Proceedings of the British Pharmacological Society at http://www.pA2online.org/abstracts/Vol17Issue1abst007P.pdf

Coupling of the human recombinant CB_2 cannabinoid receptor to extracellular signal-regulated kinase and calcium elevation in Chinese hamster ovary cells

W. Hourani, R. Roberts, S. P. Alexander. Molecular Pharmacology and Drug Discovery, University Of Nottingham, Nottingham, United Kingdom.

Background: CB_1 cannabinoid receptors are characterised as coupling via the $G_{i/o}$ family of G proteins to the inhibition of adenylyl cyclase activity and the activation of extracellular signal-regulated kinase (ERK)[1]. CB_1 receptors also inhibit voltage-gated calcium channels, activate potassium channels and stimulate calcium mobilisation[1]. CB_2 cannabinoid receptors are described to inhibit adenylyl cyclase and activate ERK, but not 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: Human recombinant CB_2 cannabinoid receptors were expressed in Chinese hamster ovary cells. ERK activation was assessed using in cell immunocytochemistry quantified with a Li-Cor Odyssey, while intracellular calcium levels were assessed using fluo-4 with a FlexStation with ATP as a positive control.

Results: A range of cannabinoid agonists both endogenous (anandamide and 2-arachidonoylglycerol), natural (THC, β-caryophyllene and caryophyllene N-oxide) and synthetic (CP55940, WIN55,212-2, fenofibrate,HU210, JWH133, and JWH015)were tested. The following agents evoked maximal responses similar to the positive control fetal bovine serum (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), 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 ATP. These responses were quantified as area under the curve: 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