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CHARACTERIZATION OF INHIBITION MEDIATED BY ADENOSINE IN THE HIPPOCAMPUS OF THE RAT *IN VITRO*

BY U. GERBER, R. W. GREENE*, H. L. HAAS† AND D. R. STEVENS
From the Harvard Medical School and VAMC, Brockton, MA 02401, USA

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SUMMARY

1. Intracellular recordings with single-electrode voltage clamp were employed to study the mechanism of adenosine-elicited inhibition of CA1 neurones of the rat *in vitro*.

2. Adenosine elicits a steady-state outward current in association with an increase in conductance. The driving force varied with external potassium concentration as predicted by the Nernst equation for a change primarily in potassium permeability.

3. Adenosine current was blocked by high concentrations of 4-aminopyridine or barium. In the majority of neurones this current was voltage insensitive. In the remainder, the current was inwardly rectifying. The rectification was blocked by tetraethylammonium.

4. When the adenosine-elicited potassium current was blocked, slow inward currents, normally carried by calcium, were unaffected by adenosine. We conclude that this adenosine inhibition is mediated by an increase in a voltage- and calcium-insensitive potassium conductance in CA1 neurones.

INTRODUCTION

There is a large body of evidence suggestive of an inhibitory neuromodulatory role for adenosine in the mammalian CNS (Phillis, Kostopoulos & Limacher, 1975; Dunwiddie & Hoffer, 1980). Studies of hippocampal CA1 neurones *in vitro* have provided electrophysiological evidence for an inhibitory tone exerted by endogenous adenosine on the activity of these cells (Greene, Haas & Hermann, 1985; Haas & Greene, 1988). This inhibition includes both pre- (Vizi & Knoll, 1976; Dunwiddie & Haas, 1985) and postsynaptic (Okada & Ozawa, 1980) sites of action but the mechanisms responsible for these actions are not fully characterized.

The most prominent postsynaptic effect observed in CA1 hippocampal neurones is a chloride-insensitive hyperpolarization associated with a decrease in input resistance (Okada & Ozawa, 1980; Segal, 1982). In the mammalian CNS, similar responses are produced by a number of agonists including serotonin (Andrade & Nicoll, 1987;

* To whom correspondence should be sent at: Neuroscience Lab 151C, VAMC, Brockton, MA 02401, USA.

† Permanent address: II Physiologisches Institut, Johannes Gutenberg-Universität, Saarstrasse 21, D-6500 Mainz, FRG.

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Colino & Halliwell, 1987; Williams, Colmers & Pan, 1988; Yakel, Trussel & Jackson, 1988), baclofen (Gähwiler & Brown, 1985), opioids (North, Williams, Surprenant & Christie, 1987; Williams, North & Tokimasa, 1988) and noradrenaline (Williams, Henderson & North, 1985). In each of these cases the agonist-gated potassium conductance was voltage sensitive, with decreasing conductance at depolarized membrane potentials (inward rectification). In the case of adenosine, the conductance mediating the response remains to be characterized with respect to its voltage and ionic sensitivities.

In CA1 neurones, adenosine also reduces Ca^{2+}-dependent action potentials (Proctor & Dunwiddie, 1983; Haas & Greene, 1984). It is not clear whether this effect is the result of a direct action of adenosine on calcium channels or a result of a shunting of calcium currents by adenosine-induced potassium conductances. Adenosine antagonism of calcium currents was observed in dorsal root ganglion cells (Dolphin, Forda & Scott, 1986; MacDonald, Skerritt & Werz, 1986) although in contrast to CNS neurones, there was no adenosine-mediated membrane hyperpolarization.

In the present study, adenosine-elicited effects on both steady-state and transient potassium and calcium currents were characterized.

METHODS

Transverse slices from the hippocampi of young adult Sprague–Dawley rats were prepared following halothane anaesthesia and decapitation using essentially the same techniques as those previously described (Greene *et al.* 1985). Media containing (in mM): NaCl, 124; KCl, 3·75; KH_2PO_4, 1·25; $NaHCO_3$, 26; glucose, 10; $CaCl_2$, 2·5; $MgCl_2$, 1·4; was maintained at 30 °C and perfused at a rate of 1·5 ml/min (dead space plus chamber volume equal to 1·2 ml).

