Adenosine Down-Regulates Giant Depolarizing Potentials in the Developing Rat Hippocampus by Exerting a Negative Control on Glutamatergic Inputs

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Safiulina, Victoria F., Alexander M. Kasyanov, Rashid Giniatullin, and Enrico Cherubini. Adenosine down-regulates giant depolarizing potentials in the developing rat hippocampus by exerting a negative control on glutamatergic inputs. *J Neurophysiol* 94: 2797–2804, 2005; 10.1152/jn.00445.2005. Adenosine is a widespread neuromodulator that can be directly released in the extracellular space during sustained network activity or can be generated as the breakdown product of adenosine triphosphate (ATP). Whole cell patch-clamp recordings were performed from CA3 principal cells and interneurons in hippocampal slices obtained from P2–P7 neonatal rats to study the modulatory effects of adenosine on giant depolarizing potentials (GDPs) that constitute the hallmark of developmental networks. We found that GDPs were extremely sensitive to the inhibitory action of adenosine (IC_{50} = 0.52 μM). Adenosine also contributed to the depressant effect of ATP as indicated by DPCPX-sensitive changes of ATP-induced reduction of GDP frequency. Similarly, adenosine exerted a strong inhibitory action on spontaneous glutamatergic synaptic events recorded from GABAergic interneurons and on interictal bursts that developed in CA3 principal cells after blockade of γ-aminobutyric acid type A (GABA_{A}) receptors with bicuculline. All these effects were prevented by DPCPX, indicating the involvement of inhibitory A1 receptors. In contrast, GABAergic synaptic events were not changed by adenosine. Consistent with the endogenous role of adenosine on network activity, DPCPX per se increased the frequency of GDPs, interictal bursts, and spontaneous glutamatergic synaptic events recorded from GABAergic interneurons. Moreover, the adenosine transporter inhibitor NBTI and the adenosine deaminase blocker EHNA decreased the frequency of GDPs, thus providing further evidence that endogenous adenosine exerts a powerful control on GDP generation. We conclude that, in the neonatal rat hippocampus, the inhibitory action of adenosine on GDPs arises from the negative control of glutamatergic, but not GABAergic, inputs.

**INTRODUCTION**

A particular feature of the developing hippocampus is the presence of network-driven spontaneous rhythmic activity called “giant depolarizing potentials” or GDPs (Ben-Ari et al. 1989). GDPs are characterized by recurrent membrane depolarizations with superimposed fast action potentials. They occur at the frequency of 0.03–0.3 Hz and can be detected both in vitro (Ben-Ari et al. 1989) and in vivo (Leinekugel et al. 2002). GDPs are generated by the synergistic action of glutamate and γ-aminobutyric acid (GABA) (Bolea et al. 1999). Early in postnatal life, GABA depolarizes and excites postsynaptic cells (Ben-Ari 2002; Ben-Ari et al. 1997; Cherubini et al. 1991) due to high intracellular [Cl\^−], which results mainly from the unbalance of two Cl− cotransporter systems, the NKCC1 and KCC2 that enhance and lower intracellular [Cl\^−], respectively (Payne et al. 2003; Rivera et al. 1999). The depolarizing action of GABA during GDPs results in calcium influx through the activation of voltage-dependent calcium channels and N-methyl-D-aspartate (NMDA) receptors (Leinekugel et al. 1997). Thus GDPs acting as coincidence detector signals for enhancing synaptic efficacy (Kasyanov et al. 2004) may contribute to the functional and structural refinement of the hippocampal network.

