A bifunctional biased μ opioid agonist - Neuropeptide FF receptor antagonist as analgesic with improved acute and chronic side effects

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Introduction

Opioid receptors and their ligands are still considered as golden standards to alleviate moderate to severe pain. Nonetheless, this potent analgesic activity is usually paired with adverse side effects such as respiratory depression, hyperalgesia, analgesic tolerance or constipation.[1] Several studies have already demonstrated that other biological pathways and systems are involved in the development of such side effects, for instance, the recruitment of β -arrestin-2 at μ -opioid receptor (MOR) [2] and the neuropeptide FF receptors (NPFFRs).[3]

Previous work from our laboratories showed that the dermorphin-derived peptidomimetic KGOP01 (Table 1) was a high efficiency ligand at MOR, which lead to efficient pain relief *in vivo*. [4] Several studies have also demonstrated that NPFF ligands are recognized through their C-terminal part, corresponding to the dipeptide RF-NH₂. [5] Herein, we discuss the design as well as *in vitro* and *in vivo* evaluation of several hybrid peptidomimetics or multitarget drugs, resulting from the merger of a MOR agonist pharmacophore and a NPFF pharmacophore, to obtain improved side effect profiles (Figure 1).

Figure 1: Example of an opioid-NPFF hybrid

Result and Discussion

In order to study the influence of changes imposed on each subunit at both MOR and the NPFFRs, several modifications were introduced in the peptide structure. Examples include the linker between the two pharmacophores (Gly or β Ala) and the basic residue within the NPFF subunit. For the latter feature, Orn and Arg derivatives were introduced based on previous studies⁶ and encompassed unnatural amino acids^[7] (Figure 2). Finally, a merged multitarget drug design was attempted, in which the two pharmacophores were completely overlapping (e.g., H-Dmt-Arg-Phe-NH₂).

Table 1: Binding affinities of selected opioid-NPFF peptidomimetics

Further *in vitro* evaluation (data not shown) indicated that all designed peptidomimetics were agonists at MOR (EC50 ranging from 1.5 to 18.2 nM), with KGFF03 being the best agonist at the NPFFRs (EC50 of 84.8 and 11 nM at NPFF1R and NPFF2R, respectively). Interestingly, KGFF09 displayed a very weak activity at NPFFRs and was subsequently even characterized as an antagonist (pA2 values of 7.25 and 7.77). Evaluation of the β -arrestin recruitment *via* a functional test (BRET1 β -arrestin-2 recruitment) showed that both KGFF03 and KGFF09 were presenting a partially biased activity at MOR toward intracellular G protein signaling, conversely to the native opioid ligand KGOP01.

Chronic administration of KGOP01 and KGFF03 resulted in a significant and persistent decrease of the basal antinociceptive threshold, thus demonstrating the development of hyperalgesia (Figure 4A). This trend was not observed with the opioid agonist - NPPFR antagonist KGFF09. On the 8th day, mice treated with KGOP01 or KGFF03 showed a decreased maximal response and antinociception efficacy by 75% compared to day 1, whereas KGFF09 maintained potent analgesia (Figure 4A). These observations demonstrate that blocking the NPPFR system by an antagonist leads to a reduced analgesic tolerance development upon chronic administration.

Figure 4: *In vivo* characterization of opioid-NPFF hybrids after chronic s.c. administration in mice. (A) Development of hyperalgesia and analgesic tolerance upon chronic treatment, (B) Effect of KGOP01, KGFF03, and KGFF09 on respiratory frequency after s.c. administration.

Finally, we studied respiratory depression, one of the major side effects that occurs upon acute opiate administration. The measurement of the respiratory frequency in mice revealed a significant respiratory depression induced by the non-biased opioid parent KGOP01, as compared to saline-treated animals (Figure 4B). This effect was not observed with either KGFF03 or KGFF09 which is in agreement with previous reports suggesting that respiratory depression is related to β -arrestin recruitment.[8]

Conclusion

Altogether, our data demonstrate the successful design of multitarget peptidomimetics merging, within a single molecule, opioid and NPFF pharmacophores. Indeed, the addition of the RF-NH₂ motif or its derivatives to our MOR ligand KGOP01 not only conferred a good NPFF1/2Rs affinity and preserved binding to MOR, but also induced a biased activity toward G protein signaling over the β -arrestin-2 recruitment. Interestingly, introducing variations of the basic residue within the NPFF pharmacophore led to two different profiles at the corresponding receptors i.e. an agonist, KGFF03, and an antagonist, KGFF09. *in vivo* experiments confirmed the benefits of one pharmacological profile, since KGFF09 displayed a potent antinociceptive activity as well as reduced respiratory depression, hyperalgesia, tolerance, and withdrawal syndrome (data not shown), when compared to the parent non-biased opioid ligand, KGOP01, or the dual opioid agonist – NPFFR agonist, KGFF03. Therefore, our data not only confirmed the relation between the NPFF system or β -arrestin-2 recruitment and opioid-induced side effects, but also demonstrate the benefits to modulate these (signaling) systems in order to obtain safer pain treatments.

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