*Proc. Natl. Acad. Sci. USA*
Vol. 84, pp. 3906–3910, June 1987
Neurobiology

# Endogenous adenosine and adenosine receptors localized to ganglion cells of the retina

(immunocytochemistry/receptor autoradiography/phenylisopropyladenosine)

KAREN M. BRAAS*, MARCO A. ZARBIN†, AND SOLOMON H. SNYDER*‡

*Departments of Neuroscience, Pharmacology and Molecular Sciences, and Psychiatry and Behavioral Sciences, The Johns Hopkins University School of Medicine, 725 North Wolfe Street, Baltimore, MD 21205; and †The Wilmer Ophthalmological Institute, The Johns Hopkins Hospital, Baltimore, MD 21205

*Contributed by Solomon H. Snyder, December 31, 1986*

ABSTRACT **Using specific sensitive antisera against adenosine, we have immunocytochemically localized endogenous adenosine to specific layers of rat, guinea pig, monkey, and human retina. Highest adenosine immunoreactivity was observed in ganglion cells and their processes in the optic nerve fiber layer. Substantial staining was also found throughout the inner plexiform layer and in select cells in the inner nuclear layer. Adenosine $A_1$ receptors, labeled with the agonists L-[$^3$H]phenylisopropyladenosine and $^{125}$I-labeled hydroxyphenylisopropyladenosine, were autoradiographically localized. The highest levels of binding sites occurred in the nerve fiber, ganglion cell, and inner plexiform layers of the retina in all the species examined. The distribution of adenosine $A_1$ receptor sites closely parallels that of retinal neurons and fibers containing immunoreactive adenosine. These results suggest a role for endogenous adenosine as a coneurotransmitter in ganglion cells and their fibers in the optic nerve.**

The purine nucleoside adenosine influences numerous physiological processes, including effects on neuronal communication within the central nervous system. These observations suggest that adenosine may serve a role as a neurotransmitter or neuromodulator. Adenosine and its analogues inhibit the release of other neurotransmitters (1–7) and depress both spontaneous and stimulated neuronal depolarization (8). The actions of adenosine are mediated by two receptor subtypes: high affinity $A_1$ receptors inhibit adenylate cyclase activity, whereas low affinity $A_2$ receptors stimulate the enzyme activity (9, 10). Immunocytochemical localizations of endogenous adenosine in neuronal perikarya and fibers in discrete regions of the brain (11) support a neurotransmitter or neuromodulator role.

A variety of neurotransmitters, including neuropeptides, amino acids, and biogenic amines, have been associated with retinal neurons. Several neurotransmitter candidates have been described for amacrine, horizontal, and bipolar cell populations, whereas possible neurotransmitters of other retinal cell types are less clearly defined (12, 13). Axons of the ganglion cells, the only retinal output neurons, enter the optic nerve and project to many structures in the brain, primarily the dorsal lateral geniculate nucleus and the superior colliculus. Ganglion cell depolarization elicits excitatory responses in the target areas, indicating that a primary neurotransmitter is stimulatory in nature (14, 15). Despite the importance of retinal ganglion cells, their neurotransmitter has not been definitively established. Adenosine and adenosine analogues modulate receptor-mediated adenylate cyclase activity in chicken and rabbit retina (16–19). In addition, retinal cells accumulate [$^3$H]adenosine in a sodium-dependent, calcium-independent manner, which is blocked by adenosine agonists (20, 21). Purine nucleosides and bases are released from retinal cells following depolarization (22, 23). Utilizing specific immunocytochemical techniques, we now describe the localization of endogenous adenosine to ganglion cells of the retina, their projections in the optic nerve, and cells in the inner nuclear layer. Parallel autoradiographic studies reveal receptors in the same retinal structures.

## MATERIALS AND METHODS

**Materials.** Reagents were obtained from the following sources: calf intestinal adenosine deaminase, L-phenylisopropyladenosine (L-PIA), D-PIA, hydroxyphenylisopropyladenosine (HPIA), Boehringer Mannheim; L-[$^3$H]PIA (49.9 Ci/mmol; 1 Ci = 37 GBq), New England Nuclear; NTB-3 emulsion, Kodak; Ultrofilm, LKB; biotinylated goat anti-rabbit IgG and avidin–biotin–peroxidase complex (ABC), Vector Laboratories. All other reagents were of analytical grade.

