Binding characterization and analgesic properties of novel A_1 adenosine receptor allosteric modulators

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Adenosine is an endogenous nucleoside modulator that mediates its effects through the activation of a family of four G-protein coupled adenosine receptors (ARs) named A_1, A_{2A}, A_{2B} and A_3ARs. A_1ARs have been identified as a potential target for the development of anti-nociceptive compounds. Allosteric modulators of A_1ARs, which act at a site distinct from the agonist binding site, could have potential therapeutic advantages over conventional direct receptor agonists. Because positive allosteric modulators enhance the function of receptors activated by endogenous agonist, they are expected to have a much lower side effect potential than direct receptor agonists, a low propensity for receptor desensitization and a high selectivity for a given receptor subtype.

The present study explores the analgesic effects of a novel A_1AR positive allosteric modulator, TRR469, in different models of acute and chronic pain in mice. To evaluate the allosteric enhancement, in vitro binding experiments were performed. It was speculated that the mechanism of action of TRR469 relies on its capability to induce a conformational change on the A_1ARs. This is evident from saturation binding assays where TRR469 increased the number of binding sites recognizable by the agonist CCPA that preferentially binds the active form of the receptor. TRR469 did not modify the binding of the antagonist DPCPX that does not discriminate between the two state of the receptor. Then the anti-nociceptive properties of TRR469 were investigated in different pain models in mice. In formalin and writhing tests, TRR469 produced robust and dose-dependent analgesic effects with ED_{50} values superimposable to those of the reference compound morphine. Furthermore, the isobolographic analysis revealed an additive interaction between TRR469 and morphine suggesting their potential combined use. To investigate the potential anti-allodynic effect of TRR469, a model of streptozotocin-induced diabetic neuropathic pain was chosen. In diabetic mice, the paw withdrawal threshold was significantly decreased in comparison to healthy mice, indicating the development of allodynia. TRR469 dose-dependently attenuated mechanical allodynia in diabetic mice completely re-establishing the pain threshold to control level. Rotarod and catalepsy tests were used to identify potential side effects and it is noteworthy that TRR469 did not produced any locomotor disturbances or cataleptic effect at anti-nociceptive doses. TRR469 enhanced the binding of the agonist radioligand [^{3}H]-CCPA and induced a 33-fold increase of adenosine affinity in spinal cord membranes. In mouse spinal cord synaptosomes, TRR469 enhanced the inhibitory effect of A_1AR activation on [^{3}H]-D-aspartate release, a non-metabolizable analogue of glutamate.

These results suggest that the anti-nociceptive effect of the novel positive allosteric modulator TRR469 could be of great potential therapeutic for the treatment of acute and chronic pain. The use of TRR469 allows for the possibility of exploiting analgesic properties of endogenous adenosine, with a minor potential to develop the various side effects often associated with the use of direct receptor agonists.