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ADENOSINE ACTIONS ON CA1 PYRAMIDAL NEURONES IN RAT HIPPOCAMPAL SLICES

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SUMMARY

1. Intracellular recordings with a bridge amplifier of CA1 pyramidal neurones *in vitro* were employed to study the mechanisms of action of exogenously applied adenosine in the hippocampal slice preparation of the rat.

2. Adenosine enhanced the calcium-dependent, long-duration after-hyperpolarization (a.h.p.) at least in part by a reduction in the rate of decay of the a.h.p. Both the reduced rate of decay and that of the control can be described with a single exponential.

3. Antagonism of the calcium-dependent potassium current (and as a result, the a.h.p.) by bath application of $CdCl_2$ or intracellular injection of EGTA (ethyleneglycol-bis-(β-aminoethyl ether)*N*,*N*′-tetraacetic acid) did not reduce the adenosine-evoked hyperpolarization or decrease in input resistance. Similarly, TEA (tetraethylammonium), which antagonizes both the voltage- and calcium-sensitive, delayed, outward rectification, had no effect on the adenosine-evoked changes in resting membrane properties.

4. Adenosine did not affect the early, transient, outward rectification. During exposure to 4-aminopyridine (4-AP) in concentrations sufficient to antagonize this early rectification, the changes in resting membrane properties evoked by adenosine were unaffected.

5. We conclude that the enhancement of the a.h.p. and accommodation by adenosine may be mediated by a change in the regulation of intracellular calcium. However, the mechanism responsible for the hyperpolarization and decrease in input resistance evoked by adenosine is both calcium and voltage insensitive. Thus, it appears distinct from that mediating the enhancement of the a.h.p. and accommodation.

INTRODUCTION

A variety of evidence suggests that adenosine has a neuromodulatory role in the mammalian central nervous system (Sattin & Rall, 1970; Shimizu & Daly, 1970; Phillis, Kostopoulos & Limacher, 1975) which could provide a means of interaction between metabolic state (Pull & McIlwain, 1972*a*, *b*; Berne, Winn & Rubio, 1982) and electrophysiological activity (Scholfield, 1978; Dunwiddie & Hoffer, 1980; Haas

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& Greene, 1984; Greene, Haas & Hermann, 1985). Adenosine can depress the spontaneous activity of neurones from many different areas of the C.N.S. (Kostopoulos & Phillis, 1977) as well as evoked synaptic activity in both peripheral nervous systems (Burnstock, 1975), and in brain slices of olfactory (Kuroda, Saito & Kobayashi, 1976; Scholfield, 1978) and hippocampal (Schubert & Mitzdorf, 1979; Okada & Ozawa, 1980; Dunwiddie & Hoffer, 1980) cortex. At the post-synaptic sites of hippocampal neurones, the mechanism for this depressant action is an increase in membrane potential accompanied by a decrease in membrane resistance (resting membrane properties), which is likely to result from an increase in potassium conductance (Segal, 1982). Recently, adenosine has also been shown to enhance accommodation and the long-duration after-hyperpolarization (a.h.p.) in hippocampal neurones (Haas & Greene, 1984). There is evidence that both these phenomena are mediated for the most part by an increase in calcium-dependent potassium conductance (Brown & Griffith, 1983; Madison & Nicoll, 1984). However, the mechanism for this presumed increase in potassium conductance evoked by adenosine is unclear, nor is it known if this mechanism is related to that responsible for the adenosine-induced hyperpolarization and conductance increase. These particular concerns are reflective of the more general question of the relationship between potassium conductances sensitive to putative neurotransmitters and those sensitive to voltage and/or calcium.

In the present study, we have extended the analysis of the effect of adenosine on the a.h.p., input resistance, and membrane potential. The sensitivities to voltage, calcium, and potassium conductance antagonists of the latter two adenosine effects were examined.

METHODS

Transverse slices were cut, 450 μm thick, with a McIlwain tissue chopper from the hippocampi of twenty-six young adult Wistar rats and transferred to a triple perfusion chamber where they were kept completely submerged. This chamber was modified from our original design (Haas, Schaerer & Vomansky, 1979), so that each of the three compartments had a volume of 50 μl and was independently perfusable. The solutions were maintained at 30 °C, and the flow rate was 0·3–0·5 ml/min in each compartment.