**Tissue Preparation for Immunocytochemical Stain.** Adult male Sprague–Dawley rats (150–250 g) or Hartley guinea pigs (250–350 g), under sodium pentobarbital anesthesia, were perfused intracardially first with Hanks' balanced salt solution and then with 2.5% glutaraldehyde in 0.15 M sodium phosphate buffer (pH 7.4). Cynamolgus monkeys (*Macaca fascicularis*) were sacrificed by intraaortic perfusion with Hanks' balanced salt solution; this was followed by perfusion with buffered 2.5% glutaraldehyde. Donated human eyes, obtained from the Medical Eye Bank of Maryland (Baltimore), were immersion-fixed in the same fixative within 24 hr of death. Following fixation, all eyes were washed with 0.32 M buffered sucrose, dehydrated, cleared, and embedded in paraffin media.

**Immunocytochemical Stain.** Paraffin tissue sections (8 μm) were stained using a modification of the avidin–biotin–peroxidase complex (ABC) technique (11, 24, 25). The production and characterization of specific antisera to adenosine using the adenosine analogue laevulinic acid ($O^{2',3'}$-adenosine acetal) has been described previously (26). Briefly, tissue was deparaffinized, rehydrated, and immunocytochemically stained as follows: (*i*) incubation with 0.2% Triton X-100 in 0.1 M sodium phosphate buffer for 20 min; (*ii*) blocking with 1:200 normal goat serum (NGS) for 15 min; (*iii*) incubation in 1:1000–1:7000 anti-adenosine for 48 hr at 4°C; (*iv*) blocking; (*v*) incubation with 1:400 biotinylated goat anti-rabbit IgG in 1:200 NGS for 90 min; (*vi*) blocking; (*vii*) incubation with 1:200 ABC in 1:200 NGS for 60 min; (*viii*) incubation with diaminobenzidine and $H_2O_2$ as peroxidase reaction substrates (diaminobenzidine at 0.3 mg/ml/0.03% $H_2O_2$/0.05 M Tris, pH 7.6) for 15 min; and (*ix*) exposure to

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: L-PIA, (−)-$N^6$-[(*R*)-phenylisopropyl]adenosine (L-phenylisopropyladenosine); HPIA, (−)-$N^6$-[(*R*)-4-hydroxyphenylisopropyl]adenosine (hydroxyphenylisopropyladenosine).

‡To whom reprint requests should be addressed.

osmium tetroxide vapors for 5–10 min to enhance the reaction product. The specificity of the immunocytochemical stain was determined by using immunoabsorption, enzymatic degradation of tissue antigen, and method controls as previously described (11).

**Tissue Preparation for Receptor Autoradiography.** Adult male Sprague–Dawley rats were placed under sodium pentobarbital anesthesia and sequentially perfused through the left cardiac ventricle with Hanks' balanced salt solution, 0.1% paraformaldehyde in 0.15 M sodium phosphate buffer (pH 7.4), and 0.32 M buffered sucrose. Monkey eyes were removed while the animals were under sodium pentobarbital sedation. Human eyes were obtained as described above. All of the eyes were embedded in brain paste and were frozen in a dry ice/ethanol slurry. Cryostat tissue sections (8 μm) were cut with a microtome, thaw-mounted onto chrome alum/gelatin-coated slides, and stored at −20°C until the autoradiographic procedure was performed.

**Receptor Labeling.** Tissue sections were brought to room temperature and pretreated in 50 mM Tris buffer (pH 7.4) containing adenosine deaminase (1 international unit, as defined by Boehringer Mannheim, per ml) for 30 min to destroy endogenous tissue adenosine. The tissue was then incubated in either 1.25 nM L-[$^3$H]PIA (49.9 Ci/mmol) (27–30) or 120 pM $^{125}$I-labeled HPIA ($^{125}$I-HPIA) (2200 Ci/mmol) (31–34) in 50 mM Tris buffer (pH 7.4) containing adenosine deaminase (1 international unit/ml) for 90 or 120 min, respectively, at 22°C. Blanks were incubated in the same solution in the presence of 10 μM (L-[$^3$H]PIA) or 1 μM ($^{125}$I-HPIA) unlabeled L-PIA. The receptor-labeled sections were washed twice for 5 min each in the same buffer at 4°C, dipped in buffer without adenosine deaminase, dipped in distilled deionized water, and dried rapidly under a stream of cool dry air.