In previous studies from our and other laboratories it was shown that GDPs are regulated by a number of neurotransmitter receptors and modulators localized on pre- and postsynaptic sites of principal cells and interneurons (Avignone and Cherubini 1999; Gaiarsa et al. 1991; Maggi et al. 2001; Mclaun et al. 1996; Strata et al. 1995; Xie et al. 1994). In turn, neurotransmitter release can be regulated by activity dependent processes like GDPs. Consistent with this, in a recent study (Safiulina et al. 2005) we demonstrated that adenosine triphosphate (ATP), a widely distributed neurotransmitter and neuromodulator (Burnstock 2004), which regulates network activity by ionotropic P2X and metabotropic P2Y receptors, can be released during GDPs. Because ATP is hydrolyzed to adenosine by ectonucleotidases (Cunha et al. 1998; Dunwiddie et al. 1997) the possibility that this neurotransmitter also regulates frequency of GDPs by activation of adenosine receptors by its breakdown product adenosine cannot be ruled out.

In the hippocampus adenosine has been shown to act on pre- and postsynaptic A1 receptors to inhibit glutamate release (Lupica et al. 1992; Wu and Saggau 1994; Yoon and Rothman 1991) and to enhance potassium conductance (Greene and Haas 1985), respectively. Altogether these effects would produce a reduction in cell excitability and firing rate (for a review see Ribeiro et al. 2003b). Here we demonstrate that, during the first week of postnatal life, endogenous adenosine, by activating A1 receptors localized mainly on glutamatergic terminals projecting to principal cells or interneurons, regulates the occurrence of GDPs.

**METHODS**

**Slice preparation**

Experiments were performed on hippocampal slices obtained from postnatal (P) P2–P6 Wistar rats (P0 was taken as the day of birth) as previously described (Maggi et al. 2001). The procedure was in accordance with the European Community Council Directive of 24 November 1986 (86/609EEC) and was approved by the local authority veterinary service. Animals were decapitated after being anesthetized with an intraperitoneal injection of urethane (2 g/kg). After the costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
decapitation the brain was removed from the skull and placed in ice-cold artificial cerebrospinal fluid (ACSF) containing (in mM): NaCl, 130; KCl, 3.5; Na$_2$HPO$_4$, 1.2; NaHCO$_3$, 25; MgCl$_2$, 1.3; CaCl$_2$, 2; glucose, 25; saturated with 95% O$_2$-5% CO$_2$ (pH 7.3–7.4). Hippocampal slices (500 μm thick) were cut with a vibratome and incubated at room temperature (22–25°C) in oxygenated ACSF. Registrations of activity were performed from an individual slice in a submerged chamber continuously perfused at 33–34°C in oxygenated ACSF at a rate of 2–3 ml/min.

**Patch-clamp whole cell recordings**

Electrophysiological experiments were performed from CA3 pyramidal cells and from GABAergic interneurons localized on stratum radiatum using the whole cell configuration of the patch-clamp technique in current-clamp mode. Neurons were visualized using infrared Nomarski optics on an upright microscope (Leica DMLFS, Wetzlar, Germany). GDPs and spontaneous (action potential–dependent and –independent) ongoing synaptic potentials (sPSPs), were recorded from a holding potential of −70 ± 5 mV with a standard patch-clamp amplifier (Axoclamp 2B, Axon Instruments; Foster City, CA). Patch electrodes, pulled from borosilicate glass capillaries (Hilgenberg, Malsfeld, Germany), had a resistance of 5–7 MΩ.

The whole cell capacitance was fully compensated and the series resistance (10–20 MΩ) was compensated at 75–80%. The stability of the patch was checked by repetitively monitoring the input and series resistance during the experiment. Cells exhibiting 15–20% changes were excluded from the analysis. Membrane input resistance was measured during recordings from the amplitude of the electrotonic potentials (300-ms duration) evoked by passing hyperpolarizing current steps of 100–200 pA across the cell membrane.

