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# Adenosine-activated potassium conductance in cultured striatal neurons

(inhibitory transmission/purinergic receptors/patch clamp/basal ganglia)

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ABSTRACT **We have examined the effect of adenosine on the membrane properties of cultured embryonic mouse striatal neurons using patch electrode techniques. Adenosine at 50 μM effectively blocked spontaneous action potential activity. Adenosine or 2-chloroadenosine caused a slow hyperpolarization of the membrane potential and, under voltage clamp, an outward current that was blocked by 1 mM theophylline. ATP also caused a hyperpolarization that was slower and weaker than the adenosine response and could be blocked by 1 mM theophylline. The current induced by adenosine appears to be carried by potassium since (*i*) an inward current was generated by adenosine when the cells were internally perfused with cesium salts and (*ii*) the reversal potential of the outward current shifted 57 mV with a 10-fold change in extracellular potassium concentration. The adenosine response is voltage dependent in that the current evoked by adenosine is reduced at holding potentials more positive than −55 mV, despite a larger driving force. Though calcium influx is not required for adenosine to activate the potassium conductance, some components of the cytosol may be essential, since the response is lost during intracellular perfusion.**

A large body of evidence demonstrates that extracellular purines and purine nucleotides have potent effects on the biochemical and electrophysiological behavior of many cell types (1). For example, electrophysiological studies on central and peripheral mammalian neurons as well as on smooth muscle and heart muscle show that adenosine has a marked inhibitory action on excitability (1, 2). The widespread distribution of purine receptors in the nervous system (3) and the possibility that ATP or even adenosine may be released from synaptic terminals (4–6) suggest that purinergic modulation of electrical activity is of fundamental importance. Behaviorally, the well-known stimulatory, and possibly addictive, properties of caffeine (an adenosine antagonist) (1) and the sedative action of drugs that block the degradation of adenosine (7) also argue for an important role for adenosine as an endogenous inhibitory agent in the central nervous system.

However, many aspects of the physiological effects of adenosine are still uncertain. What are the ionic mechanisms that underlie the inhibitory action of adenosine in the brain? It is clear that this compound can reduce transmitter release in central and peripheral terminals, possibly by interference with calcium influx or with the coupling of calcium to quantal release (8, 9). Since presynaptic currents in the central nervous system are not presently accessible to rigorous analysis, the effect of adenosine on presynaptic calcium mechanisms remains elusive. On the other hand, there is direct evidence that adenosine enhances a potassium conductance in heart muscle (10), parasympathetic ganglia (11), and hippocampal pyramidal neurons (12, 13). It is therefore likely that this conductance plays some role in mediating the inhibitory action of adenosine. Since the electrical activity of striatal neurons is inhibited *in vivo* by adenosine (14), we examined the effect of this compound on the membrane properties of striatal neurons in dissociated cell culture. Using patch electrode techniques (15), we have obtained evidence showing that adenosine blocks spontaneous action potential activity and, in a strongly voltage-sensitive manner, activates a potassium conductance. Furthermore, this action may require diffusable cytoplasmic components. A preliminary report of some of this work has appeared (16).

## METHODS

The procedure of Swaiman *et al.* (17) for culturing mouse cortex was adapted to the culture of mouse striatum. The brains of mouse embryos in their 17th day of gestation were opened along the lateral ventrical, exposing the striatal hillock. The center of the hillock was then pinched off with a fine forceps and collected for dissociation. The tissue was treated for 30 min with 0.25% trypsin in isosmolar calcium-free saline and then triturated. Cells were plated at a density of $10^6$ cells per collagen-coated 35-mm dishes (Falcon) in minimal essential medium (MEM) with 10% each of horse and fetal calf serum. Each embryo yielded approximately one dish of cells. After 5 days, the medium was changed to a medium identical to the plating medium but without calf serum and with 10 μM fluorodeoxyuridine and 50 μM uridine. The medium was changed to MEM and 10% horse serum after 2 days and subsequently changed twice weekly in the same medium. Cultures were used 1–4 weeks after plating.

