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Adenosine selectively blocks parallel-fiber-mediated synaptic potentials in rat cerebellar cortex

(adenosine/parallel fibers/cerebellum)

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ABSTRACT **Electrophysiological techniques were used to study the efficacy of adenosine in modulating synaptic transmission mediated from convergent parallel- and climbing-fiber inputs to Purkinje cells. Our results indicate that adenosine application leads to selective blocking of parallel fiber-mediated synaptic activity but not of climbing fiber activity. Adenosine does not alter the action-potential excitability properties of the parallel fibers. However, application of γ-aminobutyric acid (GABA), which directly affects Purkinje cell dendritic membranes [Malenka, R. C. & Kocsis, J. D. (1982) *J. Neurophysiol.* 48, 608–621], leads to reduction of both parallel- and climbing-fiber synaptic activity. These results support the proposals that adenosine receptors in the cerebellar cortex are selectively localized on the nonmyelinated parallel fibers and that the blocking action of adenosine is the result of a mechanism other than direct alteration of axon excitability.**

Adenosine receptors have been described at a variety of presynaptic and postsynaptic sites in the central nervous system (1–7). Much interest has focused on the ability of methylxanthines, such as caffeine and theophylline, to block adenosine receptors. Furthermore, this blockade of adenosine receptors has been linked to behavioral stimulation by the xanthines (8, 9). In a recent study (1), it was shown that the superficial (or "molecular") layer of the cerebellar cortex has an extremely large number of adenosine receptors. However, in mutant Weaver mice where granule cells are absent, and in Reeler mice where the normal migration of granule cells does not occur, adenosine binding in the molecular layer is much reduced (1). These results led to the suggestion that adenosine receptors of the molecular layer are located presynaptically on the parallel fibers (1). Indeed, in a variety of central nerve terminals, the reduction in synaptic activity associated with adenosine application is reported to be mediated presynaptically (2–7). There is evidence that adenosine presynaptic-receptor activation leads to blockade of voltage-dependent Ca^{2+} channels (5, 10, 11) and thereby prevents transmitter release.

Although adenosine blockade of synaptic transmission has been demonstrated in many regions of the central nervous system (2–7), a lack of effect on synaptic transmission in the superior colliculus has been reported (12). The effects of adenosine on convergent inputs to the same neuronal population have not been studied. In this report we compare the effects of adenosine on the convergent parallel- and climbing-fiber synaptic inputs to the Purkinje cell (PK). Adenosine application selectively blocks parallel-fiber (Pf)-mediated synaptic activity but does not block climbing-fiber (Cf)-mediated synaptic activity. Action-potential excitability properties of the Pf are unchanged by adenosine. These results indicate that adenosine is selective in its presynaptic blocking of Pf-synaptic transmission and that it does not have a generalized blocking effect on synaptic transmission. The recent histochemical (1) and electrophysiological identification of adenosine receptors in the cerebellar cortex, with its well-organized structure and superficial location, indicates that the cerebellar cortex may prove valuable for the study of functional properties of presynaptic adenosine receptors *in vivo*.

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MATERIALS AND METHODS

Wistar rats (180–250 g) were anesthetized with a combination of xylazine and ketamine (10 and 90 mg/kg of body weight, respectively). Supplemental doses of anesthetics were given every 2 hr. Body temperature was maintained with a heating pad at 36.5–37.5°C. After tracheal intubation, the animals were maintained on a respirator and their heads were stabilized in a stereotaxic frame. A hole was made in the occipital bone to expose part of the cerebellar vermis, and a perfusion pool was made around the exposed cerebellum with 3% agar in normal Krebs solution (NS), which contained 124 mM NaCl, 3 mM KCl, 2 mM $MgCl_2$, 2 mM $CaCl_2$, 26 mM $NaHCO_3$, and 10 mM D-glucose, and which was saturated with 95% O_2/5% CO_2. The NS was warmed to 37°C and continuously perfused over the exposed cerebellum. Adenosine (hemisulfate salt), 2-chloroadenosine, theophylline, or γ-aminobutyric acid (GABA) solutions of various concentrations were added to the perfusion.

