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Adenosine A₁ 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 μ M) reversibly inhibited excitatory post synaptic currents (EPSCs) recorded from subiculum neurons. These actions were mimicked by the A₁ receptor specific agonist, N⁶-cyclopentyl-adenosine (CPA, 10 nM) and blocked by the A₁ 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 A₁ receptors.

Keywords

Adenosine; subiculum; hippocampus; action potential; excitatory synaptic transmission

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: A₁, A_{2A}, A_{2B}, and A₃. Activation of the receptor subtypes by adenosine can either inhibit adenylyl cyclase activity via the G_i protein linked to the A₁ and A₃ 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, A₁ 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

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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 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 A₁ receptor subtype (Dunwiddie and Fredholm, 1989). These inhibitory actions of adenosine would be protective in neurological disorders including epilepsy (Boison, 2005a) 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 through its interaction with the A₁ 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 brain's endogenous anticonvulsant (Boison, 2005b), although this protection may be dependent upon activation of A₁ 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 back 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 A₁ 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 μ M), the glycine antagonist strychnine (50 μ M), and the NMDA-mediated glutamate antagonist APV (30 μ M), EPSCs recorded had a mean peak amplitude of -354.0 ± 67.4 pA ($n = 8$) and were completely abolished by bath application of NBQX (10 μ M; $n = 3$), indicating that they were mediated by AMPA receptors. Bath application of adenosine (10 μ M, 2 min) reversibly inhibited the AMPA-mediated EPSC in all neurons tested by an average of 66.0 ± 3.0 % (from -327.1 ± 19.9 pA to -114.6 ± 9.3 pA; $p < 0.001$; $n = 8$; Fig. 1). To determine if these changes were mediated by the A₁ receptor, the A₁ receptor-specific antagonist 8-cyclopentyl-1,3-

dipropylxanthine (DPCPX) was applied prior to adenosine perfusion at a concentration previously shown to suppress the actions of adenosine (Patel et al., 2001). Perfusion of DPCPX (500 nM, 5 min) led to an initial increase in the EPSC amplitude by an average of $44 \pm 19\%$ ($p < 0.05$; $n = 5$). In the presence of DPCPX, adenosine had no effect ($n = 5$; Table 1). The ability of DPCPX to potentiate EPSC amplitude suggests a tonic activation of the A_1 receptors by endogenous levels of adenosine. These actions of adenosine were mimicked by the A_1 receptor agonist N^6 -cyclopentyl-adenosine (CPA). Application of CPA (10 nM, 2 min) reversibly inhibited EPSCs in the subiculum ($n = 5$; Fig 2, Table 1). These effects of CPA were again inhibited by bath application of DPCPX (500 nM; 5 min, Fig 2, Table 1). In contrast, perfusion of the A_{2A} receptor antagonist ZM 241385 (50 nM, 5 min) failed to block the inhibitory actions of adenosine on EPSC amplitude, suggesting that the actions of adenosine were not mediated by the A_{2A} receptor. Adenosine inhibited the synaptic current by $63.8 \pm 7.0\%$ alone and by $56.6 \pm 12.8\%$ in the presence of ZM 241385 (50 nM; $n = 3$; Fig 3, Table 1). In contrast to the actions of the A_1 receptor antagonist DPCPX, perfusion of ZM 241385 did not increase the EPSC amplitude, further supporting the notion that A_{2A} receptors are not involved in the modulation of excitatory synaptic transmission in the subiculum.

2.2 Effects of adenosine on membrane excitability of subiculum and CA1 neurons

In order to investigate the actions of adenosine on neuronal membrane excitability of subiculum neurons, current clamp recordings of subiculum neurons were made. Subiculum neuron action potentials were evoked by stepping to predetermined hyperpolarizing and depolarizing current injections of 10 pA increments from -20 pA to 470 pA for a duration of 300 ms. Bath application of adenosine (10 μ M; 2 mins) had no significant effect on the input resistance (Fig 4B) or action potential characteristics ($n = 8$; Figure 4A; Table 2). Action potential frequency assessed at a depolarizing current injection step of 470 pA was 34.7 ± 2.7 Hz ($n = 7$) under control conditions and 34.7 ± 2.0 Hz ($n = 7$) in the presence of adenosine (Figure 4A, C).