**Drugs**

All substances were prepared as 1.000× concentrated stock solutions. ATP, adenosine, picrotoxin, and 6-(4-nitrobenzyl)thio-9-β-D-ribofuranosylpurine (NBPT) were purchased from Sigma. Bicuculline methochloride, 1,3-dipropyl-8-cyclopentylxanthine (DPCPX), 6,7-di-nitroquinoxaline-2,3-dione (DNQX), and ethynyl-9-(2-hydroxy-3-nonyl) adenine (EHNA) hydrochloride were purchased from Tocris Cookson. DPCPX and picrotoxin were dissolved in ethanol (final concentration of ethanol in solution 0.02%); NBPT was dissolved in dimethylsulfoxide (DMSO). Control experiments using a solution containing 2.5/1,000 of DMSO did not affect GDPs or sPSPs. Stock solutions were stored at −20°C. The substances to be tested were dissolved at their final concentrations in extracellular solution just before the recording session. All substances were applied through a three-way tap system. Bath volume exchange was completed within 1.5 min.

**Data acquisition and analysis**

Data were transferred to a computer after digitization with an A/D converter (Digidata 1200, Axon Instruments). Data were sampled at 20 kHz and filtered with a cutoff frequency of 1 kHz. Acquisition and analysis were performed with Clampex 7 (Axon Instruments).

The analysis of sPSPs was performed with Mini Analysis Program (Synaptosoft, Decatur, GA). General principles and details on the analysis of synaptic currents can be found elsewhere (Jo et al. 1998; Poisbeau et al. 1996).

The cumulative amplitude and interevent plots obtained for cell in controls and after drug application were compared using the Kolmogorov–Smirnov (KS) test.

For each cell, the mean GDPs frequency was calculated in control conditions and during drug application (starting 3 min after the onset of drug perfusion). Statistical comparisons were made between mean values (control vs. drug treatment). The numerical data are given as means ± SE and compared using the Student’s t-test. The differences were considered significant when $P < 0.05$.

**RESULTS**

Whole cell recordings in current-clamp mode were performed from 148 CA3 pyramidal cells and 12 interneurons localized in stratum radiatum at P2–P6. All slices exhibited spontaneous GDPs of the immature brain, which lasted 0.5–1 s and occurred at the frequency of 0.05 ± 0.06 Hz ($n = 41$). In both principal cells and interneurons GDPs were intermingled with small-amplitude action potential–dependent and –independent spontaneous synaptic potentials (sPSPs). sPSPs occurred at frequencies of 5.6 ± 0.006 ($n = 98$) and 8.5 ± 1 Hz ($n = 9$), in principal cells and interneurons, respectively.

In CA3 pyramidal cells ATP reduces the frequency of GDPs by P2 receptors and its breakdown product adenosine

Because the breakdown of ATP in the extracellular space is one important source of adenosine (Ribeiro et al. 2003b) in a first set of experiments we tested the effects of ATP on GDPs. As shown in Fig. 1A, bath application of ATP (50 μM) reversibly blocked GDPs, an effect that was associated with an increase in frequency of sPSPs. At lower concentrations (2 μM) ATP induced a reduction of frequency of GDPs from 0.06 ± 0.004 to 0.009 ± 0.001 Hz ($n = 4$, $P < 0.01$). The effect of ATP on the frequency of GDPs was dose dependent with an EC$_{50}$ of 0.78 μM (Fig. 1C). In eight out of 14 cells, ATP (50 μM) induced a membrane hyperpolarization (4.6 ± 0.8 mV) associated with an increase in membrane conductance (9 ± 1%). When ATP (50 μM) was applied in the presence of a high concentration of DPCPX (10 μM), which blocks adenosine receptors (Khakh et al. 2003), it produced a biphasic effect: an initial increase followed by a persistent decrease in frequency of GDPs (Fig. 1B; see also Safiulina et al. 2005). The depressant effect was concentration dependent with an EC$_{50}$ value of 16.2 μM (Fig. 1C). Thus at the concentration of 50 μM the reduction in frequency of GDPs produced by ATP was 43 ± 1.5%. Comparison of the two curves of Fig. 1C suggests a strong adenosine effect. Interestingly, in 10 μM DPCPX ATP (50 μM) was still able to up-regulate the frequency of sPSPs recorded from principal cells, indicating that this effect was adenosine independent (the frequency of sPSPs was 10.9 ± 0.9 and 10.8 ± 2.8 Hz in the presence of ATP and ATP + DPCPX, respectively; $n = 4$). Moreover, it should be stressed that in the presence of DPCPX, the effects of ATP on GDPs were never associated with changes in membrane potential and resistance, suggesting that the latter were probably mediated by postsynaptic A1 adenosine receptors. These data strongly suggest that endogenous ATP may also regulate the activity of GDPs by its breakdown product adenosine acting on A1 receptor subtypes.

