Dendrimeric derivatives of nociceptin/orphanin FQ: in vitro studies at recombinant human receptors

D. Malfacini[1], M.F. Bird[2], S. Salvadori[3], D.G. Lambert[2], R.Guerrini[3], G. Calo'[1]

[1]Dept. of Medical Science, Section of Pharmacology, University of Ferrara, Italy. [2]Dept. of Cardiovascular Sciences, University of Leicester, UK. [3]Dept. of Chemical and Pharmaceutical Science, University of Ferrara, Italy

Dendrimeric derivatives of the peptide nociceptin/orphanin FQ (N/OFQ) were prepared with a novel chemical strategy named peptide welding technology (PWT). Four N/OFQ sequences were linked to three different core moieties obtaining PWT1-N/OFQ, PWT2-N/OFQ, and PWT3-N/OFQ. In the present study the in vitro actions of PWT derivatives of N/OFQ were investigated at human recombinant N/OFQ receptors (NOP) in i) receptor binding, ii) stimulated [^{35}S]GTPgS binding, and iii) calcium mobilization studies. In receptor binding experiments performed in CHO_{NOP} cell membranes, N/OFQ displayed high NOP affinity (pK_i 9.42) associated to high selectivity (> 1000 fold) over classical opioid receptors (OP). PWT derivatives displayed 3-10 fold higher affinity than the natural peptide sequence and similar selectivity over OP receptors. In the same preparation N/OFQ stimulated [^{35}S]GTPgS binding with pEC_{50} and E_{max} values of 8.84 and 250 ± 20% over the basal values. All three PWT-N/OFQ derivatives produced maximal effects similar to N/OFQ displaying approximately 10 fold higher potency. In calcium mobilization experiments performed in cells stably coexpressing the NOP receptor and the Ga_{qi5} chimeric protein N/OFQ elicited a concentration dependent stimulatory effect with high potency (pEC_{50} 9.39) and maximal effects (237 ± 15% over the basal values). PWT derivatives of N/OFQ produced similar maximal effect but displayed 3 fold lower potency than the natural peptide. The competitive and NOP selective antagonist SB-612111 was able to inhibit N/OFQ stimulant effects with a pK_B value of 8.97. Similar results were obtained when the antagonist was challenged against the effect of PWT derivatives. In line with binding results, in calcium mobilization studies PWT derivatives displayed high (> 1000 fold) selectivity for NOP over OP receptors. Collectively the present findings demonstrated that PWT derivatives of N/OFQ displayed at the human recombinant NOP receptors full agonist activity, high potency, and high selectivity of action.