In contrast, synaptically evoked AP's were modulated by adenosine application. Adenosine (10 μ M, 2 min) reversibly inhibited action potentials that were evoked by stimulation of the CA1 pyramidal layer ($n = 14$; Fig 5, top traces). These effects of adenosine were abolished by bath application of DPCPX (500 nM; $n = 4$; Fig 5, middle traces), but were again not affected by bath application of the A_{2A} receptor antagonist ZM 241385 (50 nM; $n = 5$; Fig 5, lower traces), suggesting that these actions were mediated by activation of the A_1 receptor subtype and not by activation of the A_{2A} receptor subtype.

Since subiculum neurons receive their input primarily from presynaptic CA1 neurons, the effects of adenosine on membrane and intrinsic firing properties on CA1 pyramidal neurons were determined. AP's were evoked in CA1 pyramidal neurons by depolarizing current injections in a manner identical to those described for subiculum neurons. Action potential frequency was assessed at the maximum depolarizing current injection step of 470 pA. Adenosine significantly reduced the AP frequency from 26.7 ± 5.8 Hz under control conditions to 18.6 ± 5.2 Hz in the presence of adenosine ($p < 0.05$; $n = 5$; data not shown). These findings suggest that adenosine receptors are expressed in CA1 pyramidal neurons and since they provide an input to subiculum neurons, they could be involved in modulating subiculum neuron output.

2.3 Adenosine reduces frequency of mEPSCs

In order to further examine a presynaptic mechanism of action for adenosine, the effects of adenosine and CPA on miniature excitatory post synaptic currents (mEPSCs) in subiculum neurons were examined. Recordings were made in the presence of 1 μ M tetrodotoxin (TTX) to determine actions on spontaneous mEPSC's. Adenosine (10 μ M, 2 min) reduced the frequency of mEPSCs in all 6 subiculum neurons tested by an average of $51.6 \pm 6.0\%$ (from

2.5 ± 0.2 Hz in control to 1.2 ± 0.2 Hz with adenosine; $p < 0.01$; $n = 6$; Fig 6A, C). mEPSC amplitudes were not changed (from 20.3 ± 0.2 pA in control to 21.9 ± 1.6 pA with adenosine; $n = 6$; Fig 6D). Similarly, CPA (10 nM, 2 min) also reduced the frequency of mEPSCs in all 6 subiculum neurons tested by an average of 57.3 ± 5.9 % (from 2.5 ± 0.2 Hz, control to 1.1 ± 0.3 Hz, CPA; $p < 0.01$; $n = 6$; Fig 6B, C), with no effect on mEPSC amplitude (from 20.3 ± 0.2 pA, control to 22.7 ± 1.5 pA, CPA; $n = 6$; Fig 6D).

3. DISCUSSION

Adenosine is an endogenous neuromodulator capable of altering synaptic transmission within the CNS (Boison, 2008). Within the hippocampus, adenosine has been shown to inhibit excitatory synaptic transmission in CA1 pyramidal neurons (Brundage and Dunwiddie, 1996), CA1 interneurons (Li and Henry, 2000), and CA3 pyramidal neurons (Scanziani et al., 1992) via activation of A₁ receptors. Input into the hippocampus via the perforant path can also be modulated by adenosine, again via an A₁ mediated event (Mohammad-Zadeh et al., 2009). However, adenosine's ability to modulate membrane excitability of neurons that feed out of the hippocampus-EC network loop remains to be elucidated. In the present study, we have determined the actions of adenosine on synaptic currents and membrane excitability of subiculum neurons. The subiculum serves as the primary output center for the hippocampus, receiving information directly from the CA1 and projecting information out to cortical and subcortical areas including the entorhinal, perirhinal and postrhinal cortices (Witter, 2006). Modulation of the membrane excitability of these neurons would be important for regulating synaptic output from the hippocampus.

Adenosine was shown to inhibit AMPA-mediated excitatory synaptic currents and to suppress action potentials evoked by stimulation of the CA1 pyramidal layer without having any effect on action potentials evoked by depolarizing current injections into subiculum neurons. In contrast, action potentials evoked in CA1 neurons by depolarizing current injection were inhibited by adenosine. These findings suggest that subiculum neurons do not express functioning adenosine receptors and that the actions of adenosine are most likely mediated via presynaptically located adenosine receptors, presumably located on terminals of CA1. Further support for a presynaptic mechanism comes from the fact that adenosine reduced the frequency of mEPSC, but had no effect on mEPSC amplitude. Since the effects of adenosine were mimicked by the A₁ selective agonist CPA and blocked by the A₁ selective antagonist DPCPX, and unaffected by the A_{2A} receptor antagonist ZM 241387, these inhibitory actions were likely mediated through activation of the A₁ receptor subtype and not the A_{2A} receptor subtype. Interestingly, application of the A₁ receptor antagonist DPCPX alone caused a potentiation of the AMPA mediated synaptic current, implying that a level of tonic activation of adenosine A₁ receptors exists within the slice, presumably by endogenously generated adenosine. Adenosine A₁ receptors are localized at excitatory synapses within the hippocampus (Rebola et al., 2003b).

