 ± 0.2), as did THC (10-15% at 10 µM), while β-caryophyllene and caryophyllene N-oxide were inactive. 1 µM AM630, a CB_2-selective antagonist, or pretreatment with 100 ng/mL pertussis toxin blocked responses to all these agonists. Calcium ion levels were stimulated by cannabinoid agonists, albeit in a slower manner than the positive control. These responses were quantified as area under the curve over the two min incubation period: WIN55212-2 (21 ± 5 % ATP response, 7.3 ± 0.1) > HU210 (24 ± 3, 6.8 ± 0.3), CP55940 (37 ± 5, 6.7 ± 0.3), JWH015 (24 ± 3, 6.4 ± 0.1), fenofibrate (26 ± 3, 6.3 ± 0.2) > 2-AG (34 ± 6, 6.2 ± 0.2), JWH133 (29 ± 7, 6.0 ± 0.1) > AEA (11 ± 1, 5.7 ± 0.3). Responses to THC failed to reach statistical significance, while those to β-caryophyllene and caryophyllene N-oxide were not distinguishable from background. Calcium responses to cannabinoid ligands were blocked by pretreatment with 1 µM AM630 or 100 ng/mL pertussis toxin. Cannabinoid ligand-evoked calcium responses were unaltered in the presence of 10 µM U0126, a selective inhibitor of ERK activation.

Conclusions: This is the first study to determine coupling of the CB_2 cannabinoid receptor to calcium elevation in recombinant expression.

References

1. Pertwee et al. (2010). Pharmacol Rev PMID:21079038

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