Molecular basis of adenosine A3 receptor-mediated analgesic effect: an in vitro electrophysiological study on isolated rat dorsal root ganglion neurons

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Interest has been focused in recent years on the analgesic effects exerted by adenosine in different in vivo models of acute and chronic pain. Adenosine is an endogenous neuromodulator that acts on four metabotropic receptors: A1 and A3 receptors coupled to adenylyl cyclase inhibition, and A2A and A2B coupled to adenylyl cyclase stimulation. Preclinical and clinical studies demonstrate that A1 receptor and its agonists exert antinociceptive effects. However, the therapeutic utility of these compounds is limited by adverse cardiovascular (marked bradicardia and hypotension) and central (sedation) side effects. Recent preclinical observations indicate that A3 receptors (A3R), which are known to be free from cardiovascular and central side effects, proved powerful analgesic action in in vivo rodent models of experimental neuropathic pain, such as spinal nerve ligation or chemotherapy-induced peripheral neuropathy (Little et al., Brain 2015; Janes et al., BJP 2015). However, the cellular and molecular basis of A3R-mediated antinociception are still unknown.

In this study we investigate whether A3R agonists modulate excitability in dorsal root ganglion (DRG) neurons, which are the primary sensory neurons transferring nociceptive stimuli to the central nervous system (CNS). Dissociated rat DRG neurons were tested for their responsiveness to the selective A3R agonist Cl-IBMECA in the absence or presence of A3R antagonists (for methods see: Coppi et al., Neuropharmacol. 2013).

Exogenous application of the A3 selective agonist Cl-IB-MECA concentration-dependently (0.1-100 nM), inhibited voltage-gated outward currents evoked by a ramp protocol (from +80 mV to -120 mV, 800 ms duration) in medium- and small-sized DRG neurons. The I-V relationship of Cl-IB-MECA-inhibited K+ currents, investigated by applying a voltage step protocol (13 depolariziong steps from -40 to +80 mV, 200 ms, starting from a voltage of -80 mV) was consistent with the non-inactivating and depolarization-activated K+ currents gated at voltages positive to -30 mV. Cl-IB-MECA effect was mimicked by adenosine (Ado: 30 µM) and prevented in the presence of the selective A3 antagonists MRS1523 and VUF5574 (100 nM) but not by MRS1220 (0.1-1 µM). On the other hand, adenosine-mediated K+ current inhibition was only partially blocked either by MRS1523 or VUF5574. In the presence of 1 mM extracellular Cd2+, which inhibits Ca2+ entry from voltage-operated Ca+2 channels (VOCCs), the effect of Cl-IB-MECA was completely prevented, demonstrating that the K+ conductances inhibited by the A3R agonist are mediated by Ca2+-activated K+ channels (KCa).

In order to verify whether the A3R agonist directly inhibits KCa or blocks Ca2+ entry from VOCCs, we blocked all K+ channels by replacing intra- and extracellular K+ by equimolar Cs+. Under these experimental conditions, a Cd2+-sensitive inward current was revealed by the ramp protocol at voltages from -30 to +40 mV, coherently with VOCCs gating. This inward Ca2+ current was

inhibited by 100 nM Cl-IB-MECA application, demonstrating that A3R activation directly blocks Ca2+ entry from VOCCs. Furthermore, in Cs+-replacement experiments, the effect of the A3R agonist on outward currents evoked by the ramp was completely prevented, thus confirming that they are indeed K+ currents.

In conclusion, present data demonstrate that adenosine A3R activation inhibits Ca2+ entry from VOCCs and, in turn, decreases outward K+ currents evoked by a voltage ramp protocol in rat DRG neurons. These effects might justify the A3-mediated antinociceptive effects observed in vivo, since Ca+2 current inhibition would result in hampering of nociceptive neurotransmission from DRG to the brain.

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