During electrophysiological studies, the cells were maintained at 34°C in a solution containing 140 mM NaCl, 4 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, and 11 mM glucose buffered at pH 7.2 with 10 mM Hepes. Bath potassium concentrations were elevated by replacement of sodium, leaving chloride levels unchanged. Except when recording action potentials, all experiments were done in the presence of 0.5 μM tetrodotoxin. Patch electrodes contained 140 mM potassium gluconate, 1 mM $MgCl_2$, 0.5 mM $CaCl_2$, and 5 mM EGTA buffered at pH 7.2 with 10 mM Hepes. In internal ion replacement experiments, potassium gluconate was replaced with cesium or chloride salts as indicated in *Results*. Occasionally 47 mM potassium citrate with 93 mM sucrose (to maintain osmolarity) was used instead of 140 mM potassium gluconate.

Recording from striatal neurons with conventional microelectrodes was impractical because the cells were too small (cell diameters ranged from 7 to 15 μm). In contrast, recording from striatal neurons with patch electrodes was relatively easy. Membrane-electrode seals of greater than 5

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Abbreviations: GABA, γ-aminobutyric acid; *I–V*, current–voltage.

GΩ were obtained >75% of the time with patch electrodes of resistance ranging from 1 to 3 MΩ (the use of higher resistance electrodes is discussed in *Results*). After rupture of the cell membrane patch, stable resting potentials of −65 to −75 mV were common (mean ± SD = −72 ± 6 mV, $n$ = 46), while input resistances varied between 100 and 700 MΩ. The larger membrane area contributed by neurites made an effective space-clamp impossible; sodium or calcium spikes always escaped the clamp, while capacitive transients observed with voltage steps were at least 5 msec or more in duration. However, the adenosine response was a slow, weak current compared to regenerative currents and so the efficacy of the clamp was adequate for the purposes of these experiments. The series resistance of the recording (i.e., the resistance through the pipette to the cell) was determined either by the ohmic component of the voltage response to a current pulse or by the size and time course of capacitive transients obtained with voltage clamp pulses. While series resistances occasionally approached 20% of the cell input resistance, the reversal potentials measured for the adenosine response did not shift by >4 mV after correction for the series resistance. The correction was small because the adenosine-activated current reversed near the resting potential of the cell. Recordings were also corrected for an 11 mV junction potential that appeared between the patch electrode and bathing solutions. Current or voltage responses were monitored with an EPC-7 patch clamp amplifier (List Electronics/Medical Systems) and recorded on chart paper (Gould).

Current–voltage (*I–V*) relations were obtained from responses to pressure-ejected 2-chloroadenosine, where the membrane holding potential was first held constant for at least 10 sec. The peak change in membrane current produced by the agonist was then plotted against the corresponding holding potential. The response was tested at six to eight different potentials. Since the response declined during the recording (see *Results*) a control response at −70 mV was first obtained and then retested periodically between trials at other voltages. When the response to 2-chloroadenosine at −70 mV had dropped by >10% the experiment was stopped.

All chemicals were obtained from Sigma. Drugs were dissolved in the bathing solution and the pH was adjusted before each experiment. Adenosine, ATP, and 2-chloroadenosine were used at 50 μM; theophylline was used at 1 mM. Solutions of drugs were delivered by picospritzers (General Valve) with 200-msec pulses at 5–9 psi (1 psi = 6.89 kPa) applied through pipettes having a tip aperture of ≈1–2 μm and positioned within 20 μm of the cell. One or two picospritzers were used, as indicated in *Results*. Although the concentration of drug actually seen by the cell is certainly lower than that contained in the pipette, these small drug applicator tips were necessary to maintain reproducible responses.

## RESULTS

After 3–4 days in culture, striatal neurons spontaneously produced regular bursts of action potentials (18, 19). These bursts are likely to arise from the behavior of complex synaptic networks since bathing solutions containing 0.1 mM $Ca^{2+}$ and 10 mM $Mg^{2+}$ will block spontaneous spiking but not evoked spikes or spontaneous subthreshold synaptic events. Picospritzer application of adenosine (36 of 41 cells) or ATP (13 of 14 cells) at 50 μM blocked all suprathreshold activity. A representative trace is shown in Fig. 1*A*, in which a brief pulse of adenosine prevented the normal complex action potential burst from occurring, leaving instead a cluster of small synaptic potentials. Bath application of 50 μM adenosine or ATP completely blocked all spiking, and this inhibition lasted at least 1/2 hr (4 of 4 cells).