Glass microelectrodes filled with 2 M-KCl (resistance, 40–70 MΩ) were employed for sample and hold single-electrode voltage clamp recording with an Axoclamp-2A amplifier (CA, USA) at switching frequencies of 3–5 kHz. To help reduce electrode capacitance, the fluid level in the recording chamber was kept to a minimal level ($< 0·5$ mm). The head stage output was constantly monitored.

Investigation of the calcium currents required high-gain current injection to control these large-amplitude currents. In order to maintain stability, the high-frequency components of the voltage signal controlling the current injection were selectively reduced. This resulted in a small overshoot of the membrane potential in response to square-shaped voltage commands but did not affect the accuracy of the recorded membrane potential. Further, the same gain and frequency phase control settings were always employed for control, experimental and recovery conditions.

Drugs were added to the bath in known concentrations and for some experiments adenosine (5 mM) was applied by local pressure ejection via glass micropipettes with tip diameters of 10–50 μm ('puffer' application) submerged in the bath media but above the surface of the slice.

Data were digitized and stored for subsequent analysis.

RESULTS

Adenosine-evoked steady-state current

The effects of adenosine were studied on eighty-seven neurones from the CA1 region of the hippocampus. The most prominent effect observed following adenosine application by bath (10–50 μM) or puffer pipette (5 mM) was a hyperpolarization accompanied by a decrease in input resistance, in agreement with previous reports (Okada & Ozawa, 1980, Segal, 1982; Greene & Haas, 1985).

Sensitivity to extracellular potassium

The reversal potential of the adenosine (50 μM, bath; 5 mM, puffer) elicited current examined in twenty-five neurones perfused in media containing an extracellular potassium concentration ($[K^+]_o$) of 5 mM was -79 ± 10 mV (mean $\pm$ S.D.). When the

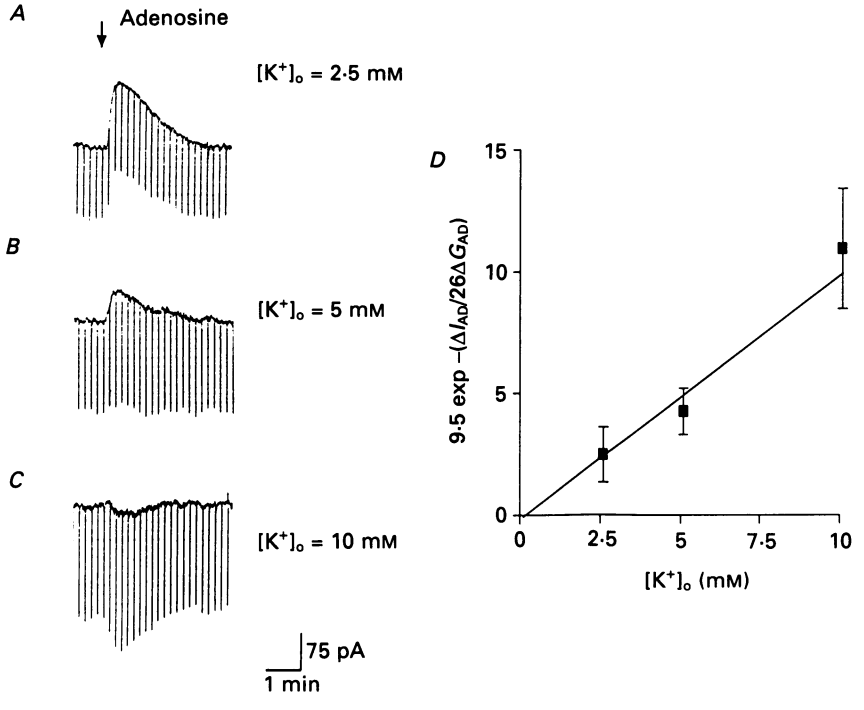

Fig. 1. Adenosine current varied with extracellular potassium as predicted by the Nernst relationship. When extracellular K^+ was increased from 2·5 (*A*) to 5 (*B*) and 10 mM (*C*) the adenosine-evoked outward current decreased and then reversed ($V_h = -70$ mV). Downward deflections resulted from a 10 mV hyperpolarizing potential shift of 300 ms duration to assess conductance. *D*, the relation of the adenosine conductance to extracellular K^+ (■; error bars indicate 1 unit S.D.) was adequately fitted by that predicted for a potassium permeability change (straight line with a *y* intercept of zero).

