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Agonist bias in delta opioid receptor signalling.

Delta opioid receptor (DOR) agonists have therapeutic potential for the treatment of a variety of conditions including chronic pain and affective disorders. Differences in the ability of G protein-coupled receptor agonists to activate different post-receptor signalling systems (biased agonism) might determine the functional profiles of individual agents. The aim of this study was to determine the post-receptor signalling characteristics of three structurally distinct DOR agonists, the peptide DADLE ([D-Ala2, D-Leu5]-enkephalin) and the non-peptides SNC80 and ADL5859.

The experiments employed CHO cells over-expressing the human DOR receptor. Activation of the MAPK/ERK pathway was assessed with an In-cell Western assay using antibodies for the phosphorylated and non-phosphorylated forms of ERK1/2. Phosphorylation was determined after 5mins of agonist exposure using an Odyssey infra-red imaging system (Li-Cor). Cyclic AMP (cAMP) inhibition was measured using an immunoassay (HitHunter; Discoverx) after stimulating cells with 1μM forskolin. Effects of agonists on B-arrestin recruitment and DOR internalization were measured using enzyme fragment complementation assays (PathHunter; Discoverx). All assays were conducted in duplicate and replicated on 3-7 occasions.

The rank orders of agonist potencies were the same for all 4 assays (DADLE>SNC80>ADL5859). Although there were only minor differences in most of the agonists' absolute potencies across the 4 assays, DADLE was markedly biased towards cAMP inhibition (IC50=0.06nM) vs ERK phosphorylation (EC50=86nM). With regard to maximal responses (Emax), SNC80 produced the greatest response in ERK, arrestin and internalization assays and the effects of the other 2 agonists were, therefore, expressed as a % of the responses to SNC80. ADL and DADLE produced complete inhibition of the cAMP response to forskolin but SNC80's Emax was only 69±8% inhibition. ADL5859 was a full agonist in cAMP and ERK assays but only partial regarding arrestin and internalization (77±4% and 75±2% respectively). Although DADLE was a full agonist in the cAMP assay, it was partial in ERK, arrestin and internalization assays (46±1%, 79±2% and 64±3% of the SNC80 Emax respectively), despite the finding that both cAMP inhibition and ERK phosphorylation were apparently Gi protein-mediated (inhibited by overnight exposure to 200nM pertussis toxin). Tolerance *in vivo* to the effects of DOR agonists is reported to be related to agonist efficacy (Codd *et al*.,2009); therefore, the potential differential ability of the three agonists to cause DOR desensitization *in vitro* was assessed by exposing DOR-expressing CHO cells to a maximally effective concentration (1μM) of each drug for 48 hrs and, thereafter, assessing their ability to inhibit forskolin-stimulated cAMP formation. However, all three agonists appeared to completely desensitize the receptor and none was able to produce any inhibition of the forskolin response.

The three agonists examined displayed significant agonist bias with regard to 4 different signalling endpoints, but whether this underpins any functional differences in wanted and unwanted pharmacological effects *in vivo* remains to be determined.

Reference:

Codd, E.E. *et al*., *J Pharmacol Exp Ther*. **329**: 241-51