Ligand-directed signaling at kappa and mu opioid receptors: identification of novel, functionally selective ligands to develop innovative analgesic drugs

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G-protein coupled receptors (GPCR) may be stabilized in different conformational states by diverse ligands, resulting in a differential modulation of the down-stream signaling cascades: some transduction pathways may be related to therapeutic responses, whereas others to adverse effects of a drug, therefore this ligand-directed signaling provides new avenues for the development of innovative, pathway-specific drugs.
Kappa-opioid receptor (KOR) is expressed in central and peripheral nervous system and modulates physiological responses as nociception, mood, stress. Therapeutically, selective KOR agonists are being explored as alternatives to mu opioid receptor (MOR) analgesics, for their low abuse potential and less gastrointestinal and respiratory side effects; however, clinical relevance of KOR agonists is still limited due to their dysphoric and stress-related effects.KOR classic agonists, in fact, trigger protein Gbeta/gamma-dependent phosphorylation of ERK1/2, leading to analgesia, as well as G-protein receptor kinase 3 (GRK3)-, arrestin 3-dependent phosphorylation of p38MAPK, determining relevant side effects like dysphoria and ion channel desensitization. Therefore, KOR agonists biased towards G-protein coupling and displaying a limited activation of GRK3-, arrestin 3-dependent signaling may represent innovative analgesics devoid of the relevant adverse effects that are currently limiting the use of KOR agonists to treat pain.
MOR agonists as morphine or fentanyl are still among the most effective analgesic drugs for the treatment of acute and chronic pain, albeit their use is limited by the development of severe side effects, thus highlighting the need to improve the therapeutic profile of opioid-based treatments. Ligand-directed signaling at MOR may be exploited for the discovery of better and safer analgesic drugs, although different classic MOR ligands, like morphine and fentanyl, albeit activating specific signaling pathways (i.e.: JNK or GRK3/arrestin), lead eventually to similar unwanted effect as receptor desensitization and tolerance.
In this frame we developed a novel series of opioid peptide hybrids of CJ-15,208 and Cyclo-EM1, being this latter an endomorphin-1 cyclic analogue with high MOR affinity and selectivity, agonist activity in vitro and antinociceptive effects in different models of pain, and the former a KOR ligand showing antagonist activity in vitro and both dose-dependent antinociception and KOR antagonist activity in vivo. We synthesized and characterized a novel library of hybrids of both sequences, aiming to identify innovative KOR-selective agonists possibly biased towards G-protein-dependent signaling on one hand, and MOR-selective agonists possibly not activating JNK- or GRK3/arrestin-dependent receptor desensitization on the other.
By competition binding assays performed in HEK-293 cells expressing KOR, MOR or DOR we found one KOR- (LOR17; Ki=1.2 nM) and one MOR-selective (EDO43; Ki=4 nM) ligand; both inhibited forskolin-induced cAMP accumulation in both HEK-293 cells expressing KOR or MOR and in U87-MG astrocytoma cells endogenously expressing both receptors, thus showing an agonist profile. Interestingly, LOR17 induced in both cell models protein Gbeta/gamma-dependent phosphorylation of ERK1/2, but not GRK3/arrestin 3-dependent phosphorylation of p38MAPK, showing a potential bias towards G protein-dependent signaling. Moreover, LOR17 determined a significant, KOR dependent antinociception in different animal models of pain (e.g.: ED_{50}=11.7 mg/kg in the tail-immersion test). Experiments supporting the hypothesis that EDO43 may modulate MOR in a biased manner (not activating JNK or GRK3) are in progress and will be presented at the Conference. We propose LOR17 as a new and selective KOR agonist, potentially biased toward G protein-dependent signaling and provided of analgesic effects in different models of pain, and EDO43 as a useful tool to explore ligand-directed signaling at MOR.