**Endogenous adenosine slows down the frequency of GDPs**

To further explore this issue, adenosine was directly applied to hippocampal slices at concentrations ranging from 0.1 to 10
M. As shown in Fig. 2A, adenosine (10 μM) completely blocked GDPs. This effect was reversible 2–3 min after adenosine was washed out. At a lower concentration (0.5 μM) adenosine reduced the frequency of GDPs from 0.06 ± 0.004 to 0.03 ± 0.002 Hz without modifying their shape (the area under GDPs was 15.9 ± 1.4 and 16.3 ± 2.2 mV s⁻¹ before and during adenosine application, respectively; P > 0.05). The effect of adenosine on GDPs was dose dependent (the EC₅₀ value was 0.52 μM; Fig. 2C). The closed circle in Fig. 2C represents the ratio between the frequency of GDPs obtained in control conditions and that achieved in the presence of a saturating concentration of DPCPX (100 nM; see Fig. 3) when the action of endogenous adenosine is neutralized. This value corresponds to the inhibitory effect of endogenous adenosine on the frequency of GDPs. In four out of seven cells, adenosine (10 μM) produced a membrane hyperpolarization (4.3 ± 0.3 mV) that was associated with an increase in membrane conductance (8 ± 1%). In contrast with ATP, adenosine did not modify the frequency of sPSPs that at P2–P6 are mainly GABAₐ mediated (Hosokawa et al. 1994). In keeping with this, adenosine (10 μM) also failed to modify the frequency of GABAₐ-mediated sPSPs when applied in the presence of the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)/kainate receptor antagonist DNQX (10 μM). In DNQX, the frequency of sPSPs was 5.7 ± 1.5 and 6.0 ± 1.7 Hz (n = 3, P > 0.05) before and during adenosine application, respectively. The effects of exogenously applied adenosine on GDPs, membrane potential, and conductance were prevented by 100 nM of DPCPX (n = 4, Fig. 2B), which at this concentration selectively blocks A₁ receptors (Giniatullin and Sokolova 1998; Ralevic and Burnstock 1998).

**FIG. 1.** Adenosine triphosphate (ATP) also inhibits giant depolarizing potentials (GDPs) by adenosine receptors. A: representative recording from a CA3 pyramidal neuron (~70 mV) demonstrating the reversible inhibitory action of ATP (50 μM) on GDPs. B: recording from another cell showing that the action of ATP was essentially attenuated in the presence of a high concentration of 1,3-dipropyl-8-cyclopentylxanthine (DPCPX; 10 μM), which blocks adenosine receptors. Note that in the presence of DPCPX, the depressant effect of ATP was preceded by an early facilitatory action. ATP-induced increase in spontaneous events (A) was still observed in the presence of DPCPX (B), indicating that it was independent of the activation of A₁ adenosine receptors. Two individual GDPs (marked with a and b) are shown below in an expanded timescale. C: depressant effects of ATP (open circles, n = 35) and ATP + DPCPX (10 μM, closed circles, n = 21) on the frequency of GDPs.