Our studies suggest that input into the subiculum can be modulated by adenosine; however, intrinsic excitability of subiculum neurons cannot be suppressed by adenosine. Since subiculum neurons are ultimately responsible for hippocampal output to cortical areas including the perirhinal and postrhinal cortices and locally onto the entorhinal cortex neurons of layer III creating a feedback loop back into the hippocampus (Witter, 2006), control of this activity could be crucial, particularly during conditions of hyper-excitability. Subiculum neurons possess the ability to fire bursts of action potentials in response to presynaptic stimulation, potentially leading to an amplification of the synaptic input. Subiculum neurons are known to have altered electrophysiological characteristics in disease states such as epilepsy. For example, seizure activity has been shown to result in a decreased afterhyperpolarizing potential (AHP) (Behr et al., 2000), an increased afterdepolarizing potential (ADP) (Wellmer

et al., 2002), and an increased persistent sodium current (I_{NaP}) (Vreugdenhil et al., 2004) in subiculum neurons, all of which could lead to hyperexcitability and an increased output.

Under normal physiological conditions, endogenously produced adenosine helps to control energy expenditure by tonically inhibiting the release of excitatory neurotransmitters (Dunwiddie and Masino, 2001), thereby, regulating neuronal excitability (Fredholm et al., 2001). This regulation can be modified by adenosine receptor antagonists, such as caffeine, which exert their excitatory effects through their interactions with adenosine receptors (Fredholm et al., 1999). Under pathological conditions such as epilepsy, adenosine levels and adenosinergic signaling are distinct. Within the hippocampus, epileptic seizure activity has been shown to drastically increase extracellular adenosine levels in rats (Berman et al., 2000) and in humans, (During and Spencer, 1992) presumably as a method of protection to reduce excitatory transmission and seizure propagation. In contrast, levels of adenosine kinase, the enzyme responsible for adenosine metabolism, may be also elevated in the epileptic condition (Gouder et al., 2004) potentially limiting adenosine levels in the brain. Moreover, adenosine receptor expression profiles as well as the G-protein coupling of these receptors may be altered in the pathological condition, again altering adenosinergic signaling and adenosine mediated regulation of synaptic transmission. Current reports of adenosine receptor expression patterns following seizure activity are conflicting. For example, increases in adenosine A_1 receptor density have been reported following bicuculline-induced seizures, and these upregulated receptors were functionally linked to their proper G-proteins (Daval and Werck, 1991). In contrast, A_1 receptor density has been shown to be downregulated in the rat hippocampus following kindling (Rebola et al., 2003a) and kainic-acid (Ekonomou et al., 2000) induced seizures. Adenosine A_{2A} receptor density has been shown to be upregulated following seizure activity (Rebola et al., 2002).

In summary, we show that adenosine can modulate AMPA-mediated excitatory synaptic transmission in subiculum neurons, but that this modulation occurs via the activation of the adenosine A_1 receptor located presynaptically, and not intrinsic to subiculum neurons. These findings support previous studies of adenosine mediated modulation of synaptic transmission in the hippocampus, while providing novel insight into the role of adenosine in controlling hippocampal output.

4. EXPERIMENTAL PROCEDURE

4.1 Brain slice preparations

Adult Sprague-Dawley rats (150–300 g) were anesthetized with isoflurane and brains removed and submerged in ice cold artificial cerebrospinal fluid (ACSF) solution containing (in mM): 125 NaCl, 2.5 KCl, 1.25 NaH_2PO_4 , 2 CaCl_2 , 1 MgCl_2 , 0.5 L-Ascorbic acid, 2 Pyruvate, 10 Glucose, and 25 NaHCO_3 . The brains were cut into 300 μm sagittal sections using a Vibratome (Vibratome, St. Louis, MO, USA), and incubated at 37°C for 30 minutes and then stored at room temperature prior to recording. During recording, slices were transferred to a perfusion chamber and constantly superfused with ACSF at a rate of 3 mL/min while being continually oxygenated with 95% O_2 , 5% CO_2 at 30° C.

