**Identification of a novel, functional cross-talk between EphB1 receptor and mu opioid receptor**

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EphBs tyrosine kinase receptors and their membrane-anchored ligands, ephrinBs, are expressed in the nervous system where they play a pivotal role in axonal guidance, regulation of neuronal progenitor positioning, controlling synapse formation, and neuronal plasticity. Mu opioid receptor (MOR) agonists like morphine, and related opioids, are still among the most powerful and widely used analgesics, but their clinical use is limited by severe side effects, including constipation, respiratory depression and immunomodulation. Repeated use of opioids such as morphine for relief of chronic pain may lead to opioid tolerance and dependence, as consequence of prolonged MOR activation that may elicit long-term neuronal alterations.

Interestingly, EphB receptors and their ligands EphrinB have been implicated in the onset and maintenance of different types of pain (neuropathic, inflammatory or chronic), in long-lasting alterations of excitability and synaptic plasticity of spinal neurons, which lead to increased sensitivity to noxious stimuli (hyperalgesia) or non-noxious stimuli (allodynia). Especially, the activation of spinal EphB1 receptor by ephrin B1-Fc (Extracellular domain of mouse ephrin B1 is fused to the C-terminal histidine tagged Fc region of human IgG1) is critical to the development of bone cancer pain, morphine tolerance as well as thermal hyperalgesia and mechanical allodynia in mice. Moreover, levels of EphB1 receptor are significantly up–regulated in the dorsal horn of the spinal cord following escalating morphine treatment, whereas spinal administration of the EphB1 receptor blocking reagent EphB2–Fc prevents or reverses bone cancer pain in animal models.

However, any cross talk between EphB1 receptor and MOR, and its potential influence on reduced opioid analgesia or tolerance to opiates, has been so far poorly investigated. The aim of this study has been to investigate any functional cross-talk between intracellular signaling pathways triggered by MOR and EphB1 receptors in different cell models co-expressing the two receptors. We used HEK–293 cells transfected with EphB1 and MOR receptors and SHSY5Y cells which endogenously express both receptors.

We found that EphB1 agonist receptor (ephrinB1-Fc) or morphine, determined a time-dependent increase of p42/44 MAPK phosphorylation only when both ligands were administered as single agents whereas their co-administration occluded p42/p44 MAPK activation. Such cross-talk as well as EphB1 and MOR expression, were modified in neuronal cells subjected to in vitro differentiation (PMA 16 nM, 5 days) or exposed to the pro-inflammatory agent TNF-α (10 ng/ml); thus, suggesting a differential role played by the functional interaction between EphB1 and MOR depending on the physiological conditions of neuronal cells.

Our study aims at investigating any signaling pathway responsible for the functional cross-talk between EphB1 and MOR and at ascertaining the ability of novel EphB1 antagonists to counteract such an occlusive interaction between these two receptors which may reduce the negative impact of ephrin system activation on pain perception and opioid efficacy; these latter studies are

currently ongoing. Data obtained in this study show, for the first time, a functional, occlusive cross talk between EphB1 and MOR receptors; this interaction is likely to play an important role in the onset and maintenance of different chronic pain states as well as may contribute to the reduction of opioid-mediated analgesia or to the development of dependence to opioids.