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

Camarda Valeria[1], Molinari Paola [2], Ambrosio Caterina [2], Kostenis Evi[3], Regoli Domenico[1], Guerrini Remo[4], Severo Salvadori[4], and Calo' Girolamo [1].

[1]Dept Exp and Clin Medicine, Section of Pharmacology, University of Ferrara, Italy;
[2]Dept of Pharmacology, ISS, Rome, Italy; [3:] Inst for Pharmaceutical Biology, Bonn, Germany; [4:] Dept of Pharmaceutical Sciences, University of Ferrara, Italy

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 $G\alpha_{qi5}$ 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-IP_3-Ca^{2+} pathway in CHO cells. $[Ca^{2+}]_i$ levels were monitored using the fluorometric imaging plate reader FlexStation II and the Ca^{2+} dye Fluo 4 AM. Concentration response curves to N/OFQ and standard ligands for classical opioid receptors were recorded in CHO cells stably expressing $G\alpha_{qi5}$ 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 $G\alpha_{qi5}$ and recombinant human receptors.

	DOP		MOP		KOP		NOP	
	pEC$_{50}$	***E$_{max}$***	***pEC$_{50}$***	***E$_{max}$***	***pEC$_{50}$***	***E$_{max}$***	***pEC$_{50}$***	***E$_{max}$***
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 (GTPγS, cAMP assays) for G_i coupled receptors and demonstrated that signalling through $G\alpha_{qi5}$ 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.