In vitro pharmacological characterization of cebranopadol a novel mixed nociceptin/orphanin FQ and opioid receptor agonist

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Cebranopadol has been recently identified by Grunenthal researchers as first-in-class potent analgesic agent with agonist activity at nociceptin/orphanin FQ (N/OFQ) and opioid receptors. The aim of the present study was to investigate in vitro the detailed pharmacological profile of cebranopadol at human recombinant opioid and N/OFQ peptide (NOP) receptors . In CHO cells coexpressing chimeric G proteins and NOP or opioid receptors N/OFQ, dermorphin, dynorphin A and DPDPE concentration dependently stimulated calcium mobilization with values of potency (pEC_{50} 9.59, 8.19, 8.54, and 8.15, respectively) and profiles of selectivity in line with literature. In parallel experiments cebranopadol mimicked the stimulatory effects of the standard agonists displaying full agonist activity at NOP, mu and delta and partial agonist activity (α = 0.55) at the kappa receptor and showing the following rank order of potency NOP = mu > kappa = delta. The stimulatory effects elicited by cebranopadol in mu and NOP receptor expressing cells were sensitive to naloxone and SB-612111, respectively. Bioluminescence resonance energy transfer (BRET) studies were performed to investigate the ability of cebranopadol to promote receptor/G protein and receptor/β-arrestin2 interaction using as donor Renilla Luciferase (linked to the receptor) and as acceptor Renilla Green Fluorescent Protein (linked to the effector). In membranes of NOP expressing cells cebranopadol stimulated NOP/G protein interaction with maximal effects similar to those of N/OFQ. However cebranopadol but not N/OFQ displayed higher potency when the incubation time was prolonged from 5 to 15 and 60 min. Similar results were obtained with membranes of mu receptor expressing cells where the effects of cebranopadol were compared to those of dermorphin. The values of potency (pEC_{50}) of cebranopadol obtained in these experiments with 60 min incubation time were 8.49 and 9.74 at NOP and mu receptor, respectively. In whole cells expressing the mu receptor cebranopadol stimulated receptor/β-arrestin2 interaction in a concentration dependent manner with maximal effects similar to that of the standard dermorphin and a value of potency of 8.36 (60 min incubation time). On the contrary in whole cells expressing the NOP receptor cebranopadol was not able to promote receptor/β-arrestin2 interaction up to micromolar concentrations while in parallel experiments N/OFQ elicited a robust and concentration dependent stimulatory effect. In conclusion the results of this study demonstrated that cebranopadol acts as a mixed NOP and opioid receptor agonist. In particular cebranopadol behaved as a slow interacting ligand displaying similar and very high potency at NOP and mu receptor. Interestingly cebranopadol acts as a G protein biased agonist particularly at the NOP protein; this might be relevant to explain some features displayed by cebranopadol in analgesiometric assays such as long lasting action and reduced tolerance liability.