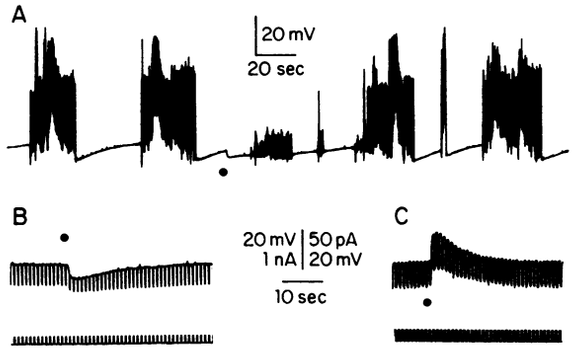


FIG. 1. Adenosine effects on electrical activity of striatal neurons. (*A*) Chart recordings of spontaneous action potential bursts. Action potential amplitude was limited by the time constant of the chart recorder. Fifty micromolar adenosine was applied with pulses of pressure for 2 sec at the time marked by the dot (●). (*B* and *C*) Current and voltage clamp records, respectively, of the response to adenosine. A 200-msec pulse of adenosine was applied at the time marked by a dot. Tetrodotoxin (0.5 μM) was present in *B* and *C* to block ongoing activity. In voltage clamp the cell was held at −70 mV.

As is apparent in Fig. 1*A*, adenosine produced a small, transient hyperpolarization of the membrane potential, typically between 2 and 10 mV in amplitude. In Fig. 1*B*, another example of this effect with current pulses to monitor membrane conductance illustrates that the hyperpolarization is associated with a substantial increase in membrane conductance (34% ± 24%, $n$ = 24 cells). Under voltage clamp conditions with the membrane potential held at −70 mV, a 200-msec pulse of 50 μM adenosine elicited an outward current that peaked 1–2 sec after the pulse ended and slowly decayed to baseline within 10–20 sec, as shown in Fig. 1*C*. The time course of this response is much slower than the time scale of drug delivery as judged by comparison with responses to γ-aminobutyric acid (GABA) and glutamate that are elicited under the same conditions and peaked in 30 msec (data not shown). Furthermore, adenosine responses are qualitatively weaker at room temperature (23°C), also in marked contrast to responses to GABA and glutamate.

The adenosine response was often variable in size from cell to cell but appeared to vary more between culture dishes. 2-Chloroadenosine, a stable analog that is not a substrate of adenosine deaminase and is not removed by adenosine uptake systems (1), also produced a strong outward current at 50 μM, indicating that the adenosine response is not due to an intracellular action or to the degradation products of adenosine (Fig. 2*A*). A total of 84% of the cells tested responded to adenosine or 2-chloroadenosine with an outward current, with the remaining cells having no response (98 cells tested). ATP also activated an outward current (Fig. 2*B*), which was substantially weaker and slower than the outward current activated by adenosine. ATP elicited this response in 14 of 20 cells first found to be sensitive to either adenosine or 2-chloroadenosine. Theophylline, a competitive antagonist of the P1 purine receptor (2), blocked all responses to ATP (6 cells) and 2-chloroadenosine (7 cells). An example of this is shown in Fig. 2*A*, in which the 2-chloroadenosine-evoked current was reduced when the cell was pulsed with 1 mM theophylline just before the adenosine pulse. Control applications of bath solution without adenosine produced either no response or, occasionally, a small outward current that decayed immediately after the end of the pressure pulse (not shown).

The increased membrane conductance associated with the outward current could be specific for potassium or chloride. Since the patch electrode effectively perfuses the cell interior (20), we altered the potassium and chloride equilibrium

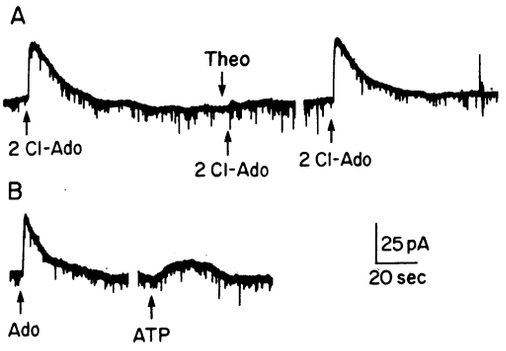


FIG. 2. Voltage clamp records of the response to adenosine agonists and an antagonist. Cells were held at −70 mV. (*A*) Outward current induced by a 200-msec pulse of 50 μM 2-chloroadenosine (2-Cl-Ado) is reversibly blocked by a 1-sec pulse of 1 mM theophylline (Theo) applied by a second picospritzer just before the agonist pulse. (*B*) In a different cell adenosine (Ado) produces a strong outward current while ATP produces only a smaller, slower response (both at 50 μM). The numerous fast inward currents seen throughout the records are spontaneous excitatory synaptic currents.

potentials by filling the electrode with different solutions and then examined the adenosine-induced current. In Fig. 3, a set of three traces illustrates that with high intracellular KCl or potassium citrate, adenosine responses under voltage clamp were outward, whereas replacement of intracellular potassium by cesium resulted in an inward response. This strongly suggests that adenosine activates a potassium conductance.