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Importantly, DPCPX per se increased the frequency of GDPs. This effect was concentration dependent and reversible because it completely recovered after washing out DPCPX. Thus DPCPX (10 nM) enhanced the frequency of GDPs from 0.08 ± 0.02 to 0.012 ± 0.004 Hz and DPCPX (100 nM), from 0.08 ± 0.02 to 0.21 ± 0.009 Hz (Fig. 3A). This effect occurred in the absence of any change in the amplitude or shape of GDPs (the area under GDPs was 7.0 ± 1.0 and 9.1 ± 1.7 mV s⁻¹ before and during application of 100 nM of DPCPX, respectively; n = 4, P > 0.05; see insets below Fig. 3A). The EC₅₀ value for the facilitatory effect of DPCPX on the frequency of GDPs was 11.9 nM (n = 25; Fig. 3B). These data indicate that endogenous adenosine by A₁ receptor subtypes contributes to regulation of GDPs.

To further evaluate the contribution of endogenous adenosine to the generation of GDPs we increased the extracellular level of endogenous adenosine by applying in the bath NBTI, which inhibits adenosine transport (de Mendonça and Ribeiro 1994; Fredholm et al. 1994; Ribeiro et al. 2003a,b), or EHNA that blocks adenosine deaminase, the enzyme that converts adenosine into the inactive metabolite inosine (Ribeiro et al. 2003a,b). NBTI, at the concentration of 20 μM, significantly decreased the frequency of GDPs from 0.07 ± 0.004 to 0.045 ± 0.002 Hz (n = 5, P < 0.01; Fig. 4A and C), in the absence of any change in GDP amplitude or shape. This effect was prevented by DPCPX (100 nM; n = 3), implying that it was mediated by adenosine acting on A₁ receptor subtypes (Fig. 4A). Similar results were obtained with EHNA (10 μM). The adenosine deaminase inhibitor reduced the frequency of GDPs was from 0.07 ± 0.002 to 0.003 ± 0.002 Hz (n = 3, P < 0.001; Fig. 4, B and C). As for NBTI, the effect of EHNA was prevented by DPCPX (100 nM; n = 4; Fig. 4B). These data
further support the idea that endogenous adenosine contributes to control the generation of GDPs.

In CA3 principal cells, NBTH and EHNA did not modify spontaneous synaptic events that at this developmental stage are mainly GABAergic mediated (see following text). The frequency of sPSPs was 4.3 ± 0.2 and 4.4 ± 0.02 Hz before and after NBTH, respectively, whereas it was 5.8 ± 0.3 and 5.7 ± 0.2 Hz before and after EHNA, respectively (data not shown).

GDPs are generated by the synergistic action of glutamate and GABA on AMPA and GABA_4 receptors, respectively (Bolea et al. 1999). Therefore they are readily blocked either by DNQX or bicuculline (Ben-Ari et al. 1989; Bolea et al. 1999).

In agreement with a previous report (Safullina et al. 2005) DPCPX did not modify the frequency or amplitude of sPSPs in principal cells (the frequency of sPSPs was 5.5 ± 0.7 and 5.4 ± 0.9 Hz, whereas the amplitude was 3.9 ± 0.4 and 3.8 ± 0.5 mV before and during DPCPX, respectively; n = 3). In our experimental conditions (symmetrical chloride solutions) sPSPs recorded at −60 mV from principal cells can be attributed to the action of GABA and glutamate acting on GABA_4 and AMPA/kainate receptors, respectively. Therefore to see whether endogenous adenosine regulates glutamate release into principal cells we blocked GABA_4 receptors with picrotoxin. However, in keeping with the late development of glutamatergic connections (Ben-Ari et al. 1989; Hennou et al. 2002; Hosokawa et al. 1994; Tyzio et al. 1999) in the presence of picrotoxin (100 μM) glutamatergic events were merely detectable, suggesting that during the first postnatal week, spontaneous activity of principal cells is mainly explained by GABA acting on GABA_4 receptors. DPCPX applied in the presence of DNQX (10 μM) failed to modify spontaneous GABA-mediated synaptic activity (the frequency of spontaneous events was 4.7 ± 0.8 and 4.8 ± 0.9 Hz in the presence or absence of 100 nM DPCPX; n = 3; P > 0.05; data not shown). These data suggest that, in control conditions, endogenous adenosine exerts its action on GDPs through glutamatergic terminals localized downstream of principal cells.

