Activity-Dependent Release of Adenosine Contributes to Short-Term Depression at CA3-CA1 Synapses in Rat Hippocampus

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Brager, Darrin H. and Scott M. Thompson. Activity-dependent release of adenosine contributes to short-term depression at CA3-CA1 synapses in rat hippocampus. J Neurophysiol 89: 22–26, 2003. 10.1152/jn.00554.2002. High-frequency stimulation results in a transient, presynaptically mediated decrease in synaptic efficacy called short-term depression (STD). Stimulation of Schaffer-collateral axons at 10 Hz for 5 s resulted in approximately 75% depression of excitatory postsynaptic current (EPSC) slope recorded from CA1 cells in rat organotypic slice cultures. An adenosine A1 receptor antagonist decreased the magnitude of STD elicited with 10-Hz stimulation by approximately 30%. The A1 receptor antagonist had no effect on STD elicited with 3-Hz stimulation. The activation of A1 receptors during 10-Hz stimulation was not due to the extracellular conversion of released ATP to adenosine, because block of 5'-ectonucleotidases did not significantly affect STD. The adenosine transport inhibitor dipyridamole did not reduce STD, indicating that adenosine was not released by facilitated transport. We conclude that 10-Hz, but not 3-Hz, stimulation causes the vesicular release of adenosine and the rapid (<3 s) activation of presynaptic inhibitory A1 receptors, which account for approximately 40% of homosynaptic EPSC depression.

INTRODUCTION

Presynaptic inhibitory receptors for a number of neuromodulatory factors regulate fast excitatory synaptic transmission in the CNS. The physiological conditions under which these receptors become activated are poorly defined.

Tetanic stimulation causes release of two modulators of glutamatergic synaptic transmission, adenosine and adenosine nucleotides (e.g., McIlwain 1972; Mitchell et al. 1993; Wieraszko et al. 1989). Activation of A1 receptors with exogenous adenosine depresses excitatory synaptic transmission and activates a postsynaptic K+ conductance in hippocampus (e.g., Greene and Haas 1991; Thompson et al. 1992, 1993; Wu and Saggau 1997). Adenine nucleotides also exert pre- and postsynaptic effects in hippocampus, mediated by their rapid extracellular conversion to adenosine, catalyzed by 5'-ectonucleotidases (Dunwiddie et al. 1997).

There are few examples of endogenously released purines affecting synaptic transmission under physiological conditions. At the frog neuromuscular junction, activation of presynaptic adenosine receptors, as the result of adenosine nucleotide release and subsequent conversion to adenosine, underlies short-term homosynaptic depression at low stimulation frequencies (Redman and Silinsky 1994). At hippocampal Schaffer collateral-CA1 cell synapses, adenosine mediates heterosynaptic depression induced with 100-Hz stimulation for 0.3–1 s (Manzoni et al. 1994; Mitchell et al. 1993). There are several potential sources of endogenous adenosine, including glia (Caciagli et al. 1988), interneurons (Manzoni et al. 1994), and pyramidal cells (Brundege and Dunwiddie 1996). Adenosine release from glia and pyramidal cells under certain conditions is not vesicular, but rather, it is mediated by facilitated transport (Brundege and Dunwiddie 1996; Caciagli et al. 1988).

In hippocampus, excitatory synaptic transmission becomes transiently depressed when presynaptic inputs are stimulated at frequencies >1 Hz. We report here that activation of presynaptic inhibitory adenosine receptors contributes to this depression, and we examine the mechanisms underlying this process.

METHODS

Preparation of organotypic hippocampal slice cultures

Organotypic hippocampal slice cultures are ideal for studying the activity-dependent release of adenosine because there is no constitutive activation of pre- or postsynaptic A1 receptors (Thompson et al. 1992). As described previously (Gähwiler et al. 1998), hippocampi were dissected from CO2-anesthetized 5- to 6-day-old rat pups and cut into 375-μm transverse slices with a McIlwain tissue chopper. Slices were attached to glass coverslips by a chicken plasma clot, placed into culture tubes with serum-containing media, and incubated in a rollerdrum at 36°C for 14 days. The University of Maryland School of Medicine IACUC approved this protocol.