**Autoradiography.** The dried, labeled tissue sections were apposed either to LKB Ultrofilm or to Kodak NTB-3 emulsion-coated coverslips (35, 36). Tissue sections from rat, monkey, and human labeled with L-[$^3$H]PIA were exposed for 28, 42, and 56 days, respectively, whereas tissue sections labeled with $^{125}$I-HPIA were exposed for 2–4 days. After the autoradiograms were developed, the tissue sections were stained with toluidine blue and mounted. Autoradiographic grains were counted over specific retinal laminae as previously described (37) by using a Zeiss Universal microscope fitted with a 0.5-mm grid squares eyepiece reticle.

## RESULTS

In initial experiments, we localized adenosine immunoreactivity to discrete neurons of the rat retina. Staining was most intense in perikarya and select fiber regions. The antisera employed in the present study have previously been shown by radioimmunoassay to be both sensitive and selective for adenosine (26). Several control experiments, as previously described for immunocytochemical staining of the rat brain (1), were conducted to ensure that the immunoreactivity observed was specific for adenosine itself (data not shown). In liquid and solid phase immunoabsorption studies or pretreatment of tissue with adenosine deaminase, retinal staining was abolished to background levels.

In rat and guinea pig retina, adenosine-like immunoreactivity was most prominent in the ganglion cells and their processes in the optic nerve fiber layer (Fig. 1 *A* and *B*). Diffuse staining was also present throughout the inner plexiform layer. Moderate staining was detected in select populations of cells within the inner nuclear layer. Immunoreactivity was absent in the outer nuclear, outer plexiform, and photoreceptor cell layers. Since the predominant adenosine staining was in retinal ganglion cell soma, much of the fiber staining in the inner plexiform layer is probably associated with ganglion cell dendritic processes.

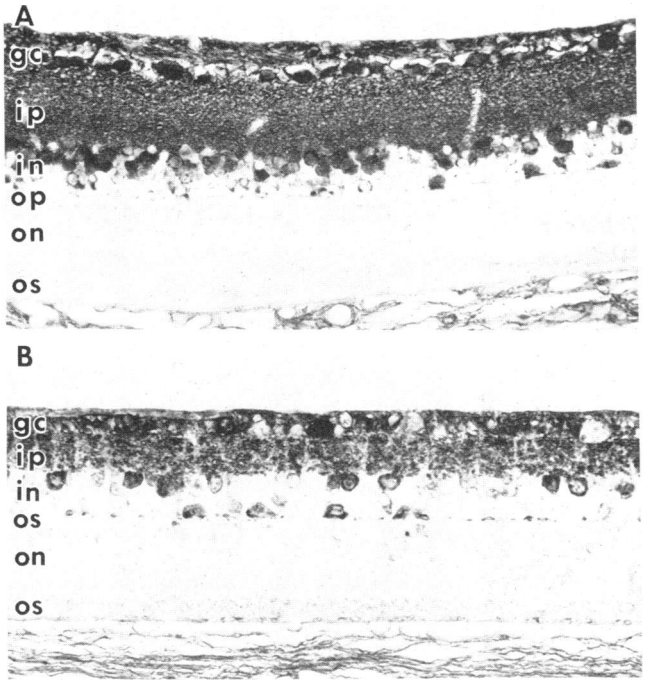


FIG. 1. Immunocytochemical staining of rat and guinea pig retina for adenosine. Rat and guinea pig retina were perfusion-fixed, dehydrated, embedded, sectioned, and immunocytochemically stained with anti-adenosine as described in *Materials and Methods*. (*A*) Adenosine immunoreactivity in rat retina. (*B*) Staining of guinea pig retina for adenosine. Abbreviations for all figures are nf, nerve fiber layer; gc, ganglion cell layer; ip, inner plexiform layer; in, inner nuclear layer; op, outer plexiform layer; on, outer nuclear layer; and os, outer segments of the photoreceptor cell layer.

Tissue sections of monkey and human retina were similarly stained, and immunoreactivity was primarily localized to ganglion cells (Fig. 2 *A–C*). Staining was also present in the inner plexiform layer and in cells of the inner nuclear layer. Unlike the rat retina, monkey and human retinal tissue possessed immunoreactive adenosine staining in a very small population of photoreceptor cells (Fig. 2*D*).