The medium contained (in mM): NaCl, 124; KCl, 2; $MgSO_4$, 1·3; $CaCl_2$, 2·5; KH_2PO_4, 1·25; $NaHCO_3$, 26; glucose, 10; at pH 7·4. Tetrodotoxin (TTX) 0·003 mM, tetraethylammonium (TEA) 10 mM, 4-aminopyridine (4-AP) 0·1 mM, and adenosine were added as indicated in the text.

Recording and current injection, with glass micropipettes containing 2 M-KCl, were performed using a high input impedance pre-amplifier and bridge circuit (WPI 707). Signals were amplified, photographed from a storage oscilloscope and stored on an FM tape recorder. Membrane potential was plotted continuously on a chart recorder. Measurements, unless otherwise noted, are given as the mean ±S.D.

In one set of experiments, the normal KCl solution in the recording electrode was replaced with one containing 2 M-K acetate and 0·5–1·0 M-ethyleneglycol-bis-(β-aminoethyl ether)N,N'-tetraacetic acid (EGTA), so that the intracellular calcium concentration could be maintained at low levels. To avoid possible interference with potassium conductances by hydrogen ions, the EGTA solution was titrated to a pH of 7·2–7·3 with KOH, which had the added advantage of increasing the solubility of the EGTA.

RESULTS

Data for this study were gathered from intracellular recordings of fifty neurones in the CA1 region of the hippocampus which remained healthy and stable for 30 min or longer. This was determined by a resting potential and input impedance of greater

than 60 mV (average, 67·5 ± 4·7 mV) and 40 MΩ (average, 63·1 ± 14·8 MΩ) respectively. Action potential overshoot was at least 10 mV.

Enhancement of the a.h.p. Adenosine at low concentrations (less than 50 μM) enhances the a.h.p. following a burst of action potentials in hippocampal CA1 neurones with little or no effect on the resting membrane properties (Haas & Greene, 1984). At higher concentrations, the a.h.p. was often reduced, an effect always associated with a decrease in input resistance of 9–40 % ($n = 17$).

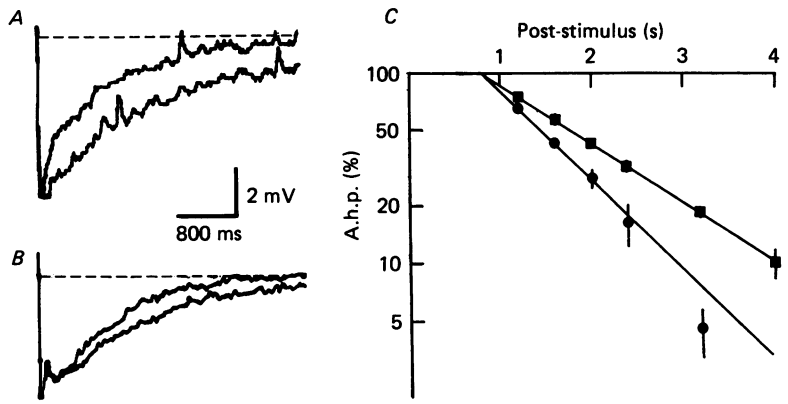

Fig. 1. Adenosine decreases the rate of return of the long-duration a.h.p. to resting membrane potential. *A* and *B* show two averages of ten a.h.p.s each, recorded before (the smaller) and during exposure to adenosine (40 μM). The averages begin at the cessation of an intracellularly applied depolarizing current pulse which evoked five action potentials. The horizontal dashed line indicates resting membrane potential. *C* is a semilog graph of the percentage amplitude of the a.h.p. *versus* time. Each data point reflects the pooled results of averaged a.h.p.s from four neurones and the vertical bars represent 1 S.D. ● symbolize data gathered prior to, and ■, during exposure to adenosine.

The time course of the decay of the long-duration a.h.p. appears to reflect the return to base line of the internal calcium concentration in the neuronal preparations where this was examined (Thomas & Gorman, 1977; Smith, McDermott & Weight, 1983). Because the enhancement of the a.h.p. by adenosine might involve altered calcium metabolism, the decay of a.h.p.s recorded before and during the perfusion of 20–50 μM-adenosine ($n = 4$) were compared (Fig. 1). The a.h.p.s were evoked by five action potentials which resulted from intracellularly applied depolarizing current pulse of 80 ms duration. Recordings of ten or more a.h.p.s were averaged for each analysis of the time course. In normal media the decay of the a.h.p. was exponential with respect to time and yielded a time constant of 841 ± 80 ms. In the presence of adenosine the time constant was significantly increased to 1345 ± 118 ms ($P = 0·05$; Wilcoxon test).