Electrophysiology

 Cultures were placed in a recording chamber and perfused with extracellular saline containing (in mM) 137 NaCl, 2.8 KCl, 2.5 CaCl2, 2.5 MgCl2, 11.6 NaHCO3, 0.4 NaH2PO4, and 5.6 glucose at approximately 1 ml/min. Extracellular stimuli (~20 to ~50 μA for 20–100 μs) were delivered in stratum radiatum at the border between area CA3 and CA1 using a 2-MΩ patch pipette filled with extracellular saline. Postsynaptic CA1 cells were voltage clamped at ~75 mV using whole cell recording techniques. Excitatory postsynaptic currents (EPSCs) were low-pass filtered at 2 kHz and digitized at 10 kHz using an Axopatch 200B amplifier and Clampex 7 software (Axon Instruments). Patch pipettes were filled with (in mM) 140 KF, 10 KCl, 0.4 HEPES, 2 MgCl2, and 1.1 EGTA (pH 7.2). The presence of fluoride ions in the pipette solution blocked GABAergic inhibitory currents in the recorded cell (Nelson et al. 1994). N-methyl-D-aspar-
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A$_1$ receptor activation during high-frequency stimulation

During 10-Hz stimulation for 5 s, EPSC slope decreased progressively before reaching a minimum of 26 ± 4% (n = 10) of control slope (Fig. 1A). STD was accompanied by a significant increase in paired-pulse ratio (PPR) and a significant decrease in the inverse square at the coefficient of variation (CV$^{-2}$) (Brager et al. 2002), indicating that a decrease in the probability of neurotransmitter release underlies STD.

Tetanic stimulation causes elevations of extracellular GABA and adenosine, both of which are potent agonists at presynaptic inhibitory receptors on glutamatergic nerve terminals (Thompson et al. 1993; Wu and Saggau 1997). We therefore tested the hypothesis that activation of presynaptic inhibitory receptors contributes to STD. The depression of EPSCs was first compared before and after blocking adenosine A$_1$ receptors with 330 nM DPCPX (Thompson et al. 1992). In acute hippocampal slices, A$_1$ receptor antagonists increase release probability as measured by an increase in synaptic responses and a decrease in PPR (Costenla et al. 1999; Dunwiddie et al. 1981). In hippocampal slice cultures, however, application of DPCPX did not significantly affect EPSC slope (control: 26.5 ± 4.1 pA/ms vs. DPCPX: 27.2 ± 3.4 pA/ms) or PPR (1.0 ± 0.1 vs. 1.1 ± 0.1; n = 5). DPCPX reduced EPSC depression significantly relative to vehicle controls (control: 19 ± 3% vs. DPCPX: 44 ± 7%; P < 0.05; Fig. 1B). We next tested whether GABA$_B_2$ receptor activation also contributes to STD by blocking GABA$_B_2$ receptors with 2 μM CGP 52432, a concentration sufficient to block the inhibition of EPSCs by 10 μM baclofen (data not shown). Application of CGP 52432 did not significantly affect EPSC slope (control: 63.2 ± 14.1 pA/ms vs. CGP: 55.5 ± 12.7 pA/ms) or PPR (1.0 ± 0.1 vs. 1.0 ± 0.3 pA/ms; n = 5). CGP 52432 had no significant effect on the depression of EPSCs (control: 33 ± 6% vs. CGP: 38 ± 6%). We conclude that the activation of adenosine, but not GABA$_B_2$, receptors occurs during 10-Hz stimulation and contributes to the depression of EPSCs. To be certain that a GABA$_B_2$-sensitive component was not revealed after block of A$_1$ receptors, we examined the effect of blocking A$_1$ and GABA$_B_2$ receptors simultaneously. We found that the depression of EPSCs in the presence of both DPCPX and CGP 52432 (43 ± 9%; n = 3) was not significantly different from DPCPX alone (44 ± 7%; n = 5).

