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RESEARCH HIGHLIGHT

In vivo neuronal co-expression of mu and delta opioid receptors uncovers new therapeutic perspectives

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Opioid receptors belong to the G protein coupled receptor family. They modulate brain function at all levels of neural integration and therefore impact on autonomous, sensory, emotional and cognitive processing. *In vivo* functional interaction between mu and delta opioid receptors are known to take place though it is still debated whether interactions occur at circuitry, cellular or molecular level. Also, the notion of receptor crosstalk via mu-delta heteromers is well documented *in vitro* but *in vivo* evidence remains scarce. To identify neurons in which receptor interactions could take place, we designed a unique double mutant knock-in mouse line that expresses functional red-fluorescent mu receptors and green-fluorescent delta receptors. We mapped mu and delta receptor distribution and co-localization throughout the nervous system and created the first interactive brain atlas with concomitant mu-delta visualization at subcellular resolution (http://mordor.ics-mci.fr/). Mu and delta receptors co-localize in neurons from subcortical networks but are mainly detected in separate neurons in the forebrain. Also, co-immunoprecipitation experiments indicated physical proximity in the hippocampus, a prerequisite to mu-delta heteromerization. Altogether, data suggest that mu-delta functional interactions take place at systems level for high-order emotional and cognitive processing whereas mu-delta may interact at cellular level in brain networks essential for survival, which has potential implications for innovative drug design in pain control, drug addiction and eating disorders.

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The opioid system acts as a major key player in reward and motivation but also regulates emotional responses and cognition. In addition, this neuromodulatory system impacts on nociception, and autonomic functions [1]. The three opioid receptors mu, delta and kappa are homologous G protein coupled receptors (GPCRs) [2] and both opioid receptors and endogenous opioid peptides are largely expressed throughout the nervous system [3]. Interestingly, several decades of opioid pharmacology have brought to light the complexity of the opioid physiology, which initiated extensive studies to determine the respective involvement of mu, delta and kappa receptors in pain control, drug abuse and mood disorders [4-7]. In particular, analyzing the effects of opioid drugs in vivo has revealed

functional interactions mainly documented for mu and delta [8]. However, whether in vivo receptor interactions occur at circuit, cellular or molecular level remains highly debated.

Numerous reports described heteromer formation taking place in transfected cells between mu, delta and kappa opioid receptors or between one of them and a non-opioid receptor [9, 10]. As a result, physical interaction between two receptors would give rise to a novel molecular entity with specific signaling and/or trafficking properties. Such heteromers would represent the molecular determinant that underlies the integrated changes observed at system level. However, mu-delta in vivo co-expression and heteromerization remain extremely difficult to tackle with existing tools [11]. In vivo co-localization has indeed only been reported in dorsal root ganglia (DRG) [12-14], spinal cord [15] and within a limited number of brain areas [16-18] but, as for most GPCRs, we still miss in-depth anatomical mapping of opioid receptors in the brain that also provides subcellular resolution.

We recently addressed in vivo mu-delta co-localization using a double mutant line (delta-eGFP/mu-mcherry) that expresses functional fluorescent forms of mu and delta receptors [19]. This mouse line was obtained by breeding delta-eGFP knock-in mice that express a functional delta receptor with a fused C-terminal eGFP instead of the native receptor [20] with a second knock-in mouse line that was generated according to a similar strategy and expresses a functional mu receptor with a fused C-terminal red fluorescent mcherry protein. The single mutant mice showed no detectable alteration of behavior and responses to drugs. Mu-mcherry and delta-eGFP fluorescent signals were mapped in the nervous system with subcellular resolution. We collected fluorescent images of coronal and sagittal sections to generate a virtual atlas that can be freely searched at http://mordor.ics-mci.fr/. In the double mutant mouse line, mu-mcherry and delta-eGFP distributions were consistent with previously published data. This designates the double fluorescent knock-in mouse as a particularly well-suited tool to map mu and delta receptor neuronal co-localization throughout the brain. In addition, co-immunoprecipitation experiments uncovered mu-delta close physical vicinity in the hippocampus and hence qualifies the use of the double knock-in animals to address the physiopathological relevance of mu-delta heteromerization in vivo.

