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Assessment of calcium mobilization induced by the cannabinoid ligands N-arachidonoyl glycine (NAGly) and delta ^{9}Tetrahydrocannabinol (Δ^{9}THC) in GPR18-transfected HEK293TR cells

The candidate cannabinoid receptor GPR18 has the potential of being a novel therapeutic target (1). NAGly has been suggested by many studies as an endogenous high potency agonist at GPR18(2,3) However, some studies have reported a lack of activation of GPR18 by NAGly (4,5).

The rationale of this study was to investigate the effect of putative GPR18 ligands NAGly andΔ^{9}THC on calcium mobilization in HEK293TR cells heterologously expressing human GPR18.

SNAP-tagged human GPR18 under the control ofa tetracycline-regulated expression system was heterologously expressed in HEK293TR cells. The transfected HEK293TR cell line was imaged on the IX Ultra confocal plate reader to determine successful expression of SNAP-tagged receptor. Calcium mobilization was assessed using fluo-4 AM dye with Flexstation. Single cell calcium imaging of fura-2 loaded cells was also used to assess GPR18 responses.

Out of a range of concentrations of NAGly (1 nM-10 μM) and Δ^{9}THC (1 nM-20 μM) examined, 10μM showed a gradual increase in intracellular calcium which was readily distinguished from that evoked by the positive control (100 μM Carbachol). Modification of the experimental conditions by inclusion of protein (1mg/ml BSA) in the assay buffer to enhance the presentation of the hydrophobic cannabinoid compounds to the GPR18 receptor did not change the result as none of the NAGly or Δ^{9}THC concentrations tested in this study showed any significant change in calcium response compared to the vehicle control (two way ANOVA with Dunnett corrections)in the presence and absence of BSA in GPR18-transfected HEK293TR with and without tetracycline induced receptor expression. NAGly, but not Δ^{9}THC, significantly increased the peak ($P<0.01$) and total ($P<0.001$) calcium response induced by 1μM carbachol in GPR18-transfected HEK293TR with and without tetracycline induction of receptor expression when compared to the vehicle control. No significant difference in calcium response was observed between tetracycline and non-tetracycline treated cells (two way ANOVA with Bonferroni corrections) indicating that this effect was not related to GPR18 receptor expression.

In this study, recombinant GPR18 receptor failed to respond to NAGly or Δ^{9}THC in terms of calcium mobilization. This suggests that GPR18 signalling may involve other pathways not examined in the current study or there is some other issue affecting GPR18 responses to these cannabinoid compounds in these cells.

Reference:

1. Alexander, SPH (2012). So what do we call GPR18 now? Br J Pharmacol 165: 2411-2413.

2. McHugh, D, Page, J, Dunn, E, Bradshaw, HB (2012).Δ^{9}9-THC and N-arachidonyl glycine are full agonists at GPR18 and cause migration in the human endometrial cell line, HEC-1B. Br J Pharmacol 165: 2414-2424.

3. Kohno, M, Hasegawa, H, Inoue, A, Muraoka, M, Miyazaki, T, Oka, K, Yasukawa, M (2006). Identification of N-arachidonylglycine as the endogenous ligand for orphan G-protein-coupled receptor GPR18. Biochem Biophys Res Commun 347: 827-832.

4. Lu, VB, Puhl, HL, Ikeda, SR (2013). N-Arachidonyl glycine does not activate G protein-coupled receptor 18 signaling via canonical pathways. MolPharmacol 83: 267-282.

5. Southern, C, Cook, JM, Neetoo-Isseljee, Z, Taylor, DL, Kettleborough, CA, Merritt, A, Bassoni, DL, Raab, WJ, Quinn, E, Wehrman, TS, Davenport, AP, Brown, AJ, Green, A, Wigglesworth, MJ, Rees, S (2013). Screening β-arrestin recruitment for the identification of natural ligands for orphan G-protein-coupled receptors. J Biomol Screen 18: 599-609.