$[K^+]_o$ was increased from 5 to 10 or 12 mM, the adenosine-evoked current was always reversed from outward to inward at a holding potential (V_h) of -70 mV ($n = 6$), suggestive of a change in potassium permeability.

A more quantitative assessment of the permeability change was made by observing the adenosine-elicited current (ΔI_{AD}) and conductance (ΔG_{AD}) at a $[K^+]_o$ of 2·5 ($n = 3$), 5·0 ($n = 4$) and 10 mM ($n = 4$) while maintaining the membrane potential at -70 mV under voltage clamp control. The ΔG_{AD} was determined using hyperpolarizing 10 mV steps (200 ms duration) (Fig. 1).

The adenosine was applied by puffer at intervals of greater than 3 min and no desensitization was observed. The theoretical plot for an adenosine-evoked change in

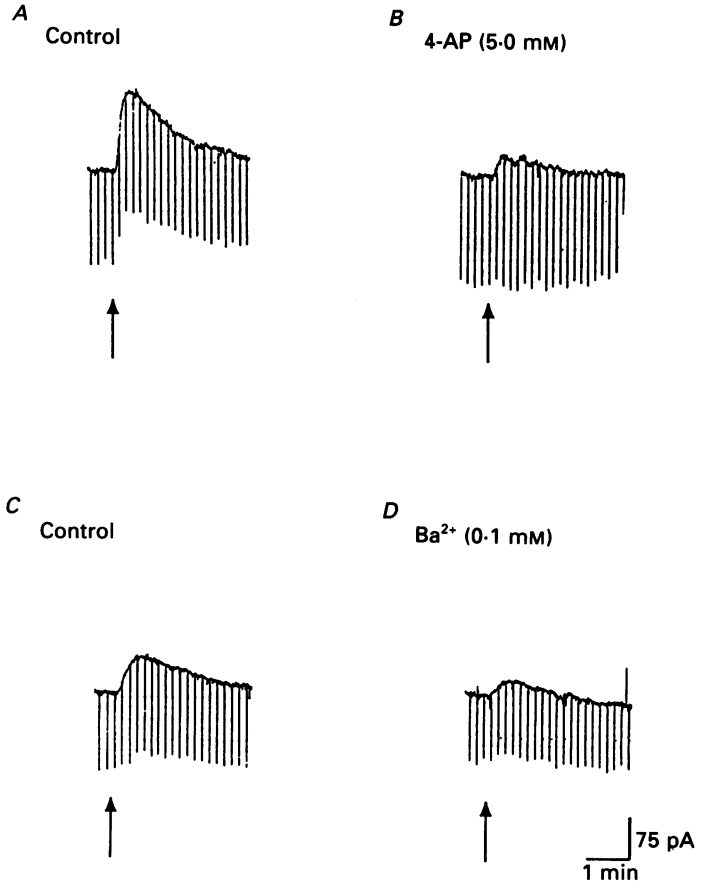

Fig. 2. Adenosine-induced current was blocked by potassium channel blockers. *A* and *B*, adenosine (5 mM) was applied by puffer for 0·5 s (arrows) before and during exposure to 4-AP (5 mM). *C* and *D*, with the same protocol as in *A*, adenosine was examined in the presence of Ba^{2+} (0·1 mM). Downward deflections were a result of 10 mV hyperpolarizing steps of 300 ms duration.

only potassium permeability is a line with a slope of 1 and a y intercept of zero (Fig. 1*D*) determined from the relationship:

$$\Delta I_{AD} = \Delta G_{AD}[V_h - (26 \ln [K^+]_o/[K^+]_i)].$$

Solving for $[K^+]_o$ gives:

$$[K^+]_o = [K^+]_i \exp[(V_h/26) - (\Delta I_{AD}/26\Delta G_{AD})].$$

When the membrane potential (V_h) is held at -70 mV and $[K^+]_i = 140$ mM (Alger & Nicoll, 1980) the equation simplifies to:

$$[K^+]_o = 9{\cdot}5 \exp -(\Delta I_{AD}/26\Delta G_{AD}).$$

This equation was used to avoid overestimation of the reversal potential, which may