4.2 Electrophysiological recordings

Whole cell patch clamp recordings were made using electrodes with resistances of 2–4 M Ω when filled with intracellular solution containing (in mM): 120 K gluconate, 10 NaCl, 2 MgCl_2 , 0.5 K_2EGTA , 10 HEPES, 4 Mg_2ATP , and 0.3 NaGTP (pH 7.2). Electrophysiological signals were digitally recorded using an Axopatch 200A patch-clamp amplifier (Molecular Devices, Sunnyvale, CA, USA). All voltage protocols were applied using pCLAMP 9 software

(Molecular Devices, USA) and a Digidata 1322A digitizer (Molecular Devices, USA). Currents were amplified and low pass filtered (2 kHz) and sampled at 33 kHz.

After establishing the whole-cell configuration, neurons were allowed to return to their natural resting membrane potential before running protocols. In current clamp configuration, action potentials were evoked by current injection steps of 10 pA from -20 pA to 470 pA for a duration of 300 ms. To evoke synaptic responses and action potentials, a digital stimulator (Digitimer Ltd., Hertfordshire, UK) producing 400 ms stimulations of varying current amplitudes to a concentric platinum iridium stimulating microelectrode (WPI, Sarasota, Florida, USA) was placed in the CA1 region of the hippocampus, approximately 2 mm from the recording site in the subiculum. The stimulation strength was 1.5 times the strength needed to evoke a response, ranging from 1–5 mA, and was reconfigured for each synapse recruited. Electrical stimulations were made every 15 s for synaptic current recordings and every 20 s for evoked action potential recordings.

For voltage clamp experiments, 50 μ M picrotoxin (GABA_A antagonist), 50 μ M strychnine (glycine antagonist), and 30 μ M AP5 (NMDA antagonist) were added to ACSF to only record AMPA mediated glutamatergic synapses. In some experiments, 5 μ M QX-314, was added to the intracellular patch pipette to suppress action potential generation. Control excitatory synaptic current amplitudes were calculated by averaging 6 synaptic currents prior to the application of drugs. Drug effects on synaptic currents were determined by averaging 6 synaptic currents at the peak of drug effect. mEPSCs were recorded in the presence of TTX (1 μ M).

For analysis purposes, input resistance was determined from the steady state voltage deflection observed as a result of a 300 ms current injection steps from -20 to 100 pA. Action potential threshold was defined as the point at which the change in voltage over time exceeded 30 mV/ms on the rising phase of the action potential. Action potential amplitude was defined as the difference between peak action potential voltage and the threshold. Action potential upstroke velocity was determined between 0 mV and 20 mV range of the rising phase of the action potential. Action potential width was defined as the amount of time between the rising and falling phase of the action potential, assessed at half of the action potential amplitude.

4.3 Drugs

Adenosine (10 μ M), N⁶-cyclopentyl-adenosine (CPA), 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) and ZM 241385 were obtained from Sigma Aldrich (St. Louis, MO, USA) and prepared as 1000 $\times$ stock solutions in DMSO. Drugs were then diluted to working concentrations directly preceding experiments. DMSO concentration did not exceed 0.02%.

4.4 Data Analysis

Electrophysiology data analysis was performed using Clampfit software (v9, Molecular Devices) and Origin (v6, OriginLab Corp). Statistical analyses were performed using the standard paired T-test (SigmaStat, SPSS Inc.). Averaged data are presented as means $\pm$ standard error of the mean (S.E.M). Statistical significance was set at $P < 0.05$.

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ABBREVIATIONS

ACSF

Artificial cerebrospinal fluid

EPSC

Excitatory post synaptic current

mEPSC

Miniature excitatory post synaptic current

AP

Action potential

EC

Entorhinal cortex

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Figure 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 μ M adenosine (B), and washout (C). **Bottom:** Bar graph representing the average EPSC amplitudes recorded during control conditions, following 2 min application of 10 μ M adenosine, and washout ($n = 8$). Error bars represent SEM. * represents $P < 0.001$.

Figure 2. Inhibition of AMPA mediated synaptic transmission by adenosine is mediated by A₁ receptors

Left: Bar graph shows that CPA (10 nM) reversibly inhibits AMPA mediated EPSC's. The inhibition was abolished by application of the A₁ receptor antagonist DPCPX (500 nM). Bars represent the average EPSC amplitudes recorded from five neurons. CPA (10 nM, $n = 5$) was applied for 10 minutes. DPCPX (500 nM, $n = 5$) was applied for 5 minutes prior to, during and after the second application of CPA (10 nM; 2 minutes, $n = 5$). **Right:** Example traces of AMPA mediated synaptic currents recorded during control (**A**), after 2 min application of 10 nM CPA (**B**), washout of CPA (**C**), after 5 min application of 500 nM DPCPX (**D**), application of CPA (10 nM) in the presence of DPCPX (500 nM; **E**), and following washout of all drugs (**F**). Note the potentiation of the synaptic current in the presence of DPCPX (**D**) and the lack of effect of CPA in the presence of DPCPX (**E**). Error bars represent SEM. * represents $p < 0.01$, † represents $p < 0.05$.

