Dendrimeric derivatives of nociceptin/orphanin FQ: in vitro studies at native animal receptors

M.C. Cerlesi[1], S. Molinari[1], S. Salvadori[2], R.Guerrini[2], and G. Calo'[1]
[1]Dept. of Medical Sciences, Section of Pharmacology, and [2]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 tree 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 in bioassay studies using the electrically stimulated mouse vas deferens. In this preparation N/OFQ elicited a concentration dependent inhibition of the electrically induced twitch response with a pEC_{50} of 7.37 and E_{max}- 88 ± 1%. All three PWT-N/OFQ derivatives produced maximal effects similar to N/OFQ displaying approximately 3 fold higher potency. It is worthy of mention that the inhibitory effect of the natural peptide was rapid in onset, and fully and immediately reversible after washing while that exerted by the PWT derivatives displayed slow onset and kinetics and was resistant to wash. However in curative protocol experiments, the addition to the organ bath of 1 μM of the NOP antagonist SB-612111 produced a rapid and full reversal of the effects of both N/OFQ (30 nM) and its PWT derivatives (10 nM). The receptor involved in the inhibitory effects of N/OFQ and PWT-N/OFQ molecules has been investigated in receptor antagonist and knockout studies. SB-612111 produced a rightward shift of the concentration response curve to N/OFQ without modifying its maximal effects; a pK_B value of 8.48 was derived from these experiments. Similar results were obtained challenge SB-612111 vs PWT derivatives of N/OFQ (pK_B range 8.02 – 8.33). In tissues taken from wild type mice (NOP(+/+)) N/OFQ inhibited the twitch response in a concentration dependent manner (pEC_{50} 7.68 and E_{max} - 92 ± 2%). On the contrary, in tissues taken from NOP receptor knockout mice (NOP(-/-)) the peptide was completely inactive up to 1 μM. PWT derivatives of N/OFQ mimicked the inhibitory effect of the natural peptide in NOP(+/+) tissues showing similar maximal effects and higher potency. In tissues taken from NOP(-/-) animals PWT-N/OFQ compounds were still able to inhibit the electrically induced twitch response being however 20 (PWT1-N/OFQ) or 100 (PWT2-N/OFQ and PWT3-N/OFQ) fold less potent than in NOP(+/+) tissues. Collectively the present findings demonstrated that PWT derivatives of N/OFQ displayed at the mouse NOP receptors full agonist activity associated with high potency. The PWT modification also produced a loss of selectivity which was more evident for PWT1 than for PWT2 and PWT3.