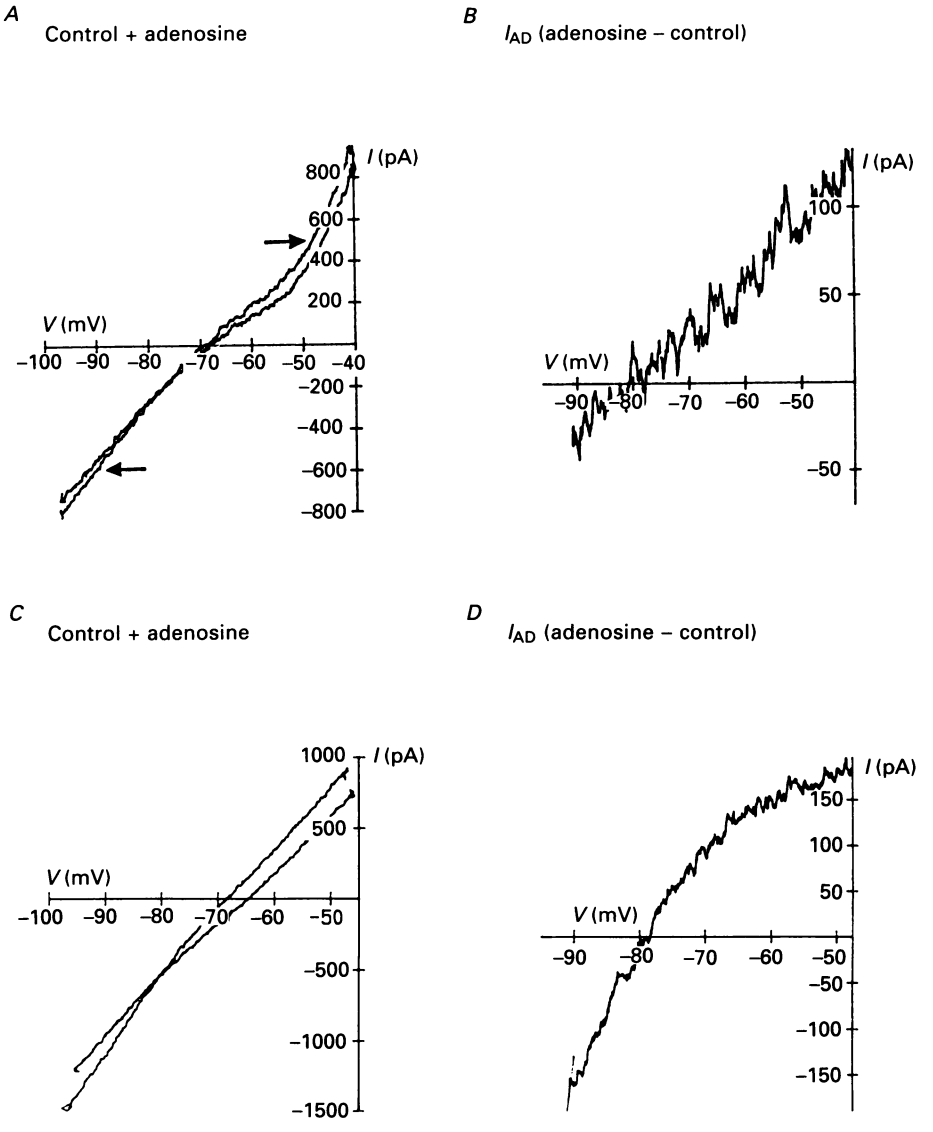

Fig. 3. The slope conductance of the adenosine current elicited in the majority of neurones was voltage insensitive. *A*, *I–V* plots of steady-state currents resulting from voltage ramps (2·5 mV/s from −100 to −50 mV) were obtained in the presence (indicated by arrows) and absence of 50 μM-adenosine. *B*, an adenosine *I–V* plot computed by digital subtraction of control from adenosine plots (shown in *A*) showed no voltage dependence. *C* and *D*, *I–V* plots as in *A* and *B*, but characteristic of a minority of neurones. Note the inward rectification.

occur to the extent that the receptor effector sites are electrotonically distant and the reversal potential is hyperpolarized relative to resting membrane potential. The ratio of $\Delta I_{AD}/\Delta G_{AD}$ is less affected by the electrotonic location of the sites. The experimentally determined values at each $[K^+]_o$ were within one standard deviation of those predicted for a change in potassium conductance alone.