Figure 3. Inhibition of AMPA mediated synaptic currents by adenosine are not mediated via activation of A_{2A} receptors

Left: Bar graph shows that adenosine (10 μ M, $n = 3$) reversibly inhibits the AMPA mediated synaptic current. This inhibition is still observed in the presence of ZM 241385 (50 nM, $n = 3$), an A_{2A} receptor antagonist. Note that application of ZM 241385 does not cause a potentiation of the synaptic current. EPSC amplitudes represent an average of three neurons. **Right:** Example traces of AMPA mediated synaptic currents recorded during control (A), after 2 min application of 10 μ M adenosine (B), after washout of adenosine (C), after 5 min application of 50 nM ZM 241385 (D), re-application of adenosine (10 μ M) in the presence of ZM 241385 (50 nM) and (E), following washout of all drugs (F). ZM 241385 was not able to block the inhibitory actions of adenosine (E). Error bars represent SEM. * represents $P < 0.01$.

Figure 4. Adenosine has no effect on action potentials evoked by depolarizing current injections
A: Action potentials in subiculum neurons evoked by current injection steps of -20 , 0 , 30 , 60 , 90 , and 100 pA. Example traces recorded in control, following 2 min of application of adenosine ($10 \mu\text{M}$), and following washout. **B:** I-V plot showing voltage deflection as a function of current injection for control conditions is not significantly different following 2 min application of adenosine ($10 \mu\text{M}$; $n = 8$). Solid line represents a linear fit of control data. Dashed line represents a linear fit for adenosine data. **C:** Frequency plot showing the number of action potentials evoked in subiculum neurons as a function of current injection for control, adenosine, and washout conditions ($n = 8$ for all conditions). Data represent means $\pm$ S.E.M.

Figure 5. Adenosine inhibits action potentials evoked by stimulation of CA1 pyramidal layer
A: Adenosine (10 μ M) application reversibly inhibits action potentials evoked by stimulation of the CA1 pyramidal layer. These inhibitory actions were abolished by application of the A₁ receptor antagonist DPCPX (500 nM; **B**), but were not changed by application of the A_{2A} receptor antagonist, ZM 241385 (50 nM). Arrows on the left of traces represent -60 mV.

Figure 6. Adenosine inhibits frequency but not amplitude of mEPSCs in subiculum neurons via an A_1 receptor mediated event

Effects of adenosine (10 μ M; A) and CPA (10 nM; B) on mEPSCs recorded in subiculum neurons. Insets show expanded timescales of mEPSCs for clarity. Bar graph showing decrease in mEPSC frequency (C) but not amplitude (D) after 2 min application of adenosine (10 μ M; n = 6) or CPA (10 nM; n = 6). Data represent means $\pm$ S.E.M. * denotes $P < 0.01$.

TABLE 1
Inhibition of AMPA EPSC's by adenosine is mediated via A₁ and not A_{2A} adenosine receptors
EPSC's were evoked by stimulation of the CA1 pyramidal layer.

	Control Antagonist (pA)	Agonist Agonist and (pA)	Wash Wash (pA)	(pA)	antagonist (pA)	(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

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.

Table 2**Adenosine has no effect on membrane properties of subiculum neurons**

Membrane properties of subiculum neurons under control (n = 8) and following 2 min application of adenosine (10 μ M; n = 8) are shown.

	Control	Adenosine (10 μ M)
RMP (mV)	-62.4 ± 0.8	-62.5 ± 1.0
Input resistance (MΩ)	76.4 ± 1.9	77.9 ± 0.8
AP threshold (mV)	-44.9 ± 0.9	-42.8 ± 1.3
AP amplitude (mV)	109.6 ± 7.3	105.7 ± 7.2
AP U-V (V/s⁻¹)	309.8 ± 10.1	307.9 ± 12.2
AP width (ms)	0.86 ± 0.03	0.84 ± 0.04

RMP, resting membrane potential; AP threshold, action potential threshold; AP amplitude, action potential amplitude; AP U-V, action potential upstroke velocity; AP width, action potential width. Values represent means $\pm$ S.E.M.