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Attenuation of Opioid Tolerance in Mice by Systemic Inhibition of the Mammalian Target of Rapamycin Complex 1 (mTORC1) Signaling Pathway.

Opioids remain the mainstay of clinical analgesia but after prolonged administration they induce therapeutic desensitization (*tolerance*) that significantly restricts their clinical usefulness. Therefore, there is a pressing need for the identification of new therapeutic strategies to improve efficacy of opioid-based treatments. While the mammalian target of rapamycin complex 1 (mTORC1), a kinase which controls protein synthesis, is well established as a regulator of chronic pain sensitivity, its role in the regulation of opioid efficacy has also been recently promoted. It was found that the activation of μ-opioid receptor by morphine in naïve rodents triggers the mTORC1 activity within the nociceptive pathway and promotes morphine-induced protein translation changes associated with morphine tolerance and hyperalgesia. However, these observations were recorded following direct mTORC1 inhibition with rapamycin, a therapy restricted due to adverse effects. For this reason, we extended these observations and focused on upstream regulation of mTORC1 activity *via* targeting 5' adenosine monophosphate-activated protein kinase (AMPK) with metformin. This drug is widely given to treat type-2 diabetes and was shown to negatively regulate translation *via* inhibition of mTORC1.

In adult male C57BL/6J mice (n=6) opioid tolerance was induced by morphine (20 mg/kg, i.p.) given twice daily at 12 h intervals for 9 consecutive days. The influence of mTORC1 inhibition was assessed by repeated injections of the rapamycin analogue CCI-779 (25 mg/kg, i.p.) or the AMPK activator metformin (200 mg/kg, i.p.) once daily, 24 h before morning morphine injection on each testing day. Pain threshold was assessed by tail-flick daily 30-60 min after morphine. To determine the effect of a single injection of CCI-779 or metformin on restoring analgesic effect of morphine, separate groups of morphine tolerant mice received a single injection of CCI-779 (25 mg/kg, i.p.) or metformin (200 mg/kg, i.p.), 24 h before subsequent morphine dose. Western blotting was used to determine changes in mTORC1 activity after treatment with CCI-779 and metformin.

Our study showed for the first time that chronic metformin blocked the development and maintenance of morphine tolerance, while a single metformin injection fully restored the analgesic effect of morphine in naïve mice (pain threshold after chronic morphine on day 9: 4.1±0.2s *vs.* in the presence of chronic metformin: 7.7±0.5s or single dose of metformin: 6.9±0.9s). In parallel studies using the direct mTORC1 inhibitor, CCI-779 showed that these effects were attributed to the inhibition of mTORC1 (pain threshold after chronic morphine on day 9: 4.9±0.2s *vs.* in the presence of chronic CCI-779: 8.1±0.4s or single dose of CCI-779: 7.8±0.3s). This mechanism was confirmed by immunoblotting showing inhibition of mTORC1 activity in the dorsal spinal cord after metformin and CCI-779 observed as a decrease in the phosphorylation levels of two mTORC1 downstream targets, P-p70 S6 kinase (68.4±16.4% decrease after metformin) and P-S6 ribosomal protein (61.6±10.1% decrease after CCI-779).

Since chronic pain and tolerance to antinociceptive effects of morphine share some common pathological mechanisms, our study may suggest that mTORC1 represents a novel and tractable target for the improvement of opioid analgesic efficacy in chronic pain. An important aspect of our observations is related to the use of metformin, a widely clinically available and relatively safe anti-diabetic drug, in contrast to other mTORC1 inhibitors that display numerous side effects (e.g. rapamycin), thus leading to an immediate novel avenue for the improvement of opioid therapy in humans.

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