Co-localization of mu-mcherry and delta-eGFP was observed in discrete populations of the DRGs similarly to previous reports[12-14] but also across all layers of the spinal cord in agreement with a previous study reporting physical mu-delta interaction in the spinal cord using co-immunoprecipitation experiments [15]. In the brain, mu-delta co-expression was predominantly observed in the hippocampus, the hypothalamus, the lateral parabrachial nucleus and vestibular nuclei. Additional regions included the piriform cortex, the auditory pathway, as well as mid- and hindbrain regions involved in the control of movement and posture or relaying somatosensory or motor information to the autonomic nervous system. Surprisingly, mu-delta co-expressing neurons were extremely scarce in the forebrain. The analysis of mu-delta co-localization in the brain raised interrogations about the possible in vivo roles of neuronal co-expression. First, the low mu-delta co-expression in forebrain networks responsible for higher-order processing was unexpected and suggests that, under basal conditions, functional interactions between mu and delta take predominantly place at circuitry level as far as mood control, reward processing and cognition are concerned. Intriguingly, mu-delta neuronal co-expression is observed in the CA1 area of the hippocampus, the piriform cortex and the nucleus of the trapezoid body. These regions operate as coincidence detector for spatial, olfactive and auditive cues respectively [21-23] suggesting critical mu-delta modulation of their processing. Mu-delta neuronal co-expression in brain areas involved in motor activity also came as a surprise and expands our current knowledge of opioid physiology. Mu-delta co-localization is indeed present in brain areas where it may exert a modulatory role on mandibular movements involved in masticatory reflexes and jaw movements associated with feeding [24]. In addition, mu-delta co-localization is also detected in brain areas involved in motor responses that can be correlated to the search for food and water, the sexual attraction or the avoidance of aversive stimuli. Indeed, the physiological impact of mu-delta neuronal co-expression seems related to the modulation of both ascending somatosensory information and corresponding descending reflex responses intended to protect the individual. Neuronal co-expression of mu and delta receptors is mainly observed in subcortical networks responding to presentation of noxious or non-noxious aversive stimuli [25, 26] as well as in brainstem nuclei tightly connected with the autonomic nervous system. Neurons co-expressing mu and delta receptors are also distributed within neuronal circuits processing food intake, NaCl and water uptake or regulating sexual activity [27-29]. Mu-delta crosstalk at cellular level may therefore operate in neural circuits involved in behaviors associated with body homeostasis and sexual activities.

Neuronal co-expression in brainstem nuclei also suggests that the two receptors may functionally contribute to several pathological conditions such as drug addiction by contributing to somatic and autonomic symptoms associated with drug withdrawal. Targeting mu-delta

heteromers may therefore constitute a promising and entirely novel approach to reduce opioid withdrawal, and possibly withdrawal signs associated with other drugs of abuse [30]. On the other hand, the high level of co-localization of mu and delta in neurons in nociceptive pathways designates mu-delta heteromers as an attractive target in pain management. This view is supported by recent data indicating that mu-delta activation with a biased agonist induces less tolerance [31]. Also, neuronal co-expression of the two receptors in orexin-positive neurons of the lateral hypothalamus critically involved in both food and drug reward [32], points to mu-delta heteromers as potential target for novel strategies to treat obesity or to reduce drug-seeking behaviors.

Overall, our data suggest that mu and delta receptors may cooperate intracellularly in neural networks essential for survival. Therefore, mu-delta heteromers distributed within neural networks associated with abnormal nociception, aversive aspects of drug withdrawal or eating disorders emerge as very appealing targets for drug design.

Conflicting interests

The authors have declared that no competing interests exist.

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