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Activation Of The Putative Cannabinoid Receptor GPR55 Involves Both Rho And ROCK

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GPR55, an orphan G protein-coupled receptor, has been proposed to be a third cannabinoid receptor although it has other ligands (1). Gα13 has been proposed to initiate its signalling (2) and, although Rho is its immediate effector, the subtype(s) of Rho and the downstream molecules involved are unknown. This study aimed to provide further insight into the signalling molecules activated by GPR55.

RT-PCR showed that the mRNAs of RhoA, RhoB, RhoC and PLCε (a direct downstream molecule of Rho activation) were expressed in HEK293 cells stably-expressing GPR55 (HEK-GPR55). Potential ligands for GPR55, LPI, CP55,940, anandamide, AM251 and rimonabant (1-100 μM, n≥60), were assessed by population Ca^{2+} imaging. Ca^{2+} release was assessed by Ca^{2+} peak height, width and time to first peak. Negative controls were performed by the application of HEPES-buffered saline (HBS) while the positive control was carbachol (10 uM). Responses were normalized and expressed as F/F0, the fold change in fluorescence, with F0 standing for the fluorescence ratio in HBS. Responding cells were defined as those demonstrating a higher F/F0 after ligand application than HBS. Statistical analyses were performed by two-way analysis of variance followed by a Bonferroni *post hoc* test.

LPI (LogEC50=-4.76), virodhamine ($LogEC_{50}$=-4.62) and AM251 ($LogEC_{50}$=-4.29) activated GPR55 as shown by Ca^{2+} release (n≥60). Inhibition of ROCK with Y-27632 (50 μM; from Tocris) reduced (P<0.001) the Ca^{2+} peak height induced by all three agonists (F/F0: Control LPI 2.06±0.06, virodhamine 3.47±0.1, AM251 1.87±0.09, carbachol, 2.31±0.09; with Y-27632, LPI 1.42±0.05, virodhamine 2.90±0.07, AM251 1.27±0.04, carbachol, 2.55±0.09), Rho inhibition (with 20μM C3 toxin; from Universal Biologicals) reduced Ca^{2+} peak height by virodhamine (F/F0: with C3, LPI 1.97±0.09, virodhamine 2.1±0.15, AM251 1.62±0.06, carbachol, 2.50±0.05). Neither of the inhibitors had a significant effect on peak width at ½ peak height. Rho inhibition appeared to reduce the rate of activation of the receptor and signalling system by delaying the time to the onset of the first Ca^{2+} peak (onset time (s): Control LPI 13.4±1.12, virodhamine 2.73±0.76, AM251 20.3±3.73, carbachol, 2.7±0.42; with C3, LPI 22.51±2.75, virodhamine 29.08±2.16, AM251 54.13±6.27, carbachol, 1.55±0.14), (P<0.001).

In summary, LPI, virodhamine and AM251 elicit Ca^{2+} release, presumably by GPR55 activation and both Rho and ROCK are involved. However, virodhamine activates GPR55 in a manner dependent on both molecules while AM251 and LPI depend more on ROCK, suggesting that diverse ligands may activate different signalling pathways and GPR55 may exhibit functional selectivity. The subtype of Rho activated awaits identification.

(1) Brown AJ & Hiley CR (2009) Vitam Horm 81: 113-137

(2) Henstridge CM, Balenga NA, Ford LA et al. (2009) FASEB J 23: 183-193