Adenosine: Autoradiographic Localization and Electrophysiologic Effects in the Cat Retina

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Autoradiography with ³H-adenosine was used to localize cells that accumulate adenosine in the cat retina. Electrophysiologic effects elicited by adenosine on DC-electroretinograms (ERG) and optic nerve responses (ONR) were studied in isolated, arterially perfused cat eyes. Subpopulations of cells localized in the ganglion cell layer and inner nuclear layer showed clear labeling for adenosine. This purine nucleoside enhanced the ERG b-wave and the standing potential; depressed the light peak; and markedly depressed the ONR, in which it reduced the amplitudes of the ON-, plateau-, and OFF-components. A vasodilatory action of adenosine was documented by an increase in perfusion flow rate. Our data suggest that adenosine in cat retina has complex modulatory effects, involving the retinal pigment epithelium, neuronal structures, blood vessels, and probably glial cells. Invest Ophthalmol Vis Sci 30:2533-2536, 1989

Evidence for a neuromodulatory function of adenosine in the central nervous system has been widely accepted over the last decade.¹ A protective role of adenosine during hypoxia has been postulated, since the concentration of this purine has been shown to increase during ischemia, producing vasodilation, depression of ganglion firing rate, stimulation of glycolysis, and an anticonvulsant effect.² A functional role for adenosine in neurotransmission within the retina can be postulated based on its presence and potential role for adenosine in neurotransmission within depression of ganglion firing rate, stimulation of glycolysis, and an anticonvulsant effect.² A functional role for adenosine in neurotransmission within the retina can be postulated based on its presence and potential role for adenosine in neurotransmission within depression of ganglion firing rate, stimulation of glycolysis, and an anticonvulsant effect.² A functional role for adenosine in neurotransmission within the retina can be postulated based on its presence and potential role for adenosine in neurotransmission within depression of ganglion firing rate, stimulation of glycolysis, and an anticonvulsant effect.² A functional role for adenosine in neurotransmission within the retina can be postulated based on its presence and potential role for adenosine in neurotransmission within depression of ganglion firing rate, stimulation of glycolysis, and an anticonvulsant effect.² A functional role for adenosine in neurotransmission within the retina can be postulated based on its presence and potential role for adenosine in neurotransmission within depression of ganglion firing rate, stimulation of glycolysis, and an anticonvulsant effect.² A functional role for adenosine in neurotransmission within the retina can be postulated based on its presence and potential role for adenosine in neurotransmission within depression of ganglion firing rate, stimulation of glycolysis, and an anticonvulsant effect.² A functional role for adenosine in neurotransmission within the retina can be postulated based on its presence and potential role for adenosine in neurotransmission within depression of ganglion firing rate, stimulation of glycolysis, and an anticonvulsant effect.²

Materials and Methods. This study adhered to the ARVO Resolution on the Use of Animals in Research.

Autoradiography: 50 µCi of ³H-adenosine (SA, 18 Ci/mmol; Amersham, Arlington, IL) were injected into each eye of an adult cat under deep anesthesia induced by a mixture of ketamine (Parke Davis, Morris Plains, NJ) and Xylazine (Bayer, Leverkusen, West Germany). Throughout the following 4-hr period, anesthesia was maintained. At 4 hr post-injection, the cat was sacrificed by decapitation. The eyes were enucleated and anterior portions discarded, and the eyecups were fixed in 2.5% glutaraldehyde (TAAB Lab, Reading, England) in cacodylate buffer (0.16 M, pH 7.4). The eyecups were stained with osmium (EM Sciences, Ft. Washington, PA), dehydrated in ethanol, and embedded in Araldite. Sections, 1 µm, were placed on clean slides, dipped in Kodak NTB-2 emulsion (Kodak, Rochester, NY), and air-dried. The slides were stored in light-boxes at 4°C. Autoradiograms were developed after 4 weeks in Kodak D-19 and lightly stained with Richardson's stain.