*I–V* relations constructed for the adenosine response (see *Methods*) also indicated that the response was a potassium current. In Fig. 4, the closed circles show an adenosine *I–V* relation for a cell in a normal bathing solution containing 4 mM potassium. The reversal potential of the response was −88 mV, which is close to the computed potassium reversal potential of −90 mV. We further tested the role of potassium in this current by determining the reversal potential with 10 mM and 40 mM external potassium. The open circles in Fig. 4 show a *I–V* plot for a different cell bathed in 10 mM potassium: elevating the bath potassium shifted the reversal potential by about +22 mV. A more complete description of the effect of potassium is shown in Fig. 5, where the adenosine current reversal potentials are plotted against the logarithm of the ratio of external-to-internal potassium concentration. Each point is the mean value for the number of cells indicated in parentheses. A 10-fold change in bath potassium concentration produced a 57 mV change in reversal potential. The solid line shows the relationship expected for a potassium current based on the Nernst equation, assuming the intracellular potassium concentration was identical to that in the patch electrode. The linearity and slope of the relation indicates that potassium is the major charge carrier in the adenosine response.

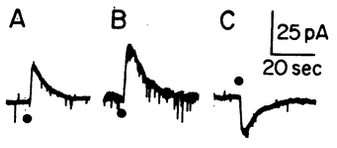


FIG. 3. Voltage clamp responses to 50 μM 2-chloroadenosine invert when cesium replaces intracellular potassium. Cells were held at −70 mV. In three different cells the major salts in the patch electrode were 47 mM potassium citrate (plus 93 mM sucrose) (*A*), 140 mM KCl (*B*), and 70 mM $Cs_2SO_4$ (plus 70 mM sucrose) (*C*). Sucrose was added to adjust the osmolarity. A 200-msec pulse of 2-chloroadenosine was applied at times marked by a dot. Other components of the patch electrode filling solution are listed in *Methods*.

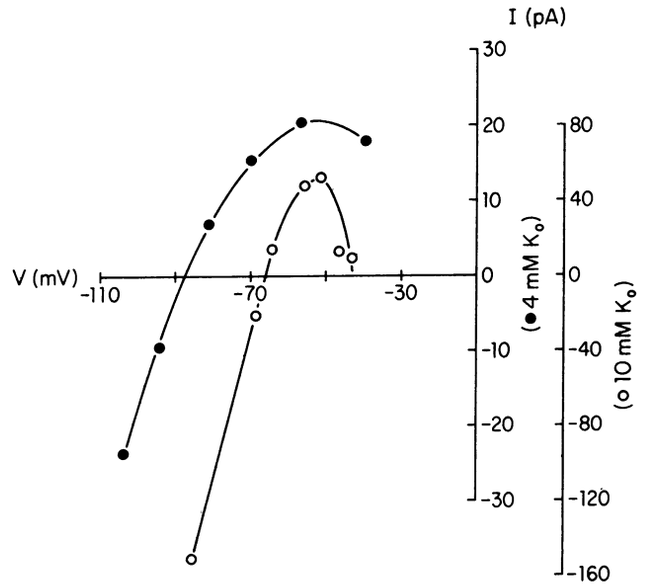


FIG. 4. Whole-cell current produced by a 200-msec pulse of 50 μM 2-chloroadenosine versus holding potential. Cells were held at each potential at least 10 sec before the adenosine was applied. To monitor any loss of responsiveness, the cells were tested periodically at −70 mV with adenosine between trials at other voltages. When the control response declined by >10% the experiment was stopped.