Blockade of adenosine current

The adenosine-mediated current has been shown to be insensitive to most potassium channel blockers including TEA, 4-aminopyridine (4-AP; at 0·1 mM) and to procedures which prevent an increase in intracellular Ca^{2+} concentration (Greene

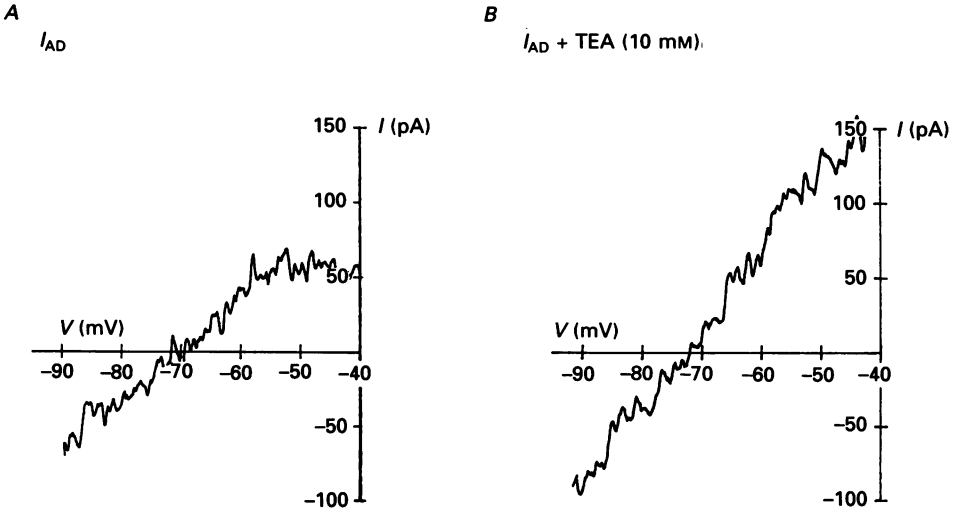

Fig. 4. TEA suppressed rectification of the adenosine-induced current. *A*, an *I–V* plot of a steady-state adenosine current which showed inward rectification was recorded from a neurone in control media. *B*, during bath application of TEA (10 mM) the rectification was absent.

& Haas, 1985). Adenosine current was blocked by 4-AP at concentrations an order of magnitude in excess of that required to block transient outward current (5·0 mM; $n = 3$). Ba^{2+} blocked more than 50% of the response at 0·1 mM ($n = 3$) and completely abolished the response at 2·0 mM ($n = 7$, Fig. 2).

Voltage sensitivity

The voltage sensitivity of the adenosine-elicited steady-state current was examined over the range of −100 to −40 mV. Under voltage clamp control and in the presence of TTX (1·0 μM) the membrane potential was shifted at a constant rate of 2·5 mV/s over this range before and during exposure to adenosine (Fig. 3*A*). The control *I–V* plot was then digitally subtracted from the adenosine *I–V* plot which provided a plot of the adenosine-elicited current as the remainder (Fig. 3*B*). In thirteen of twenty-one neurones tested, the slope conductance was constant over the range tested. However, in 38% of the neurones, the adenosine slope conductance was decreased at membrane potentials depolarized to −65 mV (Fig. 3 *C* and *D*).

Because some of the adenosine-mediated current probably originates at electrotonically distant sites on the dendrites (Lee, Reddington, Schubert & Kreutzburg, 1983), an outwardly rectifying increase in whole-cell conductance might cause the apparent adenosine current to decrease with depolarization due to a reduction of the length constant. This possibility was tested by examination of

adenosine I–V plots showing inward rectification before and during perfusion with TEA (10 mM) an antagonist of outwardly rectifying potassium currents (Stanfield, 1983). In the presence of TEA the inward rectification was reduced or abolished ($n = 3$; Fig. 4C and D).