Previously, we demonstrated an association of adenosine $A_1$ receptors with optic nerve projections to the superficial gray layer of the superior colliculus, which were abolished following contralateral enucleation (28, 29). To determine whether adenosine $A_1$ receptors are associated with ganglion cells and optic nerve fibers within the retina, similar autoradiographic localization studies were conducted using the $A_1$ selective ligands L-[$^3$H]PIA and $^{125}$I-HPIA. Autoradiographic grains associated with adenosine $A_1$ receptor labeling were apparent in the nerve fiber, ganglion cell, and inner plexiform layers as well as in the inner portion of the inner nuclear layer of the rat retina (Fig. 3). In the monkey retina, binding was more diffusely distributed from the nerve fiber layer to the outer plexiform layer (Fig. 4). Binding was observed throughout the entire human retina (Fig. 5), but it was enriched in regions of the inner retina, especially in the nerve fiber layer. The distribution of receptor sites in rat, monkey, and human closely parallels that of cells and fibers containing immunoreactive adenosine. Although the autoradiographic grain densities associated with specific retinal laminae varied between rat, monkey, and human tissues (Table 1), the ganglion cell and nerve fiber layers contain high densities of binding sites in all of the species.

Binding of $^{125}$I-HPIA (31–34) to rat retinal membranes was also assayed to characterize the adenosine receptor subtype.

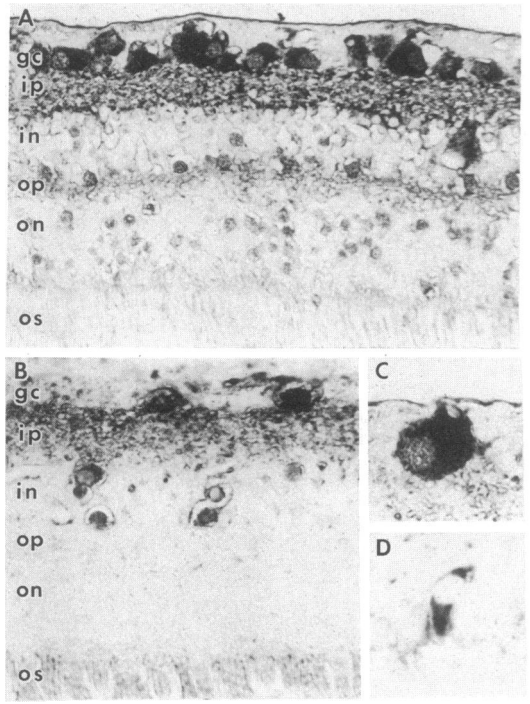


FIG. 2. Staining of monkey and human retina for adenosine immunoreactivity. Perfusion-fixed monkey eyes and immersion-fixed human eyes were dehydrated and embedded in paraffin. Tissue sections were stained with anti-adenosine as described in *Materials and Methods*. (*A*) Adenosine immunoreactivity in the human retina. (*B*) Adenosine staining in the monkey retina. (*C*) High magnification micrograph of staining for adenosine in the ganglion cells of the human retina. (*D*) High magnification micrograph demonstrating adenosine immunoreactivity in a photoreceptor cell of the monkey retina. Abbreviations are as detailed in Fig. 1.

The stereoselectivity of D-PIA and L-PIA as well as the relative potencies of adenosine analogues in the displacement of $^{125}$I-HPIA binding to retinal membranes (data not shown) is consistent with properties of adenosine $A_1$ receptors as established previously in brain homogenates (27, 31–33, 38–40).

## DISCUSSION

In this study, endogenous adenosine immunoreactivity has been selectively localized in retinal ganglion cells and their fibers extending into the optic nerve and to specific cells in the inner nuclear layer. Unlike most regions of the central nervous system (11), intense staining occurs not only in perikarya but also in a dense plexus of nerve fibers and terminals. Although adenosine is implicated in many metabolic processes in various tissues, the localization of adenosine immunoreactivity to only a few select cell types in the retina suggests a unique and specific function. Since adenosine levels increase markedly during hypoxia (41–43), we were concerned whether this selective localization of adenosine in ganglion cells and inner nuclear layer cells merely reflects a greater level of hypoxia relative to the other retinal cell types. We have previously demonstrated that the selective regional distribution of adenosine immunoreactivity in neurons of the brain is unaltered by the degree of hypoxia (11). Similarly, in preliminary experiments, varying the degree of hypoxia did not change the patterns of adenosine localization within the rat retina.

