Adenosine A\textsubscript{2A} receptors induce the formation of abnormal secondary axons in rat hippocampal neurons: a new target to arrest epileptogenesis

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Abstract: Current anti-epileptic drugs mostly control convulsions with a limited efficacy, and do not seize epileptogenesis. One of the structural-functional modifications occurring during epileptogenesis is an abnormal axonal sprouting. The hippocampal mossy fiber (MF) sprouting found in patients and in animal models of temporal lobe epilepsy (TLE) (Neuroscience 14, 375-403) generates an aberrant excitatory feedback circuit in granule cells that is thought to contribute to the hyperexcitability underlying the seizure-prone state (Neurochem. Res. 28, 1649-58). However, the mechanism underlying this aberrant rewiring is still poorly understood. There is now a growing evidence for a sustained increase in extracellular ATP levels in different pathological conditions, prompting ATP as a danger signal in the brain (Front. Neurosci. 9:148). Accordingly, we now measured a sustained increase of the evoked release of ATP from hippocampal nerve terminals of Sprague-Dawley rats subjected to Status Epilepticus (SE) induced by pilocarpine-injection. Concomitantly, we found in developing rat hippocampal neurons that the pharmacological activation of adenosine A\textsubscript{2A} receptors (A\textsubscript{2AR}s) with the selective agonist CGS21680 (30 nM) induces the formation of abnormal secondary axons. This prompted us to propose that this abnormal sprouting is due to an abnormal reactivation of the developmental-related A\textsubscript{2AR}-driven axon formation and outgrowth. We have now found in hippocampal organotypic slices that the spontaneous MF sprouting was attenuated by the pharmacological blockade of A\textsubscript{2AR}s, since the current densities (pA/pF) recorded were significantly lower in the slices cultured in the presence of the selective A\textsubscript{2AR} antagonist SCH58261(100 nM). More importantly, we found that the animals subjected to a long-term systemic treatment with SCH58261 (0.1 mg/kg, i.p.), beginning 10 hours after pilocarpine-induced SE in rats, displayed significantly less hippocampal MF
sprouting. Interestingly, CD73 knockout (CD73 KO) mice (lacking ecto-5’-nucleotidase that converts AMP into adenosine critical for the formation of ATP-derived adenosine) which have experienced SE also showed a significant lower extent of MF sprouting 15 weeks after pilocarpine administration, in comparison with wild type mice. Altogether, our data show that A2ARs contribute to the abnormal hippocampal MF sprouting, raising A2ARs as a potential therapeutic target to arrest epileptogenesis.

**Key words:** A2A receptors, Mossy fiber sprouting, Status epilepticus, CD73