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Different abilities of kappa opioid receptor agonists to inhibit cholinergic activity in human colon do not 'back-translate' to mice or recombinant receptor cell signalling assays

Kappa opioid receptor (KOR) agonists have been developed for the alleviation of pain associated with irritable bowel syndrome. Here we explore reasons for limited success by comparing the abilities of ICI204448 and asimadoline, selective KOR agonists (1,2), to reduce cholinergic contractility in human and mouse isolated colon. Mechanisms of action were studied using recombinant human and mouse receptors, measuring G protein and ß-arrestin functions, and analysis of receptor amino acid sequences.

Circular muscle strips of human colon were prepared for isometric recording in tissue baths containing Krebs solution, for electrical field stimulation (EFS; 5Hz, 50V, 10s every min) of intrinsic neurons (3). Circular muscle preparations of proximal colon were also prepared from adult C57BLK6/J mice (EFS; 5Hz, 50V, 30s every 2 min). HEK293 cells (100k cells/well) transfected with human or mouse KORs were used to assess Gi heterotrimer complex dissociation using bioluminescent (BRET) and β arrestin recruitment and internalisation using time resolved fluorescent resonant energy transfer (TR-FRET).

In human colon, responses to EFS are complex (3), consisting of cholinergically-mediated contraction or nitrergic relaxation during EFS, often followed by a predominantly cholinergic after-contraction. In preparations contracting during EFS, ICI204448 (100pM-1µM) inhibited the contractions (e.g. ascending colon: pEC_{50}=8.9±0.6, E_{max}=90±14%, n=4). After simplifying the assay by application of the nitric oxide synthase inhibitor LNAME 300µM, which changed the biphasic EFS-evoked response to a monophasic contraction in 39/70 strips in ascending colon and 32/188 strips in descending colon (3), inhibition was confirmed (ascending: pEC_{50}=8.8±0.3, E_{max}=52±4%, n=4; descending: pEC_{50}=8.0±0.4, E_{max}=57±7%, n=4-6). Asimadoline (100pM-10µM) acted similarly but was markedly less potent (e.g. in presence of LNAME: ascending: pEC_{50}=6.4±0.5, E_{max}=39±8%, n=4; descending: pEC_{50}=6.0±0.3, E_{max}=56±11%, n=4-6). In mice EFS caused tetrodotoxin-sensitive (n=9) relaxations during EFS (inhibited by LNAME, n=3) followed by after-contractions (inhibited by atropine 1µM; n=7). ICI204448 (100pM-1µM) and asimadoline (100pM-1µM) were equi-potent and equi-effective inhibitors of the after-contractions (respectively, E_{max}=49±8%, pEC_{50}=8.5±0.8, and E_{max}=51±8%, pEC_{50}=8.1±0.5; n=3-4). There were no differences in ability of asimadoline and ICI204448 to activate human or mouse recombinant KORs or signal via Gi (human: pEC_{50}=9.5 and 9.3 respectively; n=3) and ß-arrestin pathways (human: pEC_{50}=7.3 and 7.5 respectively; n=3). Human and mouse amino acid sequences differed mostly at the C terminus.

In human colon, a marked difference in activity of two KORs is not predicted by similar studies in mice or by recombinant receptor cell signalling assays. Human native tissue is needed to translate functional studies using KOR and perhaps other opioid receptors.

(1) Kumar et al. (2005) *Bioorg Med Chem Lett.* Mar 1;15(5): 1279-82.

(2) Mangel & Hicks (2012) *Clin Exp Gastroenterol.* 5: 1-10

(3) Broad et al. (2013) *Br J Pharmacol.* Nov;170(6): 1253-61.