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β_3-Adrenoceptor desensitisation in CHO cells: comparison of cAMP and ERK signalling

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Introduction: β_3-Adrenoceptors (B3AR) classically couple to cAMP formation but also to alternative pathways including phosphorylation of extracellular signal-regulated kinases (ERK). We have compared desensitization of cAMP accumulation and ERK phosphorylation in Chinese Hamster ovary (CHO) cells stably transfected with human B3AR.

Method: Cell culture and cAMP experiments were performed as reported previously[1], except that cAMP quantification was performed with the AlphaScreen assay (Perkin-Elmer, Rodgau, Germany). ERK phosphorylation was assessed using the AlphaLISA SureFire Ultra assay (Perkin-Elmer). Sample size was pre-specified as n=8. cAMP data are expressed as geometric means of fmol/25 µl, pERK as means of % of matched basal. Desensitization was assessed as changes in E_{max} and pEC_{50} based on paired t-tests with $P<0.05$.

Results: B3AR ligands (10 µM each) stimulated cAMP accumulation with a rank order of isoprenaline (255) ≈ L755,507 (247) > CL316,243 (209) > L748,337 (120) ≈ SR59,230 (108). Pre-treatment with pertussis toxin (PTX; 100 ng/ml for 24 h) did not affect the cAMP response to any ligand. Pre-treatment (10 µM for 24 h) with isoprenaline desensitized the concentration-response curves for the freshly added ligand similar to previous findings in HEK cells[1] whereas pre-treatment with L748,337 had little effect. Ligands stimulated ERK phosphorylation with a rank order of L755,507 (151%) ≈ CL316,243 (149%) ≈ isoprenaline (148%) > SR59,230 (137%) > L748,337 (122%). PTX markedly lowered basal pERK, and enhanced all agonist responses relative to this lowered basal. Pretreatment with isoprenaline lowered basal, reduced potency by 0.8 log units and increased E_{max} relative to the lowered basal (336% to 424%). In contrast, L748,337 did not change basal pERK or pEC_{50} but reduced E_{max} (171% to 146%). Neither changed total ERK.

Conclusion: We conclude that cAMP formation in CHO cells is desensitized by the full agonist isoprenaline but not the partial agonist L748,337. In contrast, pERK responses were affected in a qualitatively different manner by pre-treatment with isoprenaline and L755,507. This appears to be the first demonstration of desensitization of pERK responses for a G_s-coupled receptor.

References:

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