**Endogenous adenosine affects the glutamatergic drive to GABAergic interneurons**

To identify at the network level where the action of adenosine originated from, in additional experiments (n = 12) direct recordings from GABAergic interneurons were performed. Interneurons were localized on stratum radiatum, in close vicinity to stratum lucidum. They had a resting membrane potential ranging from −44 to −70 mV (on average −54.7 ± 2.4 mV) and input resistance ranging from 129 to 400 MΩ (on average 293 ± 23 MΩ). They discharged at high frequency (63 ± 7 Hz) in response to long depolarizing current pulses (100 pA, 400 ms; see also Safullina et al. 2005). In these cells, GABAergic and glutamatergic activities were studied in isolation in the presence of bicuculline (20 μM) and DNQX (20 μM), respectively. DPCPX, even at high concentration (10 μM), did not modify the frequency or the amplitude of GABAergic sPSPs recorded in the presence of DNQX. In three interneurons the frequency of sPSPs was 8.5 ± 1.3 and 8.2 ± 1.2 Hz, whereas the amplitude of sPSPs was 5.9 ± 0.9 and 4.8 ± 0.4 mV, in control and in the presence of DPCPX, respectively (P > 0.05).

In contrast, DPCPX (100 nM) significantly increased the frequency (but not the amplitude) of glutamatergic sPSPs recorded in the presence of bicuculline (10 μM) from 2.5 ± 0.01 to 3.7 ± 0.3 Hz; (n = 3; P < 0.05; Fig. 5, A, B, and E). DPCPX did not modify the membrane potential or the input resistance of the recorded neurons. Consistent with the expression of presynaptic A1 receptors localized on glutamatergic terminals making synapses on GABAergic interneurons, exogenous application of adenosine (10 μM) in the presence of bicuculline (10 μM) reversibly reduced the frequency (from 1.8 ± 0.06 to 0.9 ± 0.1 Hz; n = 3, P < 0.05) but not the amplitude (1.4 ± 0.3 and 0.9 ± 0.06 mV, before and after adenosine, respectively; P > 0.05) of spontaneous glutamatergic events (Fig. 5, C, D, and E). These effects occurred in the absence of any change in membrane potential and input conductance.

**Endogenous adenosine affects the release of glutamate from recurrent collaterals of CA3 principal cells**

In a recent work it has been shown that a prolonged exposure of hippocampal slices from P2–P6 rats to GABA_4 antagonists produces low-frequency interictal-like discharges that involve recurrent glutamatergic collaterals (Khazipov et al. 2004). To see whether endogenous adenosine can modulate interictal activity generated by glutamatergic recurrent collaterals in the absence of GABA_4-mediated inhibition, bicuculline (20 μM) was applied for 10–15 min. In these conditions, interictal events developed at 0.02 ± 0.004 Hz (n = 9). Further application of DPCPX (100 nM) increased the frequency of interictal bursts from 0.027 ± 0.001 to 0.04 ± 0.01 Hz (P < 0.05; n = 3; Fig. 6, B and C). In spite of their increase in frequency, the shape and the area under GDPs was unchanged (9.4 ± 2.1 and 7.1 ± 1.2 mV s⁻¹, P > 0.05 before and during DPCPX application). This indicates that A1 receptors localized on recurrent glutamatergic collaterals are involved in adenosine action. Furthermore, application of adenosine (10 μM; n = 3) completely blocked bicuculline-induced interictal bursts, an effect that was associated with a membrane hyperpolarization (5.5 ± 0.5 mV; Fig. 6A) and an increase in input conductance (8 ± 2%, P < 0.05).

