The Functional Organization of Opioid Receptors in Pain Neural Circuits

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Opioids are broadly used for the management of severe acute, perioperative, and chronic pain. However, opioids also generate numerous detrimental side effects including analgesic tolerance, opioid-induced hyperalgesia (OIH), transition to addiction, and respiratory depression. Additionally, opioid analgesics used in the clinic are limited to mu opioid receptor (MOR) agonists, and show limited utility against certain types of pain (e.g. neuropathic tactile allodynia). Strikingly, despite opioids having been used in human medicine for millenaries, the mechanisms underlying their analgesic versus detrimental properties remain largely unclear. Elucidating the functional organization of opioid receptors in neural circuits and opioids mechanisms of action is urgently needed to develop innovative analgesic therapies with limited side effects. To this aim, we combine mouse genetics, neuroanatomy, electrophysiology, opto/chemogenetics, transcriptomics, in vivo imaging and behavioral analysis. With these approaches, we identify the neural circuits and cells that express opioid peptides and the delta, kappa, mu opioid receptors (DOR, MOR, KOR), and nociceptin/orphanin FQ (NOP) receptor and elucidate receptor specific function and signaling mechanisms in circuits.

We first investigated the mechanisms underlying opioid analgesic tolerance and OIH. Tolerance and OIH counteract opioid analgesia and drive dose escalation. The cell types and receptors on which opioids act to initiate these maladaptive processes remain disputed, which has prevented the development of therapies to maximize and sustain opioid analgesic efficacy. We found that MORs expressed by primary afferent nociceptors initiate tolerance and OIH development. RNA sequencing and histological analysis revealed that MORs are expressed by nociceptors, but not by spinal microglia. Deletion of MORs specifically in nociceptors eliminated morphine tolerance, OIH and pronociceptive synaptic long-term potentiation without altering antinociception. Furthermore, we found that co-administration of methylnaltrexone bromide, a peripherally restricted MOR antagonist, was sufficient to abrogate morphine tolerance and OIH without diminishing antinociception in perioperative and chronic pain models.

We next examined the distribution of DORs and MORs in CNS pain circuits. Cellular interactions between DORs and MORs, including heteromerization, are thought to regulate opioid analgesia. However, the identity of the nociceptive neurons in which such interactions could occur in vivo remains elusive. We found that DOR-MOR co-expression is limited to small populations of excitatory interneurons and projection neurons in the spinal cord dorsal horn and unexpectedly predominates in ventral horn motor circuits. Similarly, DOR-MOR co-expression is rare in parabrachial, amygdalar, and cortical brain regions processing nociceptive information. We further demonstrate that in the discrete DOR-MOR co-expressing nociceptive neurons, the two receptors internalize and function independently. Finally, conditional knockout experiments revealed that DORs selectively regulate mechanical pain by controlling the excitability of somatostatin-positive dorsal horn interneurons.

Collectively, these results illuminate the functional organization of DORs and MORs in pain circuits and reappraise the importance of DOR-MOR cellular interactions for developing novel

opioid analgesics. Importantly, genetic and pharmacological data support the idea that MOR agonists can be combined with peripheral MOR antagonists to limit analgesic tolerance and OIH.