Calcium dependence. The changes in resting membrane properties evoked by adenosine were investigated for their sensitivity to either an influx of calcium across the membrane or an increase in intracellular calcium concentration. With respect to the former, $CdCl_2$ (100–300 μM) was added to the perfusion media to antagonize calcium current (Hagiwara & Byerly, 1981). As reported (Madison & Nicoll, 1984),

the $CdCl_2$ blocked both accommodation to depolarizing current injection and the long-duration a.h.p. The spontaneous synaptic activity was also abolished (Fig. 2). Nevertheless, there was no antagonism by $CdCl_2$ on the effects of bath-applied adenosine (100 μM; $n = 8$). Intracellular injection of EGTA can prevent an increase in intracellular calcium concentration which normally accompanies a burst of action potentials (Ahmed & Connor, 1979). It can also antagonize the associated long-duration a.h.p. (Alger & Nicoll, 1980; Schwartzkroin & Stafstrom, 1980; Hablitz, 1981) and accommodation (Madison & Nicoll, 1984) in the hippocampus, presumably by binding intracellular calcium.

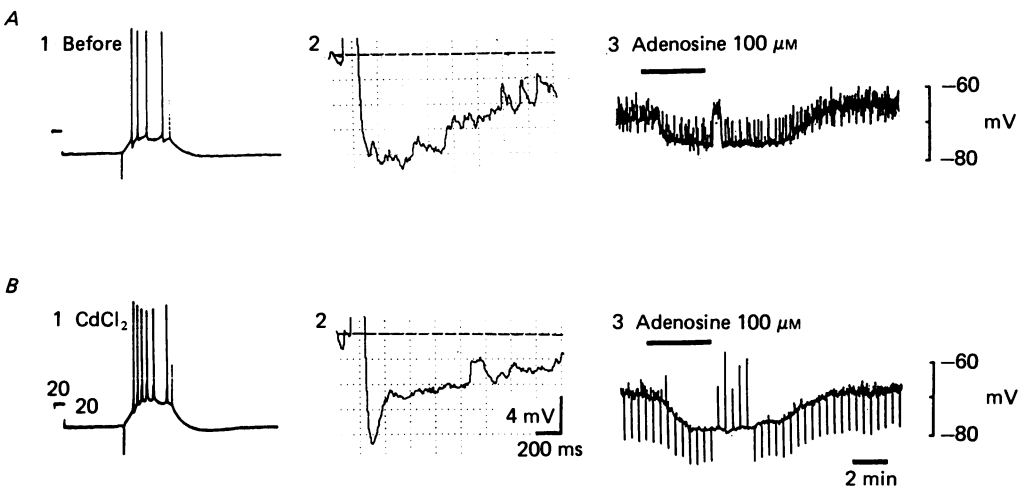

Fig. 2. Exposure to $CdCl_2$ reduces accommodation and the long-duration a.h.p. but not the membrane hyperpolarization evoked by adenosine. Recordings of the membrane potential from one neurone before (row *A*) and during (row *B*) bath application of 100 μM-$CdCl_2$. Column 1 reflects action potentials evoked by intracellularly applied, depolarizing current pulses of 100 ms duration and 200 pA amplitude (calibration is 20 ms and 20 mV). Action potential frequency was increased in the presence of $CdCl_2$. Column 2 represents two averages, of ten a.h.p.s each, which followed the action potentials generated as described for column 1. Despite the increased number of action potentials evoked during exposure to $CdCl_2$, the a.h.p. was reduced. Column 3 is of two chart records that show membrane hyperpolarization during bath application of 100 μM-adenosine (indicated by the horizontal bar) before (*A*3) and during (*B*3) exposure to $CdCl_2$. Upward deflexions in *A*3 reflect bursts of action potentials (attenuated by the low frequency response of the chart recorder) of both spontaneous and current-evoked origin (pulses of 200 pA; 200 ms, every 30 s). The large deflexions in *B*3 resulted from intracellularly applied current pulses (200 ms; 200 pA, for the hyperpolarizing pulses). Note the reduction of the fast potential fluctuations, which reflect spontaneous synaptic activity, during $CdCl_2$ exposure.