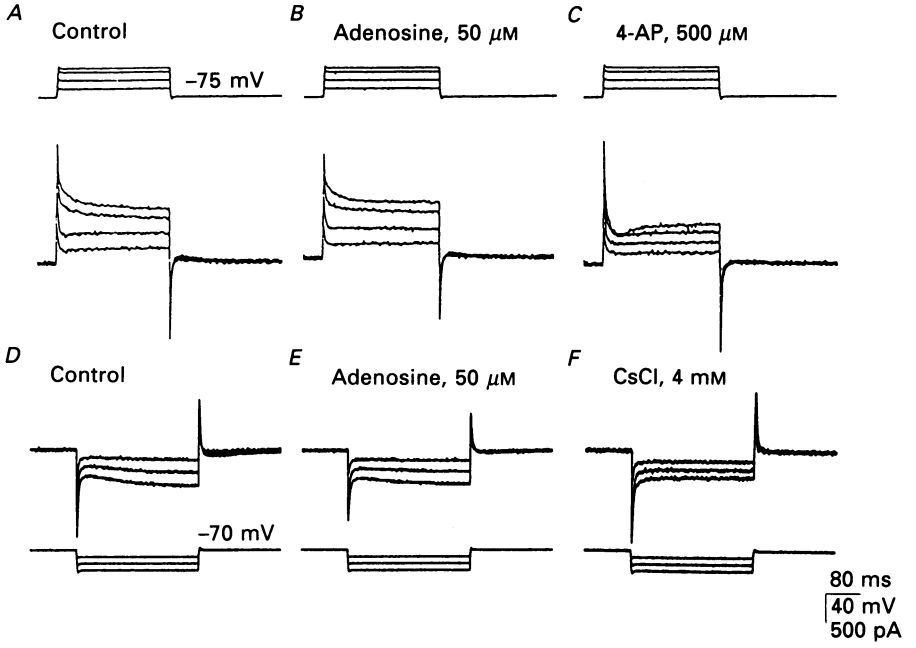

Fig. 5. Adenosine did not increase either I_A or I_Q. The current (upper row) and membrane potential (bottom row) were recorded from a neurone voltage clamped to −75 mV. In A, B and C, the membrane potential was shifted to −40 mV in 5 or 10 mV steps of 300 ms duration before and during adenosine (50 μM) exposure and in the presence of 4-AP (500 μM). In D, E and F, the holding membrane potential of −70 mV was shifted to −100 mV in 10 mV steps before and during adenosine (50 μM) exposure and in the presence of extracellular CsCl (4 mM).

Effects on voltage-sensitive outward currents

Observations under current clamp conditions with a sufficient concentration of 4-AP to block early, transient, outward rectification were inconsistent with an adenosine-mediated increase in I_A (Greene & Haas, 1985). A direct assessment of the action of adenosine (50 μM; $n = 9$) on 4-AP-sensitive, early, transient, outward current, I_A, showed no increase ($P < 0{\cdot}005$, sign test; Siegel, 1956). The last five of the nine cells examined were subjected to identical voltage steps of 300 ms duration, from a holding potential of −75 to −45 mV. The early, transient current was assessed by subtraction of the current amplitude measured at the end of the 300 ms step from the current measured 30 ms after initiation of the step (Fig. 5A–C). This gave amplitudes of 168±60 pA (mean±S.D.) for control and 132±41 pA in the presence of adenosine (50 μM).

When the I_A blocker, 4-AP (500 μM; Thompson, 1977), was applied to these three neurones, the current (measured with identical parameters) had an amplitude of 20±43 pA.