**DISCUSSION**

The present experiments clearly show that early in postnatal life, endogenous adenosine by A1 receptor subtypes, localized on glutamatergic terminals projecting mainly to GABAergic interneurons, exerts a powerful inhibitory action on network-driven oscillatory events such as GDPs. GDPs represent a hallmark of developmental networks (Ben-Ari 2002), which, as already mentioned, are generated by the neurotransmitter GABA (that at early stages of development is depolarizing and excitatory) and to a lesser extent by the excitatory transmitter glutamate (Ben-Ari et al. 1989). Our data suggest that GDPs could be completely blocked by low micromolar concentrations of adenosine, which specifically switches off only glutamatergic inputs, indicating a key role of the latter in the induction of GDPs.

The transient expression of GDPs suggests that these membrane oscillations exert a functional role for only a limited period of time during postnatal development, acting as coinci-
dent detection signals for increasing synaptic efficacy (Kas-
yanov et al. 2004). Coincidence detection would provide a
combinatorial code by which to achieve outcome that cannot
be generated by isolated stimuli (Spitzer 2004). Therefore early
membrane oscillations such as GDPs may be crucial for setting
the appropriate synaptic connections that lead to the establish-
ment of the adult hippocampal circuit. A number of neuro-
transmitters and neuromodulators whose extracellular levels
are maintained in an activity-dependent manner regulate net-
work activity. Recently, we have demonstrated that ATP
down-regulates GDPs and differently affects the release of
 glutamate and GABA within the CA3 network (Safiulina et al.
2005). In turn, GDPs would control the concentration of
endogenous ATP at synapses. In the present experiments
DPCPX-sensitive changes in ATP-induced reduction of the
frequency of GDPs demonstrate that this nucleotide exerts its
action not only through P2 but also by P1 receptors activated
by the ATP breakdown product adenosine (Cunha et al. 1998;
Dunwiddie et al. 1997). Interestingly, ATP also induced an
increase of sPSPs that was unrelated to the activation of
adenosine receptors because it persisted in the presence of
DPCPX. In accord with a previous report (Safiulina et al. 2005)
ATP-induced increase in the frequency of sPSPs was demon-
strated to be dependent on the activation of presynaptic P2Y1
receptors present on GABAergic terminals.

Another potential source of endogenous adenosine is cyclic
AMP (cAMP), which can be released from neurons and con-
verted by extracellular phosphodiesterases into AMP and
thereafter by an ecto-5'-nucleotidase to adenosine. Evidence
that this pathway is functional in the hippocampus has been
provided (Dunwiddie et al. 1997). In particular, early in post-
natal development, cAMP can be produced after activation of
metabotropic glutamate receptors by endogenously released
 glutamate (Strata et al. 1995). cAMP can exert opposite effects
on GDPs: it may up-regulate the frequency of GDPs by directly
enhancing GABA release from presynaptic nerve terminals
(Strata et al. 1995) and/or may depress GDPs by indirectly
interfering with glutamate release after having been converted
into adenosine. However, to reach a significant level, multiple
cells must release cAMP over a prolonged period of time
(Brundege et al. 1997).

On the basis of their molecular structures four different
adenosine P1 receptors have been characterized: A1, A2A, A2B,
and A3 (Ralevic and Burnstock 1998). These receptors, which
belong to the G-protein–coupled receptor family, are differen-
tially distributed in the CNS where they exert different phys-