The *I–V* relation also showed that this adenosine-activated potassium conductance is sensitive to membrane potential. As shown by the two representative cells of Fig. 4, the slope of the *I–V* is roughly linear through the reversal potential, but as the holding potential is moved positive the slope decreases and actually becomes negative past −55 mV. This voltage sensitivity was observed in every cell for which *I–V* relations were determined ($n$ = 11). However, as is evident in Fig. 4, the shape of the *I–V* relation was somewhat variable from cell

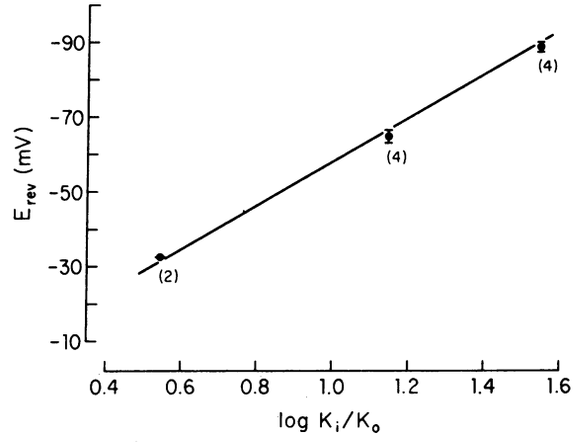


FIG. 5. Adenosine-induced current reversal potential versus the logarithm of potassium concentration ratio. *I–V* relations were constructed by determining the current responses to a 200-msec pulse of 50 μM 2-chloroadenosine at different steady-state holding potentials, including potentials in which the reversal of the response was clear. Reversal potentials were determined by interpolating to zero current, as in Fig. 4. Each point is the mean of the number of cells indicated in parentheses. In high potassium solutions, sodium was replaced with potassium, while chloride was constant. Patch electrodes contained 140 mM potassium gluconate as the major salt. The solid line indicates expected reversal potentials computed from the Nernst equation using the bath and patch electrode potassium concentrations.

to cell, especially near −40 mV. This negative-slope region is not likely to be an artefact of our recording system since *I–V* relations constructed for whole-cell voltage clamp responses to GABA were linear between −60 and 0 mV.

It is possible that adenosine only activated this potassium conductance indirectly by an enhancement of a calcium current or by the release of intracellular calcium stores. This seemed unlikely from the outset since (*i*) adenosine is thought to block calcium conductances (21, 22) and (*ii*) typical calcium and calcium-activated potassium conductances show a voltage dependence opposite in direction to that of the adenosine-sensitive conductance in question (23–25). Moreover, we have observed normal 2-chloroadenosine responses in bathing solutions containing 0 mM calcium and 5 mM magnesium. Additionally, for seven cells in this solution, we compared the current response obtained with a pulse of adenosine dissolved in a bath solution containing 2 mM calcium with the response to adenosine delivered with 0 mM calcium and 2 mM cobalt. The mean ratio (±SD) of the calcium–adenosine to cobalt–adenosine responses was 1.07 ± 0.07, and so we conclude that calcium influx has no intermediary role in the activation of the potassium conductance by adenosine. Segal (12) and Haas and Greene (13) have also found that blocking calcium currents with cobalt or cadmium had no effect on the hyperpolarization of pyramidal neurons produced by adenosine. However, a requirement for intracellular calcium, or the release of intracellular calcium stores by adenosine, cannot be excluded by any of these experiments.

One striking feature of this potassium current is that it grew smaller during the course of an experiment, sometimes disappearing entirely within 5–30 min after the first measurement. In other respects, the cells being studied appeared normal, often showing a slight enhancement of their resting potential and input resistance. This "washout" of the adenosine response could be slowed or prevented by using high-resistance (10–20 MΩ) patch electrodes for recording. These high-resistance electrodes had high series resistances (30–100 MΩ) as compared to the low-resistance (1–3 MΩ) electrodes used elsewhere in this study (with series resistances from 8 to 20 MΩ). Although high series resistances did compromise our ability to change the holding potential, they did not obscure the responses. We expect that high series resistances served as a barrier to diffusive loss of cytoplasmic macromolecules.

In Fig. 6, the time course of the loss of adenosine responsiveness is shown as a percent of the initial response. For a recording with a 2 MΩ electrode the series resistance was 14 MΩ; the response was completely lost within 15 min (filled circles). In a different experiment with a 15 MΩ electrode the series resistance was 100 MΩ; only 10% of the response was lost after 30 min (triangles). In contrast to the adenosine response, GABA-induced currents recorded with low-resistance electrodes were not lost (Fig. 6, open circles) and frequently even increased in amplitude during the recording. Since the loss of the adenosine response could be prevented with a high-resistance recording electrode, it is not likely to represent desensitization. Rather, it is probable that the adenosine response requires intracellular factors that can diffuse out through the patch electrode. A similar dependence on electrode tip size was found for the rate of disappearance of calcium currents during intracellular perfusion of snail neurons (26). Preliminary attempts to prevent washout with ATP, cAMP, low $Mg^{2+}$, altered internal pH, or different salts such as $Cs^+$, $SO_4^{2-}$, $Cl^-$, or citrate were not successful.