Are the critical adenosine receptors pre- or postsynaptic? Pretreatment of slice cultures with pertussis toxin prevented the ability of adenosine, acting at postsynaptic A$_1$ receptors, to elicit an outward current in CA1 cells (Thompson et al. 1992), without affecting its ability to inhibit EPSCs by acting at presynaptic A$_1$ receptors (data not shown). There was no significant difference in the depression of EPSCs in response to the 10-Hz stimulus train between untreated and pertussis toxin-treated cultures (control: 26 ± 4% vs. pertussis: 25 ± 4%, n = 3 cells in 3 cultures).
We next asked whether lower frequencies of stimulation would also produce significant adenosine receptor activation. DPCPX had no significant effect on the depression of EPSCs during 3-Hz stimulation (control: 64 ± 6% vs. DPCPX: 66 ± 10%; n = 5; Fig. 1C). We conclude that stimulation frequencies > 3 Hz are required for activation of presynaptic inhibitory A₁ receptors.

A₁ receptor activation is due to adenosine release

At the frog neuromuscular junction, STD is blocked by A₁ antagonists and mediated by the activity-dependent release of ATP and its extracellular conversion to adenosine (Redman and Silinsky 1994). This process could also contribute to STD in our experiments because Schaffer collateral stimulation can cause the release of ATP (Wieraszko et al. 1989). To distinguish between this possibility and the release of adenosine itself during hippocampal STD, we examined the effects of blocking 5'-ectonucleotidases with α,β methylene-ADP (α,β-ADP, 500 μM)(Dunwiddie et al. 1997; Redman and Silinsky 1994). As a positive control for the effectiveness of the α,β-ADP, we first examined the inhibition of EPSCs by ATP (50 μM). ATP potently inhibited EPSCs, and this inhibition was prevented by both 500 μM α,β-ADP, indicating that ATP is converted to adenosine by a 5'-ectonucleotidase, and by DPCPX, indicating that it acted at A₁ receptors (Fig. 2A), as shown previously (Cunha et al. 1998; Dunwiddie et al. 1997). α,β-ADP had no significant effect on the depression of EPSCs (control: 22 ± 9% vs. α,β-ADP: 29 ± 11%; n = 7) during 10-Hz stimulation (Fig. 2B). We therefore conclude that 10-Hz stimulation results in the release of adenosine and the subsequent activation of presynaptic adenosine A₁ receptors.

Brundege and Dunwiddie (1996) demonstrated that hippocampal pyramidal cells are capable of releasing adenosine via facilitated membrane transport. To determine if the adenosine released during 10-Hz stimulation occurs via facilitated transport, we examined the effect of the broad spectrum adenosine transport inhibitor dipyrudamole (Dunwiddie and Diao 2000). Dipyrudamole (10 μM) did not significantly reduce the depression of EPSCs induced by 10-Hz stimulation (control: 23 ± 6% vs. dipyrudamole: 17 ± 8%; n = 4). We conclude that adenosine is not released via facilitated transport during 10-Hz stimulation.

To better describe the contribution of adenosine activation to the overall depression during a 10-Hz stimulus train, we subtracted the DPCPX data set from the matched vehicle control data set (Fig. 3). These data show that adenosine receptor activation is responsible for a 30% decrease in EPSC slope, corresponding to 38% of the total depression, during 10-Hz stimulation. Furthermore, the subtracted data reveal that adenosine-mediated inhibition reaches maximum within 3 s.

DISCUSSION

We found that an adenosine A₁ receptor antagonist attenuated STD of neurotransmitter release in rat hippocampal slice cultures elicited with stimulation at 10 Hz. Stimulation at 3 Hz does not cause activation of presynaptic A₁ receptors because application of DPCPX had no effect on EPSC depression. STD was not affected by selective prevention of postsynaptic adenosine receptor activation with pertussis toxin treatment. We therefore conclude that a rapid decrease in release probability due to the activation of presynaptic A₁ receptors contributes to STD.