The effects of adenosine (100 μM) were examined on neurones ($n = 4$) treated with intracellular injection of EGTA (0·5–1·0 M) in quantities sufficient to antagonize the long-duration a.h.p. and accommodation. A hyperpolarization of $-8{\cdot}7 \pm 1{\cdot}2$ mV was observed in the treated neurones which is not significantly distinguishable from that of $-8{\cdot}0 \pm 2{\cdot}6$ mV, evoked by 100 μM-adenosine ($n = 12$) in untreated neurones (Fig. 3).

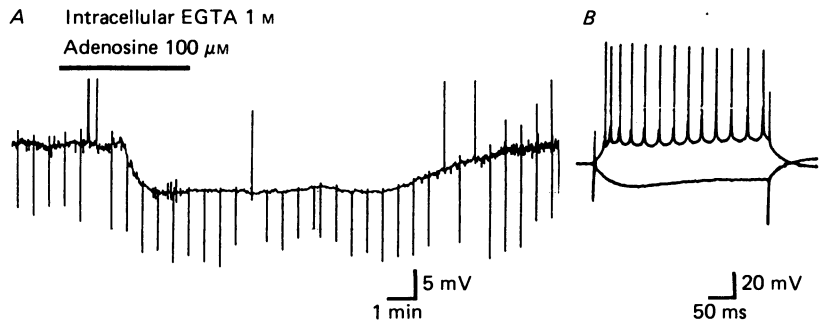

Fig. 3. Intracellular application of EGTA antagonizes accommodation and the long-duration a.h.p. but not the changes in resting membrane properties evoked by adenosine. *A* is a continuous chart recording of membrane potential before, during (indicated by horizontal bar), and after bath application of 100 μM-adenosine from a neurone injected with EGTA. Large downward and upward deflexions resulted from intracellular application of ±200 pA current pulses of 300 ms duration. *B* is two oscilloscope traces of the membrane potential taken from the same neurone as in *A*, just prior to the exposure to adenosine. In one trace, hyperpolarizing current, and in the other, depolarizing current was applied (200 pA; 300 ms). The neurone generated action potentials at approximately a constant frequency throughout the depolarizing current pulse.

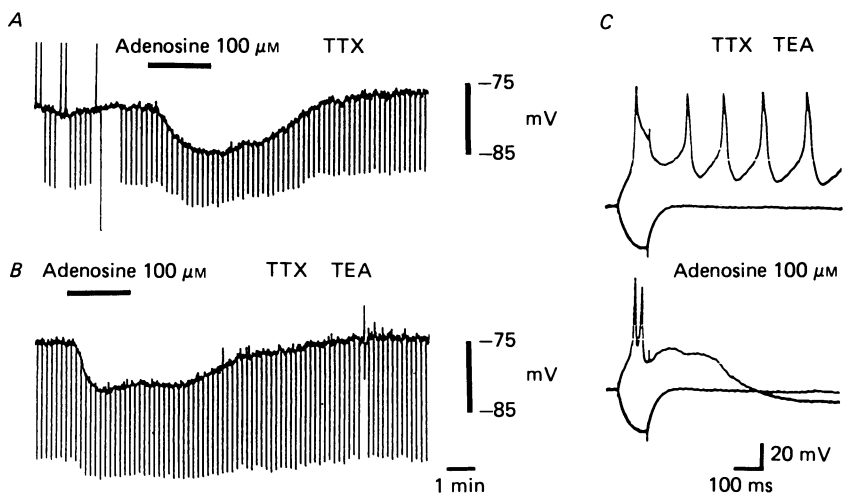

Fig. 4. The changes in resting membrane properties evoked by adenosine are not affected by the presence of TEA. *A* and *B* are continuous chart recordings of membrane potential from the same neurone treated with TTX (3 μM) before, during (indicated by horizontal bar) and after bath application of 100 μM-adenosine. *A* was recorded prior to, and *B* during, exposure to TEA (10 μM). Downward deflexions resulted from intracellular current injection of 200 pA amplitude and 100 ms duration. *C*, two sets of oscilloscope traces (two traces per set) taken at the same membrane potential, before (top) and during (bottom) exposure to adenosine. Intracellular current of ±200 pA; 100 ms, was applied.