Electrophysiology in arterially perfused eyes: Nine eyes were enucleated from anesthetized adult cats and connected to a perfusion system. The perfusate consisted of oxygenated, HEPES-buffered tissue culture medium 199, enriched with 30% newborn calf serum (for details see refs. 8 and 9). The flow rate of perfusate, usually adjusted to approximately 1.5 ml/min, was monitored by measuring the intervals between drops with an infrared beam passed through a drop chamber.⁸ Drug-induced changes in flow rate indicated changes in resistance in the vasculature of the isolated eye. The DC-electroretinogram (ERG) was recorded with an intravitreal salt-bridge electrode referenced to the posterior pole of the eye. The light-evoked optic nerve response (ONR) was recorded between a Ag–AgCl suction-electrode at the end and an Ag–AgCl electrode on the surface of the nerve. For stimulation in dark adaptation, red light (620-nm), was pulsed (pulse duration 0.4 sec, intervals 30 sec) and for rod match was attenuated by neutral density filters to stimulate selectively the rod system. To elicit light peaks we delivered pulses of diffuse white light, 60 sec in duration, to fully dark-adapted eyes. Recordings were taken before, during, and after injection of adenosine (20 experimental series), which generally was injected for 10 min at arterial concentrations ranging from 0.1 to 20 µM.

Results. Autoradiography: The accumulation of ³H-adenosine in cat eye cups showed distinct retinal labeling in both the ganglion cell and inner nuclear layers (Figures 1, 2). Labeled cell bodies in the ganglion cell layer probably corresponded to true ganglion cells and perhaps to displaced amacrine cells, while in the inner nuclear layer, the heaviest label was associated over cell bodies in the vitreal half of the layer and probably was associated with amacrine
Figs. 1 (top), and 2 (bottom). Photomicrographs of cat retinas, fixed 4 hr after intravitreal injection of 3H-adenosine (50 μCi) and developed after 4 weeks. Adenosine is significantly accumulated in ganglion cells (Fig. 2, arrows) and in cells located in the inner nuclear layer (Fig. 1, arrows). Note in Figure 2 that the black streaks among the OS are due to entrapped air in the plastic section. Photomicrographs were taken from the right (Fig. 1) and the left eye (Fig. 2) of the same animal. GC = ganglion cell layer; IPL = inner plexiform layer; INL = inner nuclear layer; OS = outer segments.

Richardson's, Bar = 20 μm.

cells. Perivascular labeling was also observed, as shown in the ganglion cell layer in Figure 1.

The discrete labeling of the pigment epithelium might have indicated specific binding, although the resolution was inadequate for conclusive differentiation from melanin granules (data not shown).

Electrophysiology: Adenosine consistently induced marked and largely reversible changes in standing potential (SP), light peak (LP), electroretinogram, flow rate of perfusate, and action potential of the optic nerve.

SP and LP: The enhancing effect of adenosine on the SP is illustrated in Figure 3. Shortly after application of 30 μM adenosine (middle trace) the SP increased by 3.1 mV, with recovery after termination of the injection. This enhancement of the SP was consistent and dose-dependent. LP were elicited (Fig. 3) before (control LP), during (middle LP), and after (recovery LP) injection of adenosine. Adenosine strongly depressed the LP; this effect was confirmed in other experiments and was reversible upon washout.

ERG b-wave: The amplitude of the dark-adapted and rod-matched ERG b-wave was reversibly enhanced (Fig. 4A) and revealed some dose-dependency only within each preparation. A concomitant increase in perfusion flow rate in the range of 7-27% (average 13%) reflected vasodilation and was only partly responsible for the initial enhancement of the b-wave. This latter finding was tested by increasing manually the perfusion flow rate to the same extent as had been reached under adenosine; this procedure led to smaller increases in b-wave amplitude (data not shown). In Figure 4A, the time course of changes in b-wave amplitudes during a 10-min injection of 2.5 μM and 5.5 μM adenosine are shown. The inset shows original ERG traces of this trial before (fine trace) and at the time of maximal effect (heavy trace) of perfusion with 2.5 μM adenosine.

