

ACTIVATION OF NOCICEPTIN/ORPHANIN FQ AND CLASSICAL OPIOID RECEPTORS IN CHO CELLS EXPRESSING THE CHIMERIC GALPHA_{qi5} PROTEIN PROMOTES CALCIUM MOBILIZATION

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G-protein coupled receptors (GPCR) are among the most promising targets for drug discovery. Nowadays, novel GPCR ligands are mainly identified using high-throughput screening techniques based on recombinant receptors and calcium measurement with fluorometric assays (i.e. FlipR). To extend this approach to G_i coupled GPCR several strategies have been evaluated including the use of chimeric G proteins. The G α_{qi5} protein was used here to force nociceptin/orphanin FQ (N/OFQ) peptide (NOP) as well as classical opioid receptors (the DOP, MOP and KOP) to signal via the PLC-IP₃-Ca²⁺ pathway in CHO cells. [Ca²⁺]_i levels were monitored using the fluorometric imaging plate reader FlexStation II and the Ca²⁺ dye Fluo 4 AM. Concentration response curves to N/OFQ and standard ligands for classical opioid receptors were recorded in CHO cells stably expressing G α_{qi5} and NOP or opioid receptors. Results are summarized in the following table.

Table 1: Effects of N/OFQ, standard opioid ligands and ATP in CHO cells expressing G α_{qi5} and recombinant human receptors.

| | DOP | | MOP | | KOP | | NOP | |
|--------------------|--------------------------|-------------------------|--------------------------|-------------------------|--------------------------|-------------------------|--------------------------|-------------------------|
| | <i>pEC</i> ₅₀ | <i>E</i> _{max} | <i>pEC</i> ₅₀ | <i>E</i> _{max} | <i>pEC</i> ₅₀ | <i>E</i> _{max} | <i>pEC</i> ₅₀ | <i>E</i> _{max} |
| Morphine | crc incomplete | | 6.61 | 130±17% | crc incomplete | | | inactive |
| DPDPE | 8.89 | 76±2 % | inactive | | inactive | | inactive | |
| Dermorphin | 6.43 | 78±3 % | 7.89 | 146±29% | inactive | | inactive | |
| Dynorphin A | 7.73 | 75±4 % | 6.67 | 121±37% | 8.95 | 222±16% | inactive | |
| N/OFQ | inactive | | inactive | | inactive | | 9.49 | 220±17% |
| ATP | 5.80 | 176±17% | 6.18 | 270±42% | 5.91 | 252±20% | 5.83 | 253±21% |

Data are mean ± SEM of at least 5 separate experiments. *E*_{max} were expressed as % over the basal fluorescence levels.

These results are in line with those reported in the literature with standard assays (GTP γ S, cAMP assays) for G_i coupled receptors and demonstrated that signalling through G α_{qi5} does not produce major modifications of the pharmacological profile of the receptor under study. This notion should be however confirmed for each receptor in further studies employing a large panel of selective agonists, partial agonists and antagonists.