John Snow Final Report – Jonathan O'Doherty

Abstract

Background

The use of opioids as analgesics is limited by their adverse effects, including tolerance. In patients requiring long term opioids, tolerance leads to a cycle of dose escalation and increased side effects, with an associated effect on quality of life. A thorough understanding of the opioid signalling pathways is required to develop the next generation of opioids, free from these adverse effects.

One aspect of opioid signalling is the activation of Mitogen Activated Protein Kinases (MAPKs). The role of MAPKs in opioid signalling poorly understood, though they have been linked to tolerance and other adverse effects such as opioid induced bowel dysfunction. In this project we examined the coupling of the MOP and NOP opioid receptors to the MAPK pathways. We compared this with two other, more conventional, measures of opioid signalling – G-protein activation assays and adenylyl cyclase inhibition assays.

Methods

Experiments were performed using Chinese Hamster Ovary cells stably expressing recombinant human MOP and NOP receptors. Activation of MAPK was assayed using Western blot for ERK1/2, p38 and JNK. Receptor activation was further assessed in cell membranes using GTPγ[^{35}S] binding (upstream signal) and in whole cells using an adenylyl cyclase inhibition assay (downstream signal).

Results

At the MOP receptor, Endomorphin-1 caused a concentration dependent activation of ERK1/2 peaking at 5-minutes with a potency (pEC50) of 8.08(8.3nM). Potency for G-protein activation and adenylyl cyclase inhibition was 6.78(166nM) and 9.81(0.15nM) respectively. There was no activation of p38 or JNK. At the NOP receptor, N/OFQ caused a concentration dependent activation of ERK1/2 peaking at 15 minutes with a potency of 9.78(0.17nM). Potency for G-protein activation and adenylyl cyclase inhibition was 9.27(0.54nM) and 10.83(0.08nM) respectively. There was a questionable activation of p38 and no activation of JNK. For a panel of MOP and NOP agonists ERK1/2 activation agonists was assessed and compared to the comparator ligands Endomorphin-1 and N/OFQ (Table 1). For this same group of ligands G-protein activation was assessed (Table 1).

MOP Receptor	αE_{max} ERK1/2 activation	αE_{max} G-protein activation
Endomorphin-1	1.00	1.00
Herkinorin	0.04±0.01 †	0.74±0.09
Dmt-N/OFQ	1.23±0.35	1.08±0.15
Buprenorphin	0.11±0.03 †	0.02±0.03
DAMGO	1.29±0.50	1.50±0.10
NOP Receptor		
N/OFQ	1.00	1.00
Ro 64-6189	1.40±0.07	1.15±0.18
Dmt-N/OFQ(1-13)	1.37±0.12	1.07±0.18
Buprenorphine	0.28±0.11 *	0.28±0.06
PWT2-N/OFQ	0.99±0.04	0.94±0.11

Table 1 – Table demonstrating the Emaxs for activation of ERK1/2 of a panel of MOP and NOP agonists relative to endomorphin-1 and N/OFQ respectively (αEmax) as well as αEmax for activation of g-proteins. Data are shown are means ± SEM. † = statistically significant vs. DAMGO (p<0.05, ANOVA). * = statistically significant vs. N/OFQ (p<0.05, ANOVA)

Conclusions

The MOP and NOP receptors demonstrated differential amplification and temporal activation profiles for the activation of ERK1/2. This suggests that these receptors activate ERK1/2 via separate mechanisms. While further study into these pathways is required, this project has highlighted differences in signalling which could be exploited in novel opioid ligands, to produce analgesics with improved adverse effect profiles. In addition, the MOP agonist herkinorin was a full agonist in terms of G-protein activation but poorly activated ERK1/2, providing a demonstrating of biased agonism.

Publications

Abstract on “Coupling of the NOP receptor to MAP Kinase” accepted at the Anaesthetic Research Society Spring Meeting