INNOVATIVE KAPPA OPIOID RECEPTOR AGONISTS WITH DIFFERENT BIAS TOWARDS G PROTEIN-DEPENDENT SIGNALING: MOLECULAR PATHWAY ANALYSIS AND PHARMACOLOGICAL EFFECTS

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Introduction: Kappa opioid receptor (KOR) agonists are investigated as alternatives analgesics for their low abuse potential and minimal gastrointestinal and respiratory adverse effects. However, KOR agonists induce dysphoria, sedation, hyperalgesia that limit their clinical use. KOR agonists determine antinociception by G protein signaling and many of their side effects through arrestin 3-dependent p38MAPK activation. However, further studies are necessary to clarify to what extent a differential biased signaling observed in vitro allows to separate side effects from therapeutic properties *in vivo*. Systems Pharmacology seeks to understand drug effects as the outcome of complex interactions between and within biological systems and uses bioinformatics and statistics techniques to integrate and interpret quantitative data related to chemical-protein, protein–protein, signaling and physiological interaction networks. Moving from these considerations we aimed at quantitatively analyzing signaling pathways and pharmacological responses induced by LOR17and CL39, two new ligands we synthesized, as compared to U50,488(classic KOR agonist).

Materials and methods: Ligands ability to activate G protein was investigated by measuring adenylyl cyclase inhibition; arrestin 3recruitment at KOR was analyzed via arrestin complementation assay in U2OS cells and through BRET assay in HEK-293cells. Activation of distinct MAPKs over others and the subsequent functional selectivity on related cellular responses were investigated in HEK-293expressing recombinant human KOR and in U87-MG astrocytoma cells and in human astrocytes endogenously expressing this receptor. Analgesia in mice was studied in the warm-water tail withdrawal test, in acetic acid-induced visceral pain and in oxaliplatin-induced neuropathic pain; effects related to sedation, motor incoordination, anhedonia were also investigated.

Results: U50,488, LOR17and CL39displayed high KOR affinity and selectivity and inhibited adenylyl cyclase in HEK-hKOR and U87-MG cells, being LOR17and CL39full and partial agonists, respectively. Conversely to U50,488, LOR17and CL39weakly recruited arrestin 3at KOR; bias factors for U50,488, LOR17and CL39were 1, 853and 65, respectively. LOR17and CL39induced early (5-15min), G protein-dependent ERK1/2phosphorylation in HEK-hKOR and U87-MG cells, but did not trigger in the same cells late (60min), arrestin-dependent ERK1/2or p38MAPK phosphorylation. Conversely, U50,488induced all the above mentioned pathways. U50,488, but not LOR17or CL39, significantly increased U87-MG cell proliferation in arrestin 3-, p38MAPK-dependent fashion. LOR17inhibited adenylyl cyclase and activated early ERK1/2, but not p38MAPK, also in normal human astrocytes; consistently, it did not alter human astrocytes cell proliferation. CL39-mediated effects in human astrocytes are currently under investigation. In vivo, LOR17, CL39and U50,488(0-30 mg/kg; 0-60 min; i.p.) induced a significant, KOR-mediated, dose-dependent analgesia in warm-water tail-withdrawal test, being LOR17and CL39full and partial agonists, respectively. Both LOR17and U50,488caused KOR-mediated antinociception in acetic acid-induced visceral pain, but only the former fully reverted, in a dose-dependent manner, thermal hypersensitivity in oxaliplatin-induced neuropathy; this without altering motor coordination, locomotor and exploratory activities or inducing anhedonia-related behaviors. CL39effects in neuropathic mice are currently under investigation.

Discussion and conclusion: Our findings highlight LOR17as an extremely G protein biased KOR full agonist, and CL39as a G protein biased KOR partial agonist; signaling pathways induced by both ligands mirror their analgesic properties with reduced adverse effects. The quantitative data obtained in this study will be exploited to implement systems pharmacology platforms aimed at analyzing and possibly predicting the effects of innovative KOR analgesics.