Antagonism of delayed outward rectification. Potassium currents responsible for both the voltage- and calcium-dependent, delayed outward rectification and the a.h.p. can be antagonized by TEA (Meech & Standen, 1975; Hermann & Gorman, 1981; Brown & Griffith, 1983). In TTX-treated hippocampal slices, the addition of TEA (10 mM) increased the excitability of the neurones so that a 100 ms depolarizing pulse could elicit slow repetitive action potential activity, often lasting longer than a second (Fig. 4*C*). However, the effects of adenosine (100 μM) on the resting membrane properties were not altered by the presence of TEA ($n = 3$, Fig. 4*A* and *B*). Accordingly, the slow action potentials were attenuated (see Haas & Greene, 1984).

Early, transient, outward rectification. Both the depression by adenosine of the transient, excitatory post-synaptic potentials (Scholfield, 1978; Dunwiddie & Hoffer, 1980) and the reported antagonism of adenosine effects by 4-AP (Perkins & Stone, 1980; Lee, Schubert & Heinemann, 1984) are consistent with an adenosine-evoked increase in the early, transient, outward current, I_A (Connor & Stevens, 1971; Neher, 1971; Thompson, 1977; Gustafsson, Galvan, Grafe & Wigstroem, 1982). Furthermore, it is conceivable that both the effects on the resting membrane properties and enhancement of the a.h.p. by adenosine could be mediated by an increase in a voltage-sensitive, steady-state I_A (Partridge & Connor, 1978).

The effects of adenosine on the transient outward rectification were investigated by applying cellular, depolarizing current pulses to TTX-treated neurones held at -80 mV by d.c. current injection. The membrane potential was measured 100 ms after initiation of the depolarizing pulse. At this point in time, a marked deviation from the passive charging curve of the membrane in the hyperpolarized direction was observed (the transient outward rectification). It was both voltage and 4-AP (100 μM) sensitive (Fig. 5*A* and *B*). However, it was unaffected by adenosine (100 μM; $n = 4$). Neither was the adenosine-evoked change in the resting membrane properties affected by 4-AP (100 μM, $n = 3$) despite the coincident antagonism by 4-AP of the transient outward rectification.

DISCUSSION

Our results confirm the presence of an adenosine-evoked enhancement of the long-duration a.h.p. (Haas & Greene, 1984) and show that this action results, at least in part, from a decrease in the rate of return of the a.h.p. to resting potential. If the enhancement of the a.h.p. was mediated by a slow potassium channel, present only during adenosine application, the rate of decay of the a.h.p. should be biphasic to reflect the additional effect of this slower channel. However, the decay rate is best described by a single exponential. The same argument applies against the possibility that a greater calcium influx slows the decay of the a.h.p. by temporarily overwhelming the calcium-uptake systems (see also Halliwell & Scholfield, 1984). An alternative mechanism is an adenosine-evoked change in the kinetics of the calcium-dependent potassium conductance. Such a change is unprecedented but cannot be excluded. However, since the decay rate appears in normal conditions to follow the intracellular calcium concentration (see references in Results), we suggest that in the presence of adenosine the slower decay rate of the a.h.p. may reflect a slower rate of return to base line of the intracellular calcium concentration. Since accommodation is probably