Another voltage-sensitive potassium (and sodium) current, I_Q, (Halliwell & Adams, 1982) was examined for adenosine-evoked increases (Fig. 5*D–F*) but none was observed ($n = 9$; $P \leqslant 0{\cdot}05$, sign test). Identical voltage steps of 300 ms duration from a holding potential of −75 to −105 mV ($n = 4$) were examined. The inward

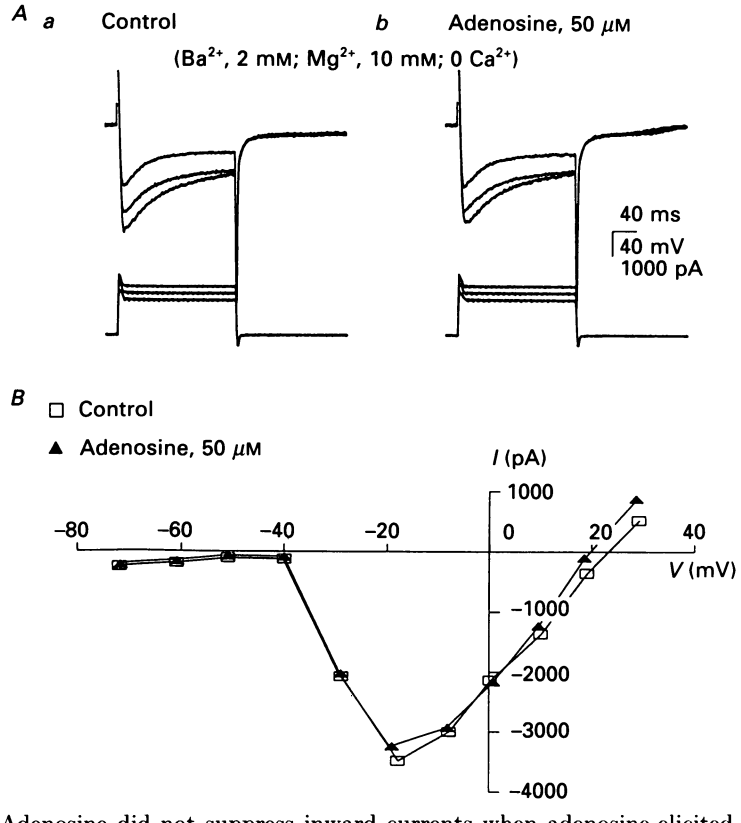

Fig. 6. Adenosine did not suppress inward currents when adenosine-elicited potassium conductance was blocked. *A a*, inward current was recorded with CsCl-filled electrodes during perfusion with 10 mM-Mg^{2+} and replacement of Ca^{2+} with 2 mM-barium. Holding potential was −70 mV. *A b*, currents from the same cell during exposure to adenosine showed there was no suppression of inward current under these conditions. *B*, *I–V* curve for the peak inward current was obtained from records including those shown in *A*; control conditions (□) and during exposure to adenosine (▲).

relaxation elicited by the voltage step was assessed by the subtraction of the current amplitude measured 30 ms after initiation of the step from the current amplitude at the end of the 300 ms step. In control conditions it was -167 ± 25 pA, in the presence of 50 μM-adenosine, -146 ± 43 pA and, in the presence of an externally applied I_Q blocker, caesium (4 mM), 33 ± 35 pA (Halliwell & Adams, 1982).

Effects on slow inward currents

The effects of adenosine on slow inward currents were examined during perfusion with barium (100–500 μM) and with caesium chloride (2 M) in the recording electrode to block outward currents so that slow inward currents, normally carried by calcium

(Brown & Griffith, 1983), might be more clearly observed (Connor, 1979; Johnston, Hablitz & Wilson, 1980). With the addition of barium, the outward current evoked by adenosine (50 μM, $n = 7$) was not eliminated but was reduced (Fig. 4). Magnesium (10 mM) was also added to reduce the amplitude of the inward currents to an amplitude controllable by the voltage clamp ($2{\cdot}6 \pm 0{\cdot}8$ nA, mean $\pm$ S.D.; $n = 14$). Peak slow inward current was reduced by 50 ± 6% in the presence of adenosine.

This reduction may have resulted from either a shunt of the inward currents by the adenosine-elicited potassium current, or a direct effect of adenosine on the inward current. A direct effect should not be affected by blockade of the adenosine-sensitive potassium channels. Replacement of Ca^{2+} with 2 mM-barium (and 10 mM-Mg^{2+}) allowed examination during blockade of the adenosine-induced current. Slow inward currents were not affected by treatment with 50 μM-adenosine ($n = 4$; Fig. 6). In two experiments, return to control media resulted in return of adenosine-induced outward current and a suppression of slow inward Ca^{2+} currents by 27 and 34%.

