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Research Report

Adenosine A_1 receptors presynaptically modulate excitatory synaptic input onto subiculum neurons

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ABSTRACT

Adenosine is an endogenous neuromodulator previously shown to suppress synaptic transmission and membrane excitability in the CNS. In this study we have determined the actions of adenosine on excitatory synaptic transmission in the subiculum, the main output area for the hippocampus. Adenosine (10 μM) reversibly inhibited excitatory post synaptic currents (EPSCs) recorded from subiculum neurons. These actions were mimicked by the A_1 receptor-specific agonist, N^6-cyclopentyl-adenosine (CPA, 10 nM) and blocked by the A_1 receptor antagonist 8-cyclopentyl-1,3-dipropylxanthine (DPCPX, 500 nM), but were unaffected by the A_{2A} antagonist ZM 241385 (50 nM). In membrane excitability experiments, bath application of adenosine and CPA reversibly inhibited action potentials (AP) in subiculum neurons that were evoked by stimulation of the pyramidal cell layer of the CA1, but not by depolarizing current injection steps in subiculum neurons, suggesting a presynaptic mechanism of action. In support, adenosine and CPA application reduced mEPSC frequency without modulating mEPSC amplitude. These studies suggest that modulation of subiculum neuron excitability by adenosine is mediated via presynaptic A_1 receptors.

1. Introduction

Adenosine is a potent endogenous neuromodulator that has been shown to suppress excitatory synaptic transmission within the CNS (Dunwiddie and Hoffer, 1980). Adenosine exerts its effects through interactions with four known G-protein coupled receptor subtypes: A_1, A_{2A}, A_{2B}, and A_3. Activation of the receptor subtypes by adenosine can either inhibit adenylyl cyclase activity via the G_i protein linked to the A_1 and A_3 subtypes or stimulate activity via the G_s protein linked to the A_{2A} and A_{2B} subtypes (Fredholm et al., 2005). Within the CNS, A_1 receptor expression is widespread, particularly at glutamatergic synapses within the hippocampus (Rebola et al., 2005), cortex, and cerebellum (Swanson et al., 1995). The A_{2A} receptor is expressed in areas with high GABAergic innervation such as the striatum (Rosin et al., 1998) and hippocampus (Cunha et al., 1994), as well as in glutamatergic nerve terminals (Rebola et al., 2005).

Adenosine's depressant effects on synaptic transmission have been extensively studied in the hippocampus (Dunwiddie and Hoffer, 1980). Adenosine has been shown to modulate synaptic transmission along mossy fibers and the Schaffer

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Abbreviations: Artificial cerebrospinal fluid, ACSF; Excitatory post synaptic current, EPSC; Miniature excitatory post synaptic current, mEPSC; Action potential, AP; Entorhinal cortex, EC

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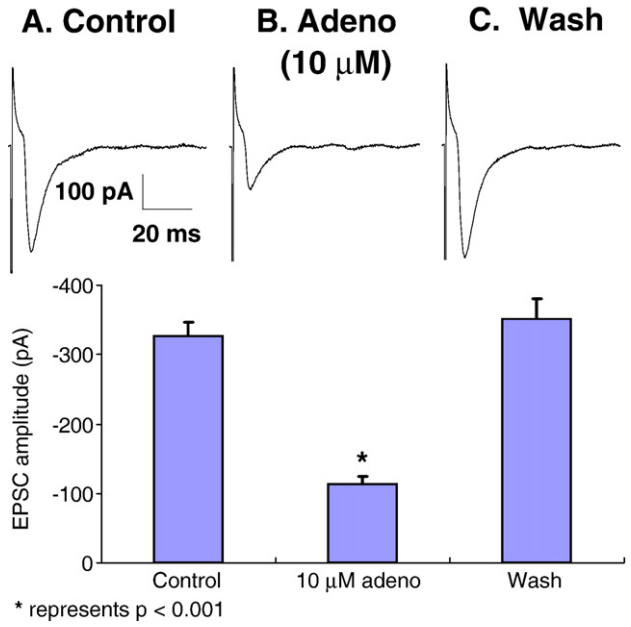

Fig. 1 – Adenosine inhibits excitatory synaptic currents in the subiculum. Top: Example traces of AMPA-mediated excitatory synaptic currents recorded during control (A), after 2 min application of 10 μM adenosine (B), and washout (C). Bottom: Bar graph representing the average EPSC amplitudes recorded during control conditions, following 2 min application of 10 μM adenosine, and washout (n=8). Error bars represent SEM.