The adenosine binding sites localized autoradiographically to select retinal laminae have the pharmacological characteristics of adenosine $A_1$ receptors (27, 31–33, 38–40). In embryonic chicken retina, there is a transiently expressed inhibition or stimulation of cAMP accumulation by adenosine and adenosine analogues (16–18). Adenosine also stimulates cAMP accumulation in rabbit retina (19), but adenosine receptors linked to adenylate cyclase in rat retina have not been established. Similarly, the presence of retinal $A_2$ receptors, localized using techniques recently developed for brain tissue (44), has not been investigated.

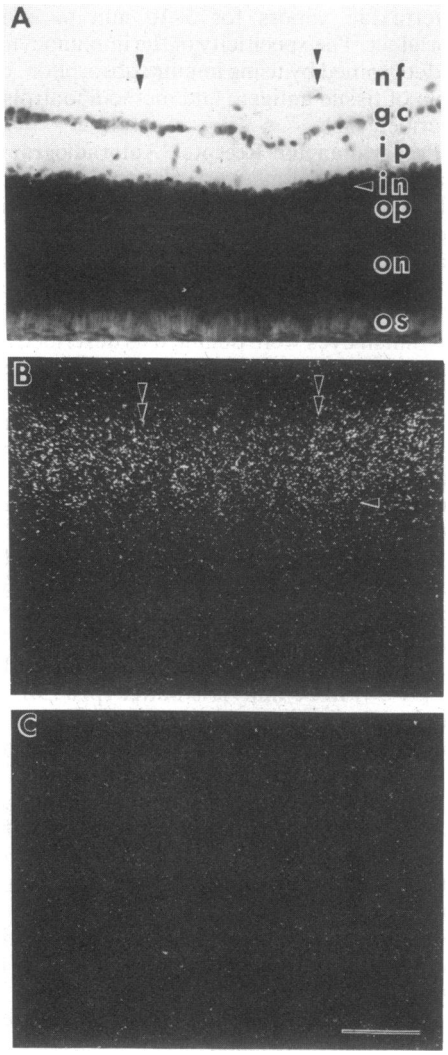


FIG. 3. Autoradiographic localization of L-[$^3$H]PIA binding to adenosine receptors in the rat retina. Rat retinal cryostat tissue sections were labeled with L-[$^3$H]PIA as described in *Materials and Methods* and apposed to emulsion coated coverslips for 28 days. After the autoradiograms were developed, the tissue sections were stained with toluidine blue and mounted. (*A*) Bright field micrograph of toluidine blue stained rat retinal cryostat tissue section. (*B*) Dark field micrograph of autoradiographic grains appearing over the tissue section shown in *A*, which was incubated with 1.25 nM L-[$^3$H]PIA. Only the autoradiographic grains, which appear white, are visible. (*C*) Dark field micrograph of an adjacent tissue section incubated with 1.25 nM L-[$^3$H]PIA in the presence of 10 $\mu$M unlabeled L-PIA. Abbreviations are as detailed in Fig. 1. Arrows indicate corresponding areas in the bright and dark field micrographs.

Several putative neurotransmitter receptors have been localized to specific retinal laminae using autoradiographic techniques (37). As is the case for adenosine receptors, many of these high affinity binding sites are associated with the inner plexiform layer of the retina. Putative neurotransmitters are also enriched in nerve terminals in this layer (12, 13). Thus far, only opiate receptors show enrichment comparable to adenosine receptors in the ganglion cell and nerve fiber layers (45). Dopamine, $\alpha_1$-adrenergic, and $\beta$-adrenergic receptors are also enriched in the outer plexiform and outer nuclear layers.