Optic nerve response: The strongest effects of adenosine were observed in the ONR, which was depressed in all components. Figure 4B illustrates the time course of changes in the ONR ON-component in response to 2.5 and 5.5 μM adenosine (same experiments as shown in Fig. 4A). The changes in amplitude of the ONR ON-component lasted longer...
Fig. 4. Time course of changes in normalized ERG b-wave amplitude (A) and ONR ON-response component (B) in response to 10-min injections of 2.5 (continuous lines) and 5.5 μM (dashed lines) adenosine. Original data of the same trial (2.5 μM) are plotted in the insets: traces were taken before (fine traces) and during (heavy traces) the maximal effect. The stimulus consisted of a pulse of dim (about 1.5 log above threshold) red light (620 nm) matched with a 400-nm pulse for the rod system and applied in full dark adaptation. It should be noted that in the inset in (A) the rod-matched ERG traces, revealing adenosine-induced increase, were recorded near b-wave threshold. Slightly higher stimulus intensities produced more transient, larger, and faster b-waves. These responses at higher stimulus intensities revealed the same increase in amplitude as illustrated here.

than the effects of adenosine on the ERG (Fig. 4A) and were dose-dependent, at least among trials in the same preparation. The inset shows a control and the test trace (at the time of the maximal effect) of ONRs with 2.5 μM adenosine. When the perfusate flow was manually increased to match the flow increase observed during the injection of adenosine, the result was a clear increase in amplitude of the ONR, in contrast to the decrease observed under adenosine.

Discussion. The distribution of adenosine-accumulating cells in the cat retina showed distinct labeling in the ganglion cell and inner nuclear layers. At both sites, subgroups of cells were labeled and were clearly distinguishable from the background. This pattern was similar to that described for other mammalian retinas, including that of the human. Blazynski et al reported that among rabbit, ground squirrel, and mouse retinas, the pattern of labeling for adenosine uptake and adenosine-like immunoreactivity is very similar. In the current study of the cat retina, uptake of adenosine was found to be identical with these previously reported patterns for the other mammalian species. It has recently been demonstrated that optic nerve transection in the rabbit results in the loss of approximately 50% of the cell bodies labeled for adenosine-like immunoreactivity, indicating that some of the label must be associated with displaced amacrine cell bodies (Blazynski et al, manuscript submitted). The similarities between mammalian species lead us to suggest that those cell bodies in the ganglion cell layer labeled by adenosine uptake also correspond to both ganglion cells and displaced amacrine cells.

Earlier electrophysiologic observations by two of the current authors (GN, BF) and Onoe to our knowledge the first reported for the retina, correspond well to the autoradiographically identified sites of adenosine uptake. The most dramatic effect was depression of the optic nerve activity, which may therefore be related to adenosine uptake in neurons of the inner nuclear and ganglion cell layers. This inhibitory action seemed to parallel that described for cerebral neurons. The adenosine-induced enhancement of the ERG b-wave indicated action in the middle layer of the retina, possibly involving Müller cells. Astrocytes have been shown to contain receptors for adenosine. The silver grain distribution, shown in Figures 1 and 2, did not indicate whether Müller cells, in addition to neurons, were labeled. In other mammalian retinas labeling of Müller cells has never been observed. Enhancement of the b-wave may have been induced by elevation in retinal glucose resulting from adenosine-induced glycogenolysis. The increase in flow rate during adenosine perfusion, a result of retinal and choroidal vasodilation, was ruled not to be the only factor enhancing the b-wave, by comparison to the results of manual flow increase without application of active agents.

The retinal pigment epithelium (RPE), although not definitively labeled with light microscopy autoradiography, revealed two distinct adenosine effects: the dose-dependent increase in the SP and the depression of the LP. An increase in c-wave amplitude (data not shown) also suggested an effect of adenosine; the c-wave increase might have depended on the same underlying mechanism as the effects of azide and of hypoxia, ie, on depolarization of the basal RPE membrane. These effects strongly support the view that adenosine, like monoamines, play a role in the communication between retina and RPE. We
would therefore expect intense labeling of the cat RPE in electron microscopic autoradiography with tritiated adenosine.

The data presented thus provide evidence for uptake and a powerful modulatory role of adenosine at multiple sites in the cat retina.

Key words: adenosine, retina, optic nerve, perfused cat eye, autoradiography

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