collaterals resulting in the suppression of burst firing in CA3 and CA1 pyramidal neurons (Moore et al., 2003; Kukley et al., 2005; Gerber et al., 1989; Proctor and Dunwiddie, 1983). These depressant effects were determined to be mediated by activation of the A_1 receptor subtype (Dunwiddie and Fredholm, 1989). These inhibitory actions of adenosine would be protective in neurological disorders including epilepsy (Boison, 2005) and cerebral ischemia (Pearson et al., 2006), making adenosine receptors a target for potential therapeutic development (Cunha, 2005). Adenosine has been shown to not only inhibit seizure activity though its interaction with the A_1 receptor subtype (Lee et al., 1984), but also to keep epileptic seizure foci localized, preventing seizure propagation to other areas of the brain (Fedele et al., 2006). Decreasing extracellular adenosine has been shown to reduce seizure activity in an *in vitro* model of epilepsy, supporting a role for adenosine in seizure suppression (Avsar and Empson, 2004). As such, adenosine has long been considered the brains endogenous anticonvulsant (Boison, 2005), although this protection may be dependent upon activation of A_1 over A_{2A} receptors (Cunha, 2008).

Although adenosine's actions within the hippocampus have been extensively studied, its modulatory role in processing synaptic network information out of the hippocampus remains to be determined. The subiculum serves as the primary output center for the hippocampus; sending projections to the deep layers of the entorhinal cortex (EC) and to other cortical and non-cortical areas including layer III of the EC, the hypothalamus, prefrontal cortex and nucleus accumbens (Naber and Witter, 1998). The subiculum consists primarily of glutamatergic pyramidal cells, approximately 60–70% of which elicit bursts of action potentials in response to stimulation (Behr et al., 1996). This bursting characteristic may allow subiculum neurons to amplify synaptic information from the hippocampus, projecting it onward to other cortical regions. The subiculum has been shown to be relatively well preserved as compared to the rest of the hippocampus in disease phenotypes such as temporal lobe epilepsy (Babb and Pretorius, 1993), implicating a possible role for the subiculum in seizure generation and spread (Wozny et al., 2005).

In the present study, we have examined the effects of adenosine on modulating excitatory synaptic transmission and membrane properties in subiculum neurons. Adenosine application inhibited evoked AMPA-mediated synaptic currents and evoked action potentials in subiculum neurons via activation of presynaptic A_1 receptors. These findings have important implications in understanding the role of adenosine in modulating synaptic input onto subiculum neurons, and ultimately out of the hippocampus to surrounding cortical regions.

2. Results

2.1. *Adenosine mediated modulation of excitatory synaptic transmission*

Stimulation of the pyramidal cell layer of the CA1 region evoked recordable synaptic currents in all subiculum neurons tested. In the presence of the $GABA_A$ antagonist picrotoxin (50 μM), the glycine antagonist strychnine (50 μM), and the NMDA-mediated glutamate antagonist APV (30 μM), EPCSs recorded had a mean peak amplitude of −354.0±67.4 pA (n=8)

Table 1 – Inhibition of AMPA EPSC's by adenosine is mediated via A_1 and not A_{2A} adenosine receptors.

	Control (pA)	Agonist (pA)	Wash (pA)	Antagonist (pA)	Agonist and antagonist (pA)	Wash (pA)
Adeno (10 μM) and DPCPX (500 nM; n=5)	−345.2±54.0	−97.6±39.4*	−324.8±55.1	−486.0±91.9†	−464.4±94.7	−481.0±53.0
CPA (10 nM) and DPCPX (500 nM; n=5)	−295.6±25.3	−153.9±16.3*	−250.4±20.1	−384.9±25.1†	−375.9±25.1	−331.9±18.7
Adeno (10 μM) and ZM 241385 (50 nM; n=3)	−236.2±15.6	−84.7±7.7*	−176.2±11.9	−173.9±10.2	−77.6±10.2¶	−177.5±7.6

EPSC's were evoked by stimulation of the CA1 pyramidal layer. Values represent means of average EPSC amplitudes±S.E.M. *represents p<0.01. †represents p<0.05. ¶represents p<0.05 when tested against antagonist.