FIG. 2. Ten-hertz stimulation results in the release of adenosine. A: application of 50 μM ATP (——) reversibly inhibits EPSCs (●). Application of 330 nM DPCPX (▲) or 500 μM α,β methylene-ADP (○) completely blocked the inhibition of EPSCs by ATP. Data are normalized to the mean EPSC slope prior to the 1st application of ATP in each condition. B: STD in response to 10-Hz stimulation before (●) and after (○) application of 500 μM α,β methylene-ADP (n = 7).

FIG. 3. The contribution of A₁ receptor activation to STD. The percent depression of EPSC slope before application of DPCPX (●) and the contribution of adenosine receptor activation (○), calculated by subtracting the percent EPSC depression in DPCPX from the control depression, are plotted as a function of time during a 10-Hz, 5-s stimulus train. Both data sets were well fit with a single exponential with a time constant of 1.1 s.
We found that a GABA_B receptor antagonist had no effect on EPSC slope, PPR, or STD of EPSCs. In contrast, even a single stimulus can release sufficient GABA to act on nearby presynaptic GABA_B autoreceptors at interneuron–pyramidal cell synapses (e.g., Davies et al. 1990). Presumably, insufficient GABA reaches presynaptic GABA_B receptors at excitatory synapses even with repetitive stimulation.

An inhibitor of 5′-ectonucleotidase, the extracellular enzyme that converts adenine nucleotides to adenosine (Dunwiddie et al. 1997), prevented exogenous ATP from inhibiting EPSCs, but did not affect STD. We therefore conclude that 10-Hz stimulation causes the release of adenosine itself, rather than an adenine nucleotide precursor. In contrast, homosynaptic depression at the frog neuromuscular junction results from the release of adenine nucleotides that are then converted to adenosine by a 5′-ectonucleotidase (Redman and Silinsky 1994).

What is the source of the adenosine in our experiments? Adenosine can be released via facilitated transport from hippocampal pyramidal cells (Brundege and Dunwiddie 1996; Jonzon and Fredholm 1985) and/or glial cells (Caciagli et al. 1988). An antagonist of adenosine transport did not reduce homosynaptic depression in our experiments, however, consistent with earlier studies of hippocampal heterosynaptic depression (Mitchell et al. 1993). Although our evidence is indirect and derived by excluding alternative hypotheses, taken with previous demonstrations of Ca^2+-dependent adenosine release (e.g., Manzoni et al. 1994; Pull and McIlwain 1973), we suggest that high-frequency stimulation causes the exocytosis of adenosine-containing vesicles, possibly as a co-transmitter. Ca^2+-dependent release of adenosine from hippocampal interneurons requires NMDA receptor activation (Manzoni et al. 1994). The presence of AP5 in our experiments suggests that there are either multiple means by which synaptic activity triggers the Ca^2+-dependent release of adenosine or that high-frequency stimulation can result in release of adenosine from pyramidal cells.

Our data revealed that presynaptic inhibition mediated by adenosine accounts for 38% of the EPSC depression elicited with 10-Hz stimulation and that this effect occurs within approximately 1 s (see also Mitchell et al. 1993). The EPSC depression that remains in the presence of DPCPX presumably results from the depletion of readily releasable synaptic vesicles (e.g., Brager et al. 2002; Dobrunz and Stevens 1997; Wu and Borst 1999). At other CNS synapses (e.g., Motley and Collins 1983; von Gersdorff et al. 1997), presynaptic inhibitory receptors also participate in homosynaptic STD, but their contribution is considerably smaller than the effect of adenosine reported here. We demonstrated previously that excitatory synaptic transmission recovers exponentially after STD and that this recovery is insensitive to DPCPX (Brager et al. 2002). These results suggest that the inhibitory action of A_1 receptors has both a rapid onset and termination.

In conclusion, short-term homosynaptic depression reflects processes that depend not only on the activity of individual nerve terminals, such as vesicle depletion, but also on activity in populations of cells, such as the release of adenosine. Although the importance of adenosine release in ischemic/hypoxic insults has previously been emphasized (e.g., Fowler 1990; Gribkoff et al. 1990), our results indicate that adenosine can also mediate a rapid modulation of excitatory synaptic transmission at physiological levels of neuronal activity.

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REFERENCES


