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β3-Adrenoceptor desensitisation in HEK cells: a role for biased agonism?

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Introduction: β3-Adrenoceptor (B3AR) coupling to cAMP formation undergoes agonist-induced desensitisation in some cell types such as human embryonic kidney (HEK) cells but not in others. In some cell types, B3AR can also couple to Gi proteins, leading to dampened cAMP formation and activation of extracellular signal-regulated kinases (ERK). Using a panel of structurally different B3AR ligands, we wished to explore whether B3AR-mediated ERK activation also undergoes agonist-induced desensitisation in HEK cells transfected to express physiological levels of human B3AR.

Method: Cell culture and cAMP experiments were performed as previously described1, except that cAMP quantification was performed with the AlphaScreen assay from Perkin-Elmer (Rodgau, Germany). ERK activation was assessed as phosphorylation using the AlphaLISA SureFire Ultra assay (Perkin-Elmer) using the one-plate protocol. With a pre-specified sample size of n = 8 experiments, statistical analysis of cAMP data was based on log-transformed values using ANOVA with $P < 0.05$ considered significant.

Results: B3AR ligands (10 μM each) stimulated cAMP accumulation (fmol/25 μL) with a rank order of isoprenaline (414 ± 258) ≥ L755,507 (360 ± 219) ≥ CL316,243 (306 ± 180) ≈ solabegron (272 ± 174) > SR59,230 (153 ± 101) ≥ L748,337 (100 ± 89), whereas the response to 100 μM forskolin was 282 ± 196. Pre-treatment with pertussis toxin (100 ng/ml for 24 h) did not affect the cAMP response to any ligand. Pre-treatment with 10 μM of the each B3AR ligands except L 748,337 for 24 h reduced cAMP responses to freshly added isoprenaline or forskolin by 73-85% and 63-78%, respectively; in contrast, L 748,337 reduced cAMP formation by isoprenaline and forskolin by only 37% and 30%, respectively. Pre-treatment with isoprenaline, solabegron and SR 59,230 also desensitised the concentration-response curves for the freshly added ligand, albeit with different pattern with regard to effect on Emax, potency and forskolin responses. Despite testing numerous experimental conditions, we were unable to detect robust ERK phosphorylation in HEK cells by any ligand.

Conclusion: We conclude that agonists for cAMP formation with different efficacies also differ in their ability to induce desensitisation of cAMP formation. HEK cells lack B3AR coupling to Gi and, accordingly, ERK phosphorylation, not allowing to study a role for biased agonism in desensitisation.

References:

1. Michel-Reher MB et al. (2013). Naunyn-Schmiedebergs Arch Pharmacol 386: 843-851