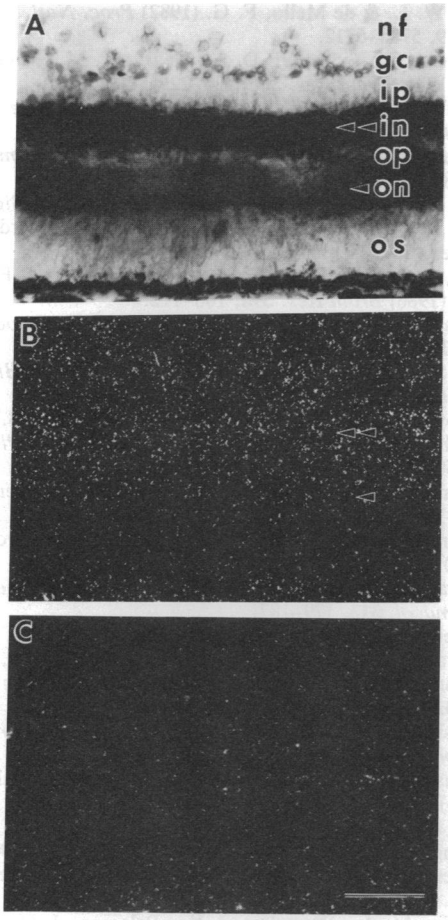


FIG. 4. Distribution of L-[$^3$H]PIA binding to adenosine receptors in the monkey retina. Cryostat tissue sections of the monkey retina were labeled with L-[$^3$H]PIA as described in *Materials and Methods* and apposed to emulsion coated coverslips for 42 days. (*A*) Bright field micrograph of the monkey retinal cryostat tissue section stained with toluidine blue. (*B*) Dark field micrograph of the autoradiographic grains observed over the tissue section shown in *A*. (*C*) Micrograph of an adjacent tissue section incubated with 1.25 nM L-[$^3$H]PIA in the presence of 10 $\mu$M unlabeled L-PIA. Abbreviations are as in Fig. 1. Arrows indicate corresponding areas in the bright and dark field micrographs.

The colocalization of adenosine immunoreactivity and adenosine binding sites suggests that the sites are physiologically relevant receptors. The species differences in binding site distribution are matched by changes in the distribution of adenosine immunoreactive neurons in rat, monkey, and human retina. The distribution of binding sites for the adenosine uptake site inhibitor [$^3$H]nitrobenzylthioinosine correlates well, although not completely, with the distribution of adenosine immunoreactivity and L-[$^3$H]PIA binding sites (K.M.B. and S.H.S., unpublished results). Similarly, the distribution of [$^3$H]adenosine accumulation in retina demonstrated by autoradiography also exhibits similar localizations with species variations (21). In contrast, the histochemically localized distribution of 5′-nucleotidase activity, which may be involved in adenosine biosynthesis, is primarily in the outer portions of mouse retina (46). Species variations may account for this discrepancy, although 5′-nucleotidase may serve other metabolic functions. Since adenosine binding has been detected in glial cells in culture (9, 47), some binding of L-[$^3$H]PIA to glial cells in the present studies may be possible.

Table 1. Adenosine receptor binding grain densities in individual layers of rat, monkey, and human retina

| Layer | Grain density, grains/95 $\mu$m$^2$ | | |
|---|---|---|---|
| | Rat (6) | Monkey (3) | Human (5) |
| Nerve fiber layer | 18 ± 3 | 13 ± 2 | 28 ± 2 |
| Ganglion cell layer | 21 ± 3 | 10 ± 1 | 15 ± 1 |
| Inner plexiform layer | 23 ± 1 | 8 ± 2 | 13 ± 2 |
| Inner nuclear layer | 2 ± 1 | 7 ± 1 | 14 ± 2 |
| Outer plexiform layer | 1 ± 1 | 7 ± 1 | 13 ± 1 |
| Outer nuclear-outer segment layer | 2 ± 1 | 3 ± 1 | 9 ± 2 |

Cryostat tissue sections of rat, monkey, and human retinas were incubated with 1.25 nM L-[$^3$H]PIA as described in *Materials and Methods* and were apposed to emulsion-coated coverslips for 28, 42, or 56 days, respectively. For each tissue section, grains were counted over each of the designated retinal layers. Data are the mean ± SEM for the number of animals (pairs of eyes) given in parentheses.

Enucleation experiments in the rat strongly suggest that some of the adenosine receptors are associated with ganglion cells (29, 48, 49). Opiate receptors are also enriched in the ganglion cell and nerve fiber layers, and enucleation experiments have confirmed a ganglion cell association for these binding sites (50). We have previously demonstrated that after enucleation, adenosine binding in the contralateral thalamus is unchanged, whereas binding in the superficial superior colliculus is decreased (29). In contrast to cat X and Y classes of ganglion cells, which project heavily to the lateral geniculate body, W ganglion cells have small cell bodies and large dendritic arbors, are slowly conducting (i.e., thinly myelinated or nonmyelinated), and project heavily to the superior colliculus (51–53). The adenosine receptors might, therefore, be associated with ganglion cells similar to W cells. Supporting this notion are observations in the central nervous system that adenosine receptors are associated with unmyelinated fibers (3, 29). Other adenosinergic markers, including adenosine uptake sites (54–56), endogenous aden-