After the dura was cut, two Teflon-coated bipolar stainless-steel electrodes were placed on the cerebellar surface. These were used to stimulate the Pf. Another pair of stimulating electrodes was positioned stereotactically in the medulla between the olivary nuclei to activate Cf where they decussate (intersect) in the brainstem. Field potentials were measured with glass microelectrodes filled with 3 M NaCl. The signals were amplified and either photographed or digitized and stored on magnetic tape. (Further details of experimental methods can be found in refs. 13 and 14.)

RESULTS

The arrangement of the stimulating and recording electrodes used in these experiments is shown in a schematic of a longitudinal section through a folium of the cerebellar cortex (Fig. 1*a*). Stimulation of the cerebellar surface leads to a well-known field potential that can be recorded along the longitudinal axis of a folium (Fig. 1*b*; refs. 14–16). The early positive–negative–positive (P1–N1–P2) components of the field potential correspond to the action-potential activity of the Pf. The N2 component of the field potential corresponds to

Abbreviations: Pf, parallel fiber(s); Cf, climbing fiber(s); PK, Purkinje cell(s); GABA, γ-aminobutyric acid; P1 and P2, first and second positive voltage deflections, respectively; N1 and N2, first and second negative voltage deflections; NS, normal Krebs solution (124 mM NaCl/3 mM KCl/2 mM $MgCl_2$/2 mM $CaCl_2$/26 mM $NaHCO_3$/10 mM D-glucose, saturated with 95% O_2/5% CO_2).