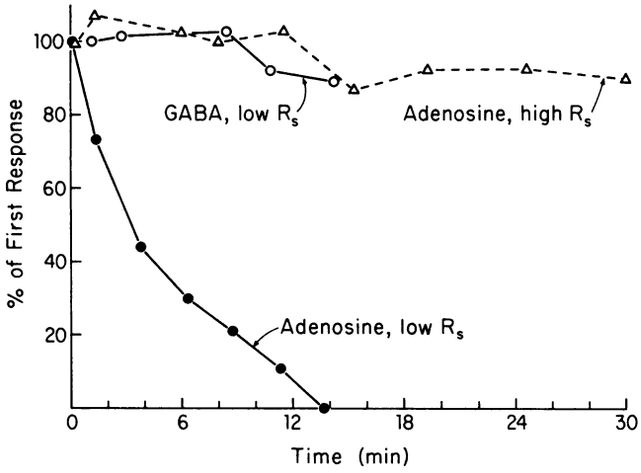


FIG. 6. Responses to adenosine and GABA as a function of time after establishing intracellular recording. △, Adenosine responses in which the recording electrode had a 15 MΩ resistance and the series resistance ($R_s$) was 100 MΩ. In this recording the adenosine responses declined only slightly in 30 min. The circles are responses of a single cell to GABA (○) and adenosine (●) using a 2 MΩ recording electrode in which the series resistance was 14 MΩ. With the low-resistance electrode the adenosine response declined to zero in 14 min while the GABA response changed only slightly.

## DISCUSSION

Evidence drawn from studies in the central nervous system and peripheral tissues strongly suggests that adenosine activates a potassium conductance and may also directly inhibit calcium-mediated events (8–12). The mechanism of inhibition of transmitter release is unclear, although it is of interest that changing external calcium concentration does not affect the magnitude of the reduction of transmitter release by adenosine in central or peripheral synapses (8, 9). It should be emphasized that we do not know if the block of spontaneous activity in our cultures by adenosine is due solely to activation of the potassium conductance described above. The possibility exists that adenosine has additional actions on another receptor in this preparation. The size of the conductance change and the voltage sensitivity would argue that this potassium mechanism might alter excitability only to small or brief depolarizations. However, if these potassium channels and their receptors are more densely localized at nerve terminals and postsynaptic dendritic regions, the effect on synaptic potentials could be more profound.

The voltage dependence of the adenosine-activated conductance probably reflects a direct voltage sensitivity of the potassium channel gating mechanism. In this regard it is similar to other chemically or voltage-activated ion channels. For example, enkephalin in rat locus coeruleus (27), baclofen in rat hippocampus (33), and acetylcholine in frog cardiac ganglion (28) all activate a potassium conductance with a similar voltage sensitivity. Some other potassium conductances are reduced with depolarization, specifically the A current and the anomolous rectifier, both of which exist in cultured striatal neurons (unpublished observations). Recent evidence suggests that A current (29) and anomalous rectifier (30) channel behavior may be regulated by neurotransmitter-sensitive phosphorylation mechanisms. It will therefore be of interest to see how similar the adenosine response is to these two currents in terms of gating kinetics, pharmacological sensitivity, and channel conductance.

Also of interest is the possibility that adenosine activates the potassium conductance through intracellular second messengers, a possibility raised by the slowness of the response, its temperature dependence, and its washout during recordings made with low-resistance patch electrodes. It is also possible that washout occurs simply from a mild cellular trauma. However, in homogenates of rat striatum (31) or cultured mouse striatal neurons (32), adenosine

potently and specifically activates a membrane-bound adenylate cyclase. Furthermore, this response is blocked by theophylline, as is the adenosine activation of the potassium current. Since cyclic nucleotides are an important part of a cell's biochemical response to adenosine, it may be that inclusion of a protein kinase in the patch electrode could prevent the washout. The adenosine response of cultured striatal neurons may therefore present a useful system for studying the intracellular modulation of ion channel gating mechanisms.

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