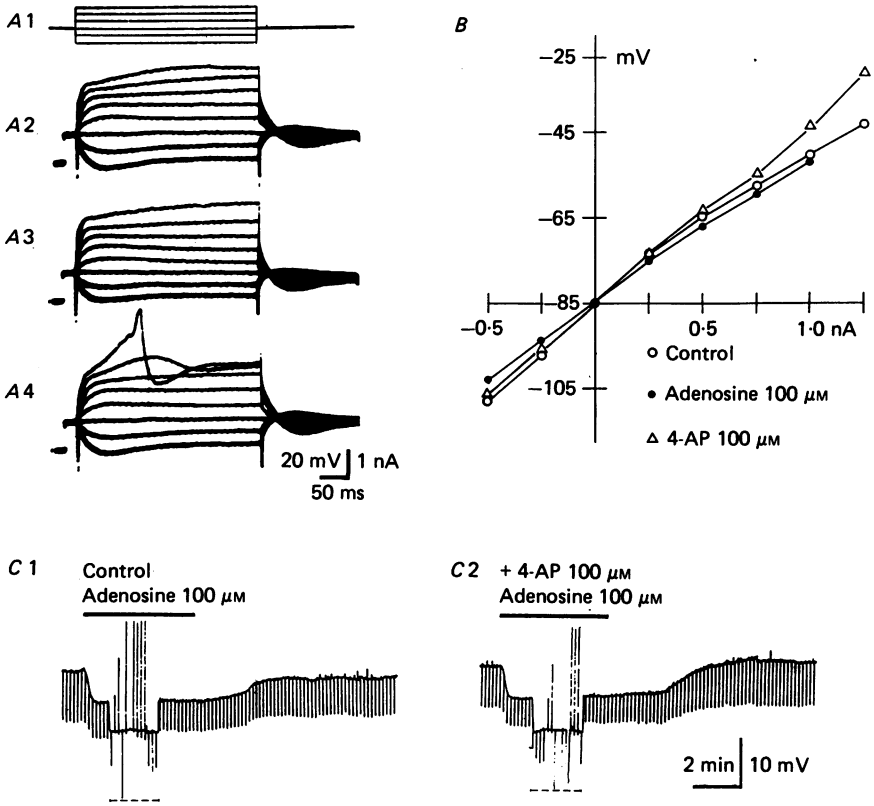

Fig. 5. Adenosine does not affect the early transient outward rectification. *A*, four series of superimposed oscilloscope traces showing intracellularly applied current pulses (*A*1) and the resultant changes in membrane potential in control perfusion media with 3 μM-TTX (*A*2); with the addition of 100 μM-adenosine (*A*3); or with the addition of 100 μM-4-AP (*A*4). In all cases, base-line membrane potential was maintained at −85 mV by d.c. current injection. *B* is a graph of the membrane potential (shown in *A*) taken 100 ms after initiation of the current pulse *versus* the amplitude of the current pulse. The most marked variation from the control occurred in the presence of 4-AP at membrane potentials of less than −60 mV; the region of the control curve where outward rectification is most apparent. The curve from potentials recorded in the presence of adenosine reflects a voltage-insensitive decrease of input resistance relative to the control curve. *C*, two chart records of membrane potential which illustrate the changes of the resting membrane properties evoked by bath application of 100 μM-adenosine (indicated by horizontal bar) before (*C*1) and during (*C*2) exposure to 100 μM-4-AP. During the time indicated by the horizontal dashed line, the membrane potential was maintained at −85 mV by d.c. current injection. Large downward deflexions resulted from intracellularly applied current pulses of 300 ms duration and 200 pA amplitude.

mediated by the same mechanism as that responsible for the long-duration a.h.p. (Madison & Nicoll, 1984; Brown & Griffith, 1983; but see also Lancaster & Adams, 1984), it follows that its enhancement by adenosine (Haas & Greene, 1984) could also result from the same proposed change in intracellular calcium metabolism.

In three experimental procedures, including bath perfusion with $CdCl_2$ and TEA, and intracellular injection with EGTA, the long-duration a.h.p. was markedly

reduced. In none of these circumstances was the adenosine-evoked hyperpolarization or decrease in input resistance affected. Thus, it is unlikely that an increase in calcium-dependent potassium conductance can be responsible for these two effects of adenosine. This does not rule out the mediation of all the adenosine effects by some other voltage-sensitive, steady-state potassium current such as I_A. However, we have shown that the decrease of input resistance following application of adenosine is both voltage and 4-AP insensitive. This is inconsistent with a mechanism of action involving I_A or some other voltage-sensitive potassium conductance.

In conclusion, adenosine appears to exert its effects by two distinct mechanisms of action. The changes in resting membrane potential properties are insensitive to both calcium and voltage, and thus can effect a steady-state reduction in neuronal excitability. In contrast, adenosine also prolongs the time course of the calcium-dependent a.h.p. which can reduce neuronal excitability to long-, but not short-duration excitatory input. This action is particularly relevant to the antagonism by adenosine of both penicillin (Dunwiddie, Hoffer & Fredholm, 1981) and low calcium/high magnesium (Haas, Jefferys, Slater & Carpenter, 1984) -induced spontaneous epileptiform activity.

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