In vitro pharmacological characterization and comparison of NOP receptor non peptide agonists

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Nociceptin/orphanin FQ (N/OFQ) modules several biological functions via selective activation of the N/OFQ peptide (NOP) receptor. Potent and NOP selective non peptide agonists are valuable tools for investigating the therapeutic potential of drugs interacting with the NOP receptor. The aim of this study was the in vitro pharmacological characterization and comparison of the following non peptide NOP agonists MCOPPB, Ro 65-6570, Ro 2q, SCH-221510, AT-202 and AT-403. The compounds were assayed in calcium mobilization studies performed in cells stably coexpressing NOP or classical opioid receptors and chimeric G proteins, in bioluminescence resonance energy transfer (BRET) studies for investigating NOP/G protein and NOP/β arrestin 2 interaction, and in the electrically stimulated mouse vas deferens bioassay (the tissues were taken from CD-1 and from wild type (NOP(+/+)) and NOP knockout (NOP(-/-)) mice). In the calcium assay, all compounds mimicked the stimulatory effect of N/OFQ showing similar maximal effects, the following rank order of potency MCOPPB>AT-403>Ro 65-6570=Ro 2q>SCH-221510>AT-202, and moderate (approximately 60 fold AT-202 and SCH-221510) to high (1000 fold MCOPPB and AT-403) NOP selectivity over classical opioid receptors. The NOP antagonist SB-612111 displayed similar values of potency against N/OFQ (pA_2 8.75) and the non peptide agonists. In the BRET assay, the compounds mimicked the stimulatory effects of N/OFQ both at G protein and β arrestin 2 showing the same rank order of potency as in the calcium mobilization assay and a moderate bias toward G protein (3-10 fold) with the exception of AT-403 that displayed no bias. In the electrically stimulated mouse vas deferens all compounds mimicked the inhibitory effect of N/OFQ showing however a very slow kinetic of action. In tissues taken from NOP(-/-) animals, N/OFQ was inactive while non peptide agonists were still able to inhibit the electrically induced twitch response even if with lower potency. In line with findings obtained at human recombinant receptors, in the mouse vas deferens assay MCOPPB and AT-403 were the most potent and selective NOP agonists. The present results suggest that MCOPPB and AT-403 are the best pharmacological tools to be used in future in vitro and in vivo studies aimed to investigate the therapeutic potential of selective NOP agonists.