FIG. 5. Endogenous adenosine depresses the glutamatergic drive to GABAergic inter-
neurons. A: glutamatergic sPSPs recorded from a GABAergic interneuron in the presence
of bicuculline (20 μM) in control (top) and during application of DPCPX (100 nM, bot-
tom). Note that DPCPX increased the fre-
quency of glutamatergic events. B: cumulative
distributions of interevent intervals (left) and
amplitude (right) of glutamatergic sPSPs be-
fore (continuous line) and during (dashed line)
application of DPCPX for the cell shown in A.
Note that DPCPX increased the frequency but
not the amplitude of glutamatergic events. C:
 glutamatergic sPSPs in control (top) and dur-
ing application of adenosine (bottom). D: cu-
mulative distributions of inter event intervals
(left) and amplitude (right) of glutamatergic
sPSPs before (continuous line) and during
(dashed line) application of adenosine for the
cell shown in A. Adenosine produced strong
inhibition of the frequency but did not change
the amplitude of glutamatergic events. E: each
column represents the mean frequency of
sPSPs (as percentage of control), obtained in
DPCPX (100 nM, n = 3) and adenosine (10 μM); F: each column represents the mean
amplitude of sPSPs (as percentage of control),
obtained in DPCPX (100 nM, n = 3) and
adenosine (10 μM, n = 3), **P < 0.01.
Adenosine (10 μM) reduced the frequency of interictal discharges, indicating activation of A1 receptors localized on recurrent collaterals of CA3 pyramidal cells. Interictal bursts marked with a and b are shown in an expanded timescale. C: each column shows the mean frequency of interictal discharges in control and in the presence of DPCPX (100 nM, n = 3). *P < 0.05.

Although at least in the cerebral cortex of immature animals [3H] DPCPX binding sites represent only 23% of those found in the adult brain, the present results clearly show that in the hippocampus A1 receptors are present and already functional in the adult brain, the present data clearly show that in the immature hippocampus adenosine affected only glutamatergic synaptic transmission, as suggested by the reduction in frequency of AMPA/kainate-mediated sPSPs in GABAergic interneurons. Therefore by modulating the glutamatergic drive to GABAergic interneurons adenosine exerts a powerful control on the generation of GDPs. Endogenously released adenosine also produced a strong inhibitory effect on recurrent glutamatergic synapses in the CA3 area, thought to be critical for generating bursting activity (Miles and Wong 1983), as indicated by the reduction of bicuculline-induced interictal discharges. In line with the powerful anticonvulsant action of purinoceptor agonists (Dunwiddie 1999), a potent inhibitory role of adenosine on bicuculline-induced bursting activity in adult hippocampal slices has been described (Ault and Wang 1986). As for adenosine-induced down-regulation of AMPA/kainate sPSPs, adenosine-induced depression of interictal discharges observed after block of GABA<sub>A</sub>-mediated inhibition can be attributed to a reduction of calcium entry through a decrease or an increase in calcium and potassium conductances present on glutamatergic nerve terminals, respectively (Trussell and Jackson 1987).

The concentration of endogenous adenosine produced during GDPs is unknown. However, the high sensitivity of GDPs to submicromolar concentrations of exogenous adenosine (IC<sub>50</sub> = 0.52 μM) together with the strong facilitatory effect of DPCPX suggests that the concentration of endogenous adenosine is probably in the range of the IC<sub>50</sub> value and perhaps could be increased during large depolarizations such as GDPs. In keeping with this, raising the extracellular concentration of adenosine with the adenosine transport inhibitor NBTI or with the adenosine deaminase blocker EHNA (Ribeiro et al. 2003b) induced a reduction in frequency of GDPs, thus providing further evidence that endogenous adenosine exerts a powerful control on the generation of GDPs.

In previous work, using a biochemical assay we noticed that the concentration of ATP present in the extracellular space diminished significantly in the presence of TTX, implying that ATP was released in an activity-dependent manner. Because adenosine is formed as the breakdown product of ATP from nerves, this implies that the adenosine concentration is crucially linked to the ongoing neuronal activity. Thus an increase in the frequency of GDPs may lead to the production of ATP and adenosine, which in turn may exert a negative feedback on GDPs by high-affinity presynaptic A<sub>1</sub> receptors.

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REFERENCES