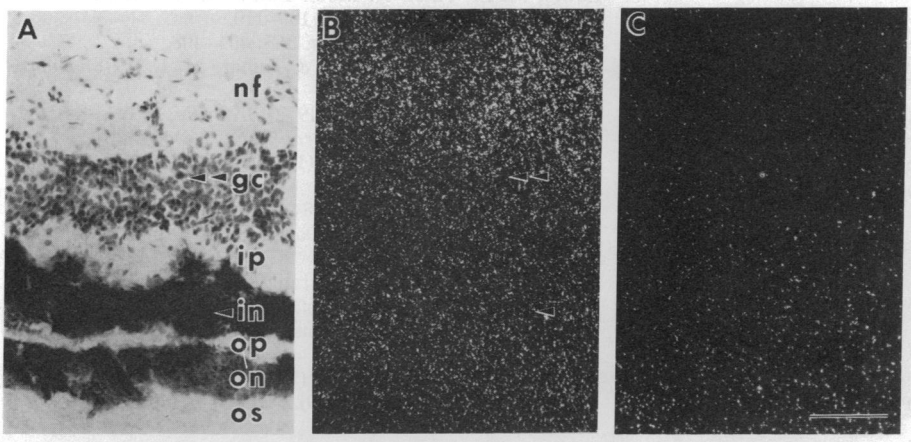


FIG. 5. Localization of L-[$^3$H]PIA binding in the human retina. Human retinal cryostat tissue sections were labeled with L-[$^3$H]PIA as described in *Materials and Methods* and were apposed to emulsion coated coverslips for 56 days. (*A*) Bright field micrograph of a cryostat tissue section of the human retina stained with toluidine blue. (*B*) Dark field micrograph of the autoradiographic grains appearing over the tissue section shown in *A*. (*C*) Dark field micrograph of a tissue section incubated with 1.25 nM L-[$^3$H]PIA in the presence of 10 $\mu$M unlabeled L-PIA. Abbreviations are as detailed in Fig. 1. Arrows indicate corresponding areas in the bright and dark field micrographs.

osine immunoreactivity (11), and adenosine deaminase (57), are present in the superior colliculus. This further corroborates the existence of an important adenosinergic retinocollicular projection. In addition, stimulation of optic tract terminals in the superior colliculus promotes adenosine release, and adenosine analogues inhibit neuronal activity in this region, although species variations exist (5, 58, 59).

The specific functions of the endogenous adenosine in retinal ganglion cells are unclear. Since the major neurotransmitter of ganglion cells is presumed to be excitatory, adenosine might serve as a coneurotransmitter that modulates the effects of stimulatory substances. The apparent localization of adenosine $A_1$ receptors to the same retinal layers as the endogenous adenosine immunoreactivity suggests a possible mode of action. Released adenosine may act upon receptors on the neuron that released the adenosine, or upon closely adjacent neurons, to inhibit further release of excitatory neurotransmitters and/or adenosine. This is consistent with the well characterized ability of adenosine to inhibit the release of neurotransmitters, including neuropeptides, amino acids, and biogenic amines (1–7), and agrees with the localization of adenosine receptors in regions of optic nerve terminals in the superior colliculus (28, 29). The roles of adenosine in cells of the retinal inner nuclear layer remains unclear. Conceivably, adenosine modulates release of neuropeptide, amino acid, and biogenic amine neurotransmitters that occur in amacrine cells of the inner nuclear layer (12, 13).

The authors are grateful to Dr. Victor May for help in the synthesis of $^{125}$I-HPIA and for useful comments throughout this work and manuscript preparation and Naomi Taylor for excellent technical assistance. We thank Dr. Andrew Newby for providing the adenosine antisera, Drs. Harry Quigley and William Smiddy for providing us with monkey material, and Dr. William Green for assistance in obtaining the human eyes. This work is supported by Public Health Service Grants DA-00266 and NS-16375, a Research Scientist Award DA-00074 to S.H.S., and a grant from the International Life Sciences Institute.

1. Fredholm, B. B. & Hedqvist, P. (1980) *Biochem. Pharmacol.* **29,** 1635–1643.
2. Harms, H. H., Wardeh, G. & Mulder, A. H. (1979) *Neuropharmacology* **18,** 557–580.
3. Kocsis, J. D., Eng, D. L. & Bhistkul, R. B. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 6531–6534.
4. Fastbom, J. & Fredholm, B. B. (1985) *Acta Physiol. Scand.* **125,** 121–123.
5. Kostopoulos, G. K. & Phillis, J. W. (1977) *Exp. Neurol.* **55,** 719–724.
6. Kostopoulos, G. K., Limacher, J. J. & Phillis, J. W. (1975) *Brain Res.* **88,** 162–165.
7. Phillis, J. W., Edstrom, J. P., Kostopoulos, G. K. & Kirkpatrick, J. R. (1979) *Can. J. Physiol. Pharmacol.* **57,** 1289–1312.
8. Phillis, J. W. & Wu, P. H. (1981) *Prog. Neurobiol.* **16,** 187–239.
9. Cooper, D. M. F., Londos, C. & Rodbell, M. (1980) *Mol. Pharmacol.* **3,** 598–601.
10. van Calker, D., Muller, M. & Hamprecht, B. (1979) *J. Neurochem.* **33,** 999–1005.
11. Braas, K. M., Newby, A. C., Wilson, V. S. & Snyder, S. H. (1986) *J. Neurosci.* **6,** 1952–1961.
12. Brecha, N. (1983) in *Chemical Neuroanatomy*, ed. Emson, P. C. (Raven, New York), pp. 85–129.
13. Ehinger, B. E. J. (1985) *Biol. Bull.* **168,** 333–349.
14. DiChiara, G. & Gessa, G. L., eds. (1981) *Glutamate as a Neurotransmitter* (Raven, New York).
15. Canzek, V., Wolfenberger, M., Amsler, U. & Cuenod, M. (1981) *Nature (London)* **293,** 572–574.
16. Paes de Carvalho, R. & de Mello, F. G. (1982) *J. Neurochem.* **38,** 493–500.
17. Paes de Carvalho, R. & de Mello, F. G. (1985) *J. Neurochem.* **44,** 845–851.
18. de Mello, M. C. F., Ventura, A. L. M., Paes de Carvalho, R., Klein, W. L. & de Mello, F. G. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 5708–5712.
19. Blazynski, C., Kinscherf, D. A., Geary, K. M. & Ferrendelli, J. A. (1986) *Brain Res.* **366,** 224–229.
20. Schaeffer, J. M. & Anderson, S. M. (1981) *Life Sci.* **29,** 939–946.
21. Ehinger, B. & Perez, M. T. R. (1984) *Neurochem. Int.* **6,** 369–381.
22. Perez, M. T. R. & Ehinger, B. (1986) in *Retinal Signal Systems, Degenerations and Transplants*, eds. Agardh, E. & Ehinger, B. (Elsevier, Amsterdam), pp. 163–172.
23. Perez, M. T., Ehinger, B. E., Lindstrom, K. & Fredholm, B. B. (1986) *Brain Res.* **398,** 106–112.
24. Childs, G. & Unabia, G. (1982) *J. Histochem. Cytochem.* **30,** 713–716.
25. Hsu, S.-M., Raine, L. & Fanger, H. (1981) *J. Histochem. Cytochem.* **29,** 577–580.
26. Newby, A. C. & Sala, G. B. (1982) *Biochem. J.* **208,** 603–610.
27. Murphy, K. M. M. & Snyder, S. H. (1982) *Mol. Pharmacol.* **22,** 250–257.
28. Goodman, R. R. & Snyder, S. H. (1982) *J. Neurosci.* **2,** 1230–1241.
29. Goodman, R. R., Kuhar, M. J., Hester, L. & Snyder, S. H. (1983) *Science* **220,** 967–969.
30. Schwabe, U. & Trost, T. (1980) *Naunyn-Schmiedebergs Arch. Pharmacol.* **313,** 179–187.
31. Linden, J. (1984) *Mol. Pharmacol.* **26,** 414–423.
32. Schwabe, U., Lenschow, V., Ukena, D., Ferry, D. R. & Glossmann, H. (1982) *Naunyn-Schmiedebergs Arch. Pharmacol.* **321,** 84–87.
33. Munshi, R., Hansske, F. & Baer, H. P. (1985) *Eur. J. Pharmacol.* **111,** 107–115.
34. Ukena, D., Furler, R., Lohse, M. J., Engel, G. & Schwabe, U. (1984) *Naunyn-Schmiedebergs Arch. Pharmacol.* **326,** 233–240.
35. Young, W