Characterisation of Changes in the Mammalian Target of Rapamycin Complex 1 (mTORC1) Signalling Pathway after Acute and Chronic Morphine in Mice

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Opioid analgesic tolerance is a pharmacological phenomenon that restricts the clinical use of opioids. While neuronal circuits implicated in opioid-mediated effects are well identified, the mechanisms that cause the neuroadaptive changes affecting these circuits are still poorly understood. Better understanding of the complexity of these mechanisms may directly lead to more effective clinical use of opioids as neural plasticity associated with the development of opioid tolerance may also activate a pronociceptive mechanism that could counteract the analgesic effects of opioids (ie, opioid-induced hyperalgesia) and/or lead to the reduced opioid analgesic efficacy in chronic opioid therapy. Recently, protein translation-mediated neuroplasticity was shown to regulate opioid effects. The mammalian target of rapamycin complex 1 (mTORC1) controls most protein translation and its role as a modulator of chronic pain-related neuroplasticity is well documented. Therefore, in the present study we investigated mTORC1-mediated adaptive changes in protein translation in the nervous system that may contribute to the development and maintenance of opioid-induced tolerance.

In adult male C57BL/6J mice (n≥6) opioid tolerance to morphine analgesic effect was developed by systemic (intraperitoneal, i.p.) injection of morphine (20 mg/kg) twice daily for 8 consecutive days (1). To determine the effect of a single injection of morphine acting at an analgesic dose, a separate group of mice (n≥6) received a single i.p. injection of morphine (20 mg/kg). Immunohistochemistry was employed to determine localization of P-mTOR and μ-opioid receptor, and Western blotting to determine changes in mTORC1 activity after acute and chronic morphine treatment.

In response to chronic morphine injections, immunohistochemical staining revealed neurones expressing P-S6 ribosomal protein (P-S6 RP; mTOR downstream effector) in several brain regions, such as prefrontal cortex, nucleus accumbens, caudate putamen, hypothalamus, thalamus, amygdala, habenula, hippocampus, periaqueductal gray, and ventral tegmental area. Further examination of the co-localization of P-S6 RP with MOR revealed that P-S6 RP was co-expressed in approximately 60% of MOR-positive neurons in the brain. Activity of mTORC1 in response to morphine treatment in the brain and spinal dorsal horn was assessed by quantifying the phosphorylation level of mTOR and its downstream targets p70-S6 kinase (p70S6 Kin), S6 RP and 4EBP1/2. Chronic, but not acute, systemic treatment with morphine increased phosphorylation of S6 RP in the prefrontal cortex (70%), caudate putamen (173%), hippocampus (92%), ventral tegmental area (70%) and in spinal dorsal horn (45%). Analysis also showed a significant increase in 4EBP1/2 phosphorylation in hypothalamus (27%) and thalamus (68%), as well as mTOR phosphorylation in thalamus (62%). A significant increase in p70S6 Kin phosphorylation was observed in the amygdala (53%). In contrast, S6 RP phosphorylation in chronic morphine treated mice decreased significantly in nucleus accumbens (37%) and habenula (40%).

Together, these findings confirm a role for mTORC1 signalling in mediating neuroadaptation to opioids and provide evidence that chronic, but not acute, morphine treatment activate mTORC1 pathway in regions of nervous system associated with reward, pain and/or addiction. This suggests that mTORC1 may be potentially a new target for strategies to improve the efficacy of chronic opioid therapy.

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1. Osikowicz M *et al*. (2008) *Pain* **139**: 117-126.