DISCUSSION

In summary, inhibition of CA1 neurones by adenosine was mediated primarily by an increase in a voltage- and calcium-insensitive potassium conductance which was blocked by 4-AP and Ba^{2+}. Adenosine did not increase transient outward current or Q-current nor was a direct effect on Ca^{2+} currents observed.

The potassium current elicited by adenosine in adult CA1 neurones appeared distinct from the inwardly rectifying potassium current elicited by adenosine in cultured neurones (Trussel & Jackson, 1985, 1987). In adult CA1 neurones, no voltage sensitivity was observed with the exception of a minority of neurones which did show some inward rectification. However, in these cases the rectification could be accounted for by a shunt due to a TEA-sensitive conductance.

The adenosine-mediated current has a number of similarities with the 'S' current described in the invertebrate central nervous system (Siegelbaum, Camardo & Kandel, 1982; Pollack, Bernier & Camardo, 1985). Both currents result from a modulation of a voltage- and calcium-insensitive potassium conductance by neurotransmitters (Brezina, Eckert & Erxleben, 1987; Brezina, 1988). They also share a pharmacological sensitivity to the potassium channel blockers 4-AP, at high concentration (5 mM), and Ba^{2+} (Shuster & Siegelbaum, 1987; Brezina, 1988).

Adenosine has been shown to reduce calcium-dependent action potentials (Proctor & Dunwiddie, 1983; Haas & Greene, 1984). Although adenosine reduces Ca^{2+} currents, we find no evidence that this effect is a direct action on Ca^{2+} channels. Block of adenosine-mediated potassium conductance by barium prevented the reduction of Ca^{2+} currents. Halliwell & Scholfield (1984) have reported that adenosine had no action on Ca^{2+} currents in CA1 pyramidal neurones and olfactory cortex. Their experiments in the hippocampus were conducted in the presence of 1 mM-barium which probably blocked most of the adenosine-induced outward current. We conclude that the observed reduction in Ca^{2+} currents by adenosine in our studies resulted from shunting of this current by the adenosine-mediated potassium conductance. While we cannot exclude the possibility of a direct action of adenosine

on Ca^{2+} conductance, particularly at electrotonically remote sites, this effect must be small relative to the effect of shunting by potassium conductance.

The holding potential employed in this study was probably of sufficient magnitude (−70 mV) to at least partially remove inactivation of the transient calcium currents (Fox, Nowycky & Tsien, 1987) since the divalent cation concentration was increased by the addition of Mg^{2+} (10 mM) in these experiments. Further, a decay of the calcium current observed during the depolarizing command step was consistent with a transient component. Thus, neither transient nor long-lasting types of Ca^{2+} currents appear affected by adenosine.

The mechanism of an indirect reduction of calcium currents by the adenosine-elicited potassium conductance observed in CA1 neurones may be responsible for the presynaptic adenosine inhibition of the EPSP evoked by stratum radiatum stimulation (Dunwiddie & Haas, 1985). Consistent with this possibility, we have observed an adenosine-elicited increase in potassium conductance with no direct effect on calcium currents in CA3 neurones which are presynaptic to CA1 (unpublished observations, D. R. Stevens & R. W. Greene).

In conclusion, the most effective mechanism observed for adenosine-mediated inhibition in the mammalian CNS was an increase in steady-state potassium conductance which can indirectly reduce calcium currents. In addition, adenosine has been shown to increase the current responsible for the long-duration after-hyperpolarization (Haas & Greene, 1984; Greene & Haas, 1985) providing a mechanism selective for inhibition of long-duration excitatory input. The adenosine-elicited increase in steady-state potassium current produced a non-selective inhibition of neuronal activity which may be important in the maintenance of homeostasis between the metabolic demand of neuronal activity and metabolite availability, especially under pathological conditions such as epilepsy, hypoxia or hypoglycaemia when cerebrospinal fluid levels of adenosine are known to increase (Pull & McIlwain, 1972; Berne, Winn & Rubio, 1982; Zetterstrom, Vernet, Ungerstedt, Tossman, Jonzon & Fredholm, 1982).

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