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Major ligand-dependent differences in efficacy between kappa opioid receptor agonists in human, but not mouse colon

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Opioid receptors are subject to biased agonism, in which G-protein- and arrestin-coupled pathways are differentially activated to evoke different cellular actions. Such mechanisms are likely to show species-dependency. Human and rodent κ opioid receptors (KORs) for example, share ~94% amino acid sequence homology, but residues involved in intracellular signalling are not conserved (1). To explore this concept further, hitherto studied only using cell-based assays, we examined the abilities of KOR agonists to inhibit cholinergic functions in human and mouse isolated colon.

Macroscopically normal human colon was obtained from surgery for cancer with informed consent (14 ascending colon, 28 descending colon; 24 male 18 female; median age = 64.5 (35-90)), and mucosa-free circular muscle strips suspended in tissue baths for electrical field stimulation (EFS) as described previously (2). Loops of ascending and descending mouse colon (3 month C57BL6 males; 2 mm wide) were suspended similarly (Krebs; 5% CO_2 in O_2; 37°C; 1g tension; isometric recording). EFS was applied at 5Hz (human: 0.5ms pulse width, 50V, 10s every 1 min; mouse: 0.5ms, 80V, 30s every 2 min) and drugs were applied non-cumulatively. Data were, analysed using GraphPad 5.0, plotted as 3-parameter concentration response curves and expressed as means ± error. Differences between drugs were analysed using 2-way ANOVA with Bonferroni post-tests, and effects on carbachol were analysed using 1-sample T tests.

Contractions (64%) and relaxations (34%) of human colon were evoked during EFS, often followed by after-contractions (85%; 42 patients; 400 strips). In mouse colon relaxations during EFS were often followed by after-contractions (68%; 115 loops). All responses to EFS were abolished by TTX 1µM (n=4, 3; human and mouse respectively). Relaxations during EFS were abolished by L-NAME 300µM and EFS-evoked contractions became monophasic (n= 30, 3). After-contractions were abolished by atropine 1µM in mouse colon (n=3), whereas in humans, contractions during EFS were abolished and the after-contractions attenuated and further reduced by NK_{1-3} receptor antagonists (2). In both regions of mouse colon, the KOR agonists asimadoline (3) and ICI204448 (4) concentration-dependently inhibited EFS-evoked after-contractions (e.g. in ascending colon, asimadoline E_{max} 52±7%, pEC_{50} 7.8±0.4; ICI204448 E_{max} 44±8%, pEC_{50} 7.8±0.5, n=3 each concentration tested; P>0.05). In human colon, asimadoline and ICI204448 inhibited the contractions during EFS in an approximately concentration-dependent manner but with marked differences in efficacy (asimadoline E_{max} 53±6% EFS, pEC_{50} 6.9±0.4, n=3-4; ICI204448 E_{max} 103±34%, pEC_{50} 8.4±0.6, n=3-4; P=0.03) and a trend towards a delay in the time to maximal effect (respectively 33±8 and 14±4 min P=0.08; unpaired t test); neither

compound consistently changed after-contraction amplitudes. A similar difference in efficacy was observed in the presence of L-NAME 300μM (respectively, E_{max} 16±6 and 45±10%, estimated pEC_{50} 8.6±1.7 and 8.5±0.7, n= 3-4 each; P=0.02). Asimadoline and ICI204448 had no effects on submaximally-effective contractions evoked by carbachol 1μM in human (n=2 each; P>0.05) or mouse colon (n=3 each; P>0.05). Differential ligand- and species-dependent functions of KORs must now be further explored using human tissues to determine the chemical structures for optimal therapeutic activity in the bowel and elsewhere.

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