In vitro pharmacological characterization of the non peptide NOP receptor agonists Ro 65-6570, SCH-221510 and Compound 6d

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The neuropeptide nociceptin/orphanin FQ (N/OFQ) via selective activation of the N/OFQ peptide (NOP) receptor regulates several biological functions both at central and peripheral sites. Selective non-peptide NOP ligands have been developed to investigate the role played by this peptidergic system in pathophysiology. As far as NOP agonists are concerned, most of the available data have been generated using the chemically related compounds Ro 64-6198 and Ro 65-6570.

Recently other non-peptide NOP agonists have been identified including SCH-221510 and Compound 6d. In the present study the *in vitro* pharmacological profile of these molecules has been investigated in comparison with that of the Roche compound Ro 65-6570. Calcium mobilization experiments were performed with CHO cells co-expressing chimeric G proteins and human recombinant NOP receptors. Bioassay experiments were performed with electrically stimulated tissues (mouse and rat vas deferens) expressing native animal NOP receptors.

In calcium mobilization studies N/OFQ displayed high potency (pEC_{50} 9.30) and maximal effects (approximated 200% over the basal levels). Non-peptide agonists mimicked the stimulatory effect of the peptide showing similar maximal effects but lower potency (20-50 fold). In the mouse vas deferens N/OFQ inhibited the electrically induced twitch response with a pEC_{50} of 7.55 and an E_{max} of -68%. The non-peptide agonists mimicked the effects of N/OFQ with lower potency (5-50 fold) but higher maximal effects. The effects of N/OFQ were sensitive to the NOP selective antagonist J-113397 (pA_2 7.88) and no longer evident in tissues taken from NOP knockout mice (NOP(-/-)). In contrast, the actions of non-peptide agonists were resistant to J-113397 and unchanged in NOP(-/-) tissues. In the rat vas deferens N/OFQ inhibited the electrically induced twitch response with a pEC_{50} of 7.25 and an E_{max} of -80%. Ro 65-6570 and SCH-221510 mimicked the peptide action with similar maximal effects and similar or reduced potency, respectively. Compound 6d was inactive in the nanomolar range of concentrations while it increased the electrically induced twitch at micromolar concentrations. The inhibitory effect of N/OFQ was antagonized by J-113397 with a pA_2 of 7.73. J-113397 displaced to the right the concentration response curve to SCH-221510 and significantly reduced the maximal effects of Ro 65-6570.

Collectively these results confirmed the NOP agonist properties of Ro 65-6570, SCH-221510 and Compound 6d. However the NOP selectivity of these compounds appears to be lower than that of the natural peptide, particularly in the mouse. Thus, caution should be adopted in interpreting the *in vivo* actions of these molecules and receptor antagonist and/or knockout studies should always be performed to demonstrate the involvement of the NOP receptor in their *in vivo* effects.