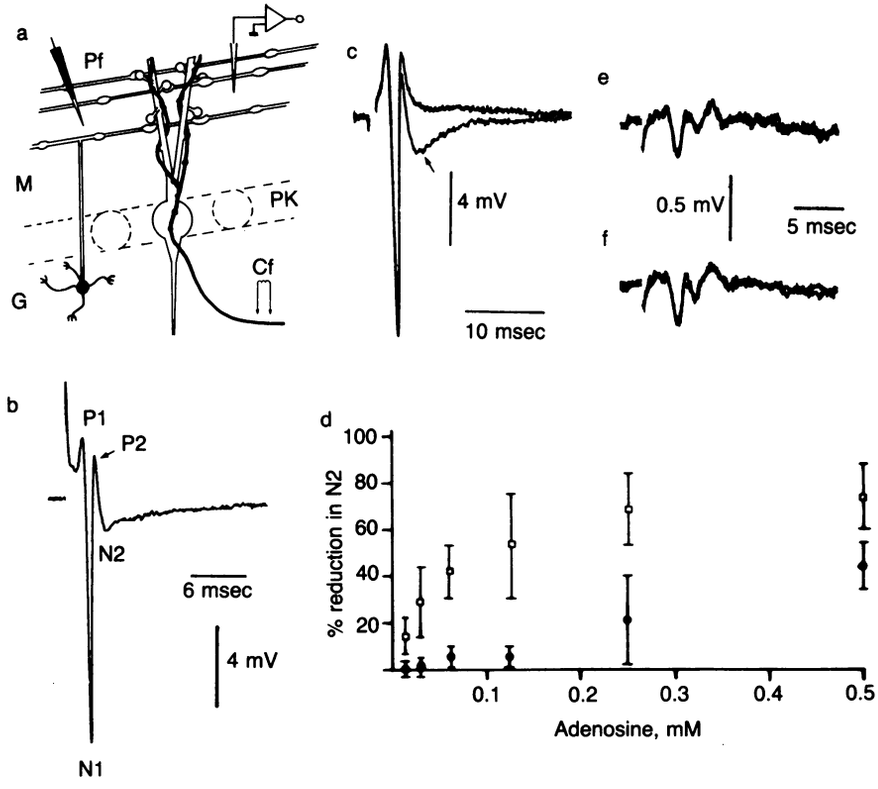

FIG. 1. (*a*) Schematic showing arrangement of stimulating and recording electrodes in the cerebellar cortex. The Pf were stimulated on the surface and the Cf were stimulated in the medulla. G, granule cell; M, molecular layer; PK, Purkinje cell. (*b*) A field potential induced by Pf stimulation. P1, N1, and P2 correspond to action-potential activity in the Pf. The N2 component is the result of synaptic activation of molecular layer dendritic elements. (*c*) Pf-induced field potentials recorded before (arrow) and after application of adenosine (100 μM). Note the reduction of N2 (synaptic component) and the lack of effect on N1 (Pf volley). (*d*) Dose–response curve showing the reduction in N2 amplitude vs. adenosine concentration in the absence (□) and presence (●) of 0.2 mM theophylline. The mean and standard deviation from three experiments are shown. There is no appreciable change in the field potential induced by Cf stimulation before (*e*) or after (*f*) adenosine application (1.0 mM). Positive voltage deflections are upward in this and subsequent figures.

synaptic activation of molecular-layer dendritic elements mediated by the Pf. Stimulation of the oliva activates Cf and leads to another well-known field potential (17).

Adenosine selectively blocks Pf-mediated synaptic activation (N2) but not the Cf-mediated response. The superimposed traces shown in Fig. 1*c* were recorded before and after addition of 200 μM adenosine to the superfusion pool. The N2, or synaptic, component of the field potential was much reduced in the presence of adenosine; however, the latency and amplitude of N1 (the Pf action potential) did not change in the presence of adenosine. 2-Chloroadenosine had effects similar to those of adenosine. The block of the N2 component was dose-dependent and antagonized by theophylline as shown in the dose–response curves in Fig. 1*d*.

In contrast to the Pf-mediated synaptic transmission, the Cf-mediated response was not reduced by adenosine. The field potential responses in Fig. 1*e* and *f* were recorded after Cf activation in NS and in NS containing 1 mM adenosine, respectively. The recordings were obtained within the superficial 100 μm of the molecular-layer. Cf activation elicits a field potential responses in Fig. 1 *e* and *f* were recorded after sponds to bursts of action potentials in PK as shown by intracellular recordings (13, 17, 18). The initial negative deflection represents a somatic spike, but the later components of the field represent dendritic activity (13, 18). Adenosine did not block the somatic or the dendritic activity (Fig. 1*f*).

The blocking action of adenosine on Pf synaptic transmission is a rapid and reproducible effect. The time course of action for five consecutive applications of adenosine is shown in Fig. 2*a*. Within seconds after adenosine enters the perfusion pool, the N2 component of the field potential is reduced in amplitude. The reversal of this effect by washing with NS was equally rapid and complete. However, the amplitude of the Cf-induced field potential was not significantly altered by adenosine application (Fig. 2*b*).

The excitability of the fine-caliber Pf is very much influenced by previous impulse activity and by changes in the extracellular ionic environment (13, 14, 19, 20). Changes in Pf electrical excitability could lead to changes in Pf-mediated synaptic transmission. The basic excitability properties of the Pf were not influenced by adenosine. The amplitude, waveform, and conduction velocity of the Pf volley were unaffected by adenosine application (see Fig. 1*c*). This indicates that the reduction in N2 was not mediated secondarily to the blocking of Pf action-potential activity.

To study Pf excitability, recovery curves were obtained in paired-stimulation experiments. The interstimulus interval was varied and the latency variation of the conditioned response was compared to control latency. It is well established that Pf latency is increased during the relative refractory period and decreased during the supernormal period (14, 20). Superimposed traces of a conditioned and unconditioned Pf response in NS at an interstimulus interval of 10 msec are shown in Fig. 3*a*. Note the reduction in latency (i.e., increased conduction velocity) of the conditioned response. The relative refractory and supernormal periods are not appreciably altered by adenosine (Fig. 3*b*), although N2 is virtually eliminated by adenosine. The recovery curves in Fig. 3*c*, which were obtained before and after additions of adenosine, show that the recovery properties of the Pf are not altered by adenosine. The stabilities of Pf waveform, conduction velocity, and excitability properties after adenosine application indicate that a mechanism other than reduction of presynaptic action-potential excitability accounts for the blocking action of adenosine on Pf-induced synaptic transmission.

