In vitro pharmacological characterization of novel nociceptin/orphanin FQ receptor ligands

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In this study we characterized the in vitro pharmacological profile of novel nociceptin/orphanin FQ (N/OFQ) receptor (NOP) non peptide ligands (AT-001, AT-004, AT-035, AT-090 and AT-127) in the following assays: calcium mobilization, receptor / G-protein and arrestin interactions, and electrically stimulated mouse vas deferens. In calcium mobilization studies performed in CHO cells stably coexpressing the $G\alpha_{qi5}$ chimeric protein and the human NOP receptor, the endogenous peptide ligand N/OFQ evoked a concentration dependent stimulation of calcium release with high potency (pEC_{50} 9.74). The standard non peptide NOP agonist Ro 65-6570 mimicked N/OFQ action showing similar maximal effects but reduced potency (pEC_{50} 8.79). Similar results were obtained with the AT-compounds that displayed the following rank order of potency AT-090=AT-127>AT-035>AT-001>AT-004. The NOP receptor antagonist SB-612111 (100 nM) produced a rightward shift of the concentration response curve to N/OFQ, Ro 65-6570, AT-090, and AT-127 without modifying the agonist maximal effects and displaying similar high potency (pK_B range 8.49 – 8.72). The selectivity of action of AT compounds has been assessed over classical opioid receptors. All the AT compounds were inactive at opioid receptors up to micromolar concentrations, with the exception of AT-001, which displayed similar potency at the NOP and kappa receptors. Bioluminescence resonance energy transfer (BRET) studies were performed in cells coexpressing the NOP receptor linked to RLuc and either the $G\beta_1$ or β-arrestin2 linked to RGFP. In cell membranes, N/OFQ and Ro 65-6570 promoted NOP/G-protein interaction in a concentration-dependent manner with values of potency of 8.52 and 7.77, respectively. AT-090 and AT-127 displayed moderate potency (7.19 and 6.81, respectively) associated with reduced maximal effects (α 0.49 and 0.69) thus behaving as partial agonists. The other AT compounds were only poorly active in promoting NOP/G-protein interaction. In whole cells expressing NOP/RLuc and β-arrestin2/RGFP, N/OFQ promoted NOP/β-arrestin2 interaction in a concentration dependent manner with high potency (8.00) while Ro 65-6570 displayed low potency (6.37). AT-090 and AT-127 behaved as NOP partial agonists with pEC_{50} of 6.96 and 6.38, respectively. In the electrically-stimulated mouse vas deferens N/OFQ inhibited the twitch response in a concentration dependent manner (pEC_{50} 7.68). Ro 65-6570 mimicked N/OFQ action, showing lower potency (6.73) but higher maximal effects. Similar results were obtained with AT-090 and AT-127. In this preparation the other AT ligands were inactive up to 10 μM. To test the selectivity of action of AT-090 and AT-127, antagonism and knockout studies were performed. The NOP antagonist SB-612111 shifted to right the concentration response curve to N/OFQ without changing its maximal effects (pA_2 8.69). SB-612111 was also able to counteract the action of Ro 65-6570, AT-090, and AT-127 but showing lower potency (pK_B range 7.09 – 7.95). In knockout experiments, N/OFQ inhibited the electrically-induced contractions of the mVD taken from NOP(+/+) mice but not from NOP(-/-) mice. On the contrary, Ro 65-6570, AT-090 and AT-127 displayed only a slight reduction in potency (3 – 10 fold) when tested in NOP(-/-) tissues. In conclusion, this study demonstrated that AT compounds are novel NOP receptor ligands. In particular AT-090 and AT-127 act as relatively potent NOP receptor partial agonists.