GABA can powerfully block both Pf-mediated and Cf-mediated synaptic transmission (13). The mechanism may be the opening of dendritic Cl^- channels that "clamp" the PK dendrites at the Cl^- equilibrium potential so that the PK dendrites are hyperpolarized. In contrast to the lack of effect of adenosine on Cf-induced activation of the PK, GABA blocked the Cf response. In Fig. 4*a*, superimposed traces show the loss of the Pf-induced N2 component recorded in the molecular layer after adenosine application. GABA application has a similar blocking effect on N2 (Fig. 4*b*). A Cf-induced response in NS, recorded at the same site as the response in Fig. 4*b*, is shown in Fig. 4*c*. In contrast to adeno-

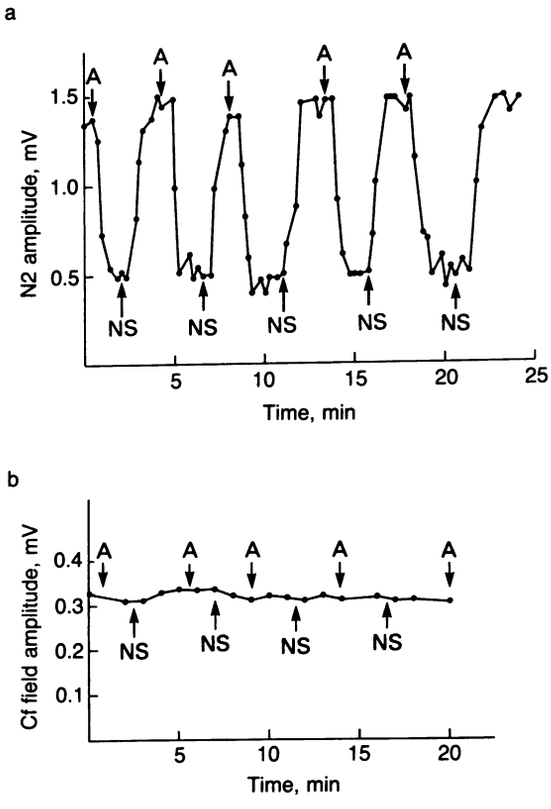

FIG. 2. (*a*) N2 amplitude (i.e., the synaptic component of the Pf-induced field) was plotted vs. time during alternating perfusions with 100 μM adenosine in NS (A) and NS. After adenosine application the amplitude of the field potential was rapidly reduced. Washing with NS led to a rapid reversal of this effect. (*b*) Alternating washes of adenosine and NS did not lead to appreciable changes in the Cf-induced field potential. Arrows indicate the times at which perfusions were changed.

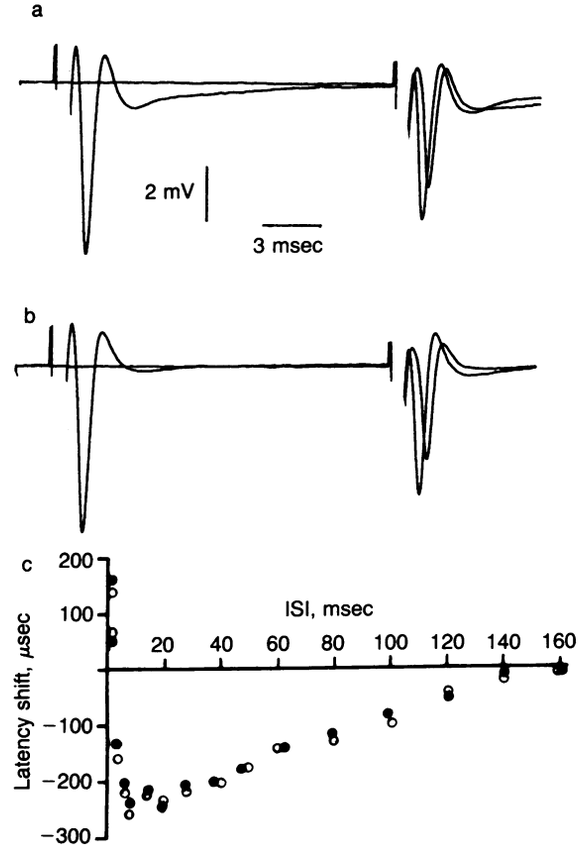

FIG. 3. Response induced from paired Pf stimulation [10-msec interstimulus interval (ISI)] in NS (*a*) and after adenosine application (*b*). Responses elicited by paired stimuli and from a single stimulus are superimposed. The shorter latency response is the conditioned response. The reduction in latency corresponds to an increase in conduction velocity and is indicative of the supernormal period. (*c*) Recovery curves plotting the latency variation of P1 (first positive deflection) before (○) and after (●) adenosine application.

sine, GABA causes a large reduction in the Cf-induced response (Fig. 4*d*). Perfusion with NS containing 1 mM adenosine (Fig. 4*e*) restores the Cf-induced field potential to that seen in NS alone (Fig. 4*f*). These results further support the proposal that adenosine action is specific to Pf–PK synaptic transmission in the cerebellum and does not have a generalized effect on all synaptic transmission.

DISCUSSION

The molecular layer of the cerebellar cortex has a high density of adenosine receptors that may be located presynaptically on the Pf (1). Adenosine has been shown to reduce synaptic transmission in a number of regions in the central nervous system (2–7), but synaptic transmission in the superior colliculus is unaffected by adenosine (12). To our knowledge, adenosine action on convergent synaptic inputs to the same neuronal population has not been previously studied. We have shown that adenosine selectively blocks the Pf-mediated synaptic transmission but does not block Cf-mediated synaptic activity of PK. Thus, adenosine does not have a generalized blocking effect on synaptic transmission. Furthermore, Pf action-potential characteristics and excitability properties are unaffected by adenosine. These results support the proposal that adenosine receptors are located on presynaptic membranes of Pf (1) and indicate that the presynaptic blocking effect of adenosine is not the result of reduced excitability of afferent Pf.

The mechanism of the adenosine-induced reduction of transmission is not understood. A proposed mechanism is that adenosine receptor activation leads to a blockade of voltage-dependent Ca^{2+}-conductance at the terminal (5, 10, 11). Adenosine-induced blockade of the Ca^{2+}-conductance of terminals could regulate synaptic transmission by reducing Ca^{2+} entry into the terminals and thereby reducing transmitter release. In the case of the Pf–PK spine synapse, this regulation would be of an excitatory synaptic input whose transmitter may be glutamate (21). The adenosine action is not likely to be a simple competitive block of Ca^{2+} channels, as is the case with extracellular divalent cation application, because Cf synaptic activation is unaffected by adenosine. This also indicates that the PK were not appreciably affected by adenosine since the Cf field potential reflects activity generated postsynaptically in the PK.

There are no morphological descriptions of axo-axonic connections on Pf. Therefore, the adenosine receptors on the Pf are not likely to be at subsynaptic sites. Perhaps circulating adenosine can activate the Pf adenosine receptors. Physiological changes in levels of adenosine might result in subtle changes in Pf–PK transmission and possibly in motor performance. Goodman *et al.* (1) have proposed that the tremor associated with caffeine ingestion may be related to adenosine-receptor block of Pf transmission.

Studies in the peripheral nervous system indicate that adenosine (in the form of its nucleoside triphosphate) may be contained within the same vesicles as the primary transmitter and released as a cotransmitter (5, 22). It is possible that, in the cerebellum, adenosine is released as a cotransmitter along with glutamate at the Pf–PK synapse. Whereas gluta-

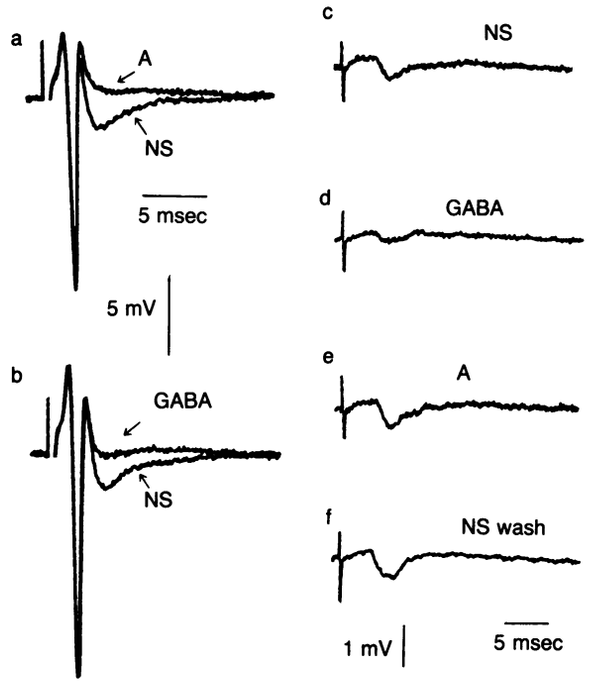

FIG. 4. (*a*) Adenosine (A, 1 mM) blocks the N2 component of the Pf field potential. (*b*) GABA (2 mM) also blocks the N2 component. (*c*) Cf-induced field potential recorded in NS. (*d*) GABA leads to reduction in the Cf field potential, (*e*) adenosine (A) does not.

mate may act primarily to influence postsynaptic PK membranes, adenosine may act on the Pf terminal. This feedback by adenosine to the Pf terminal could provide an effective means of activity-dependent modulation of Pf synaptic transmission.

Adenosine could also be a cotransmitter not within Pf terminals but within Cf terminals. It has been reported in several studies that Cf activation of the PK can lead to relatively long-term modulation of Pf-mediated synaptic transmission. Changes in PK activity have been implicated in motor plasticity in the cerebellum (23–26). If adenosine is released during Cf activation of the PK, it might influence Pf transmitter release via Pf adenosine receptors.

Another form of activity-dependent regulation of transmitter release in the cerebellum occurs due to changes in the extracellular ionic environment (13, 14, 24). Pf excitability is exquisitely sensitive to changes in extracellular K^+ concentration (13, 14, 19). Small increases in extracellular K^+ lead to increased Pf excitability, and larger increases lead to decreased excitability (11). The source of much of the activity-dependent extracellular K^+ is the PK (27, 28). Therefore, as action-potential activity in the PK increases, associated changes in extracellular K^+ occur and influence the excitability and transmitter output of the adjacent Pf. Unlike extracellular K^+, which changes the action-potential excitability properties of the Pf action potential, adenosine does not alter the basic excitability and conduction properties of the Pf. Both of these effects, activity-dependent changes in the extracellular ionic environment and activation of terminal adenosine receptors, may allow regulation of Pf–PK synaptic transmission without involvement of a conventional synapse.

The simultaneous study of presynaptic and postsynaptic activity is difficult in the intact central nervous system. However, the cerebellar cortex is well suited for such study. Its laminar organization allows recording of well-defined presynaptic and postsynaptic field-potential components (15–17), and the superficial location of dendritic synaptic activity allows the application of drugs and changes in the ionic environment to be made (13, 14, 19, 25). The cerebellar cortex *in vivo* can also be probed by systemic drug application. The high density of adenosine receptors in the cerebellar cortex (1), the accessibility of the cerebellar cortex to electrophysiological study, and the demonstration that adenosine is selective in its action on the Pf make the cerebellar cortex a very useful neuronal model for further analysis *in vivo* of the functional properties of presynaptic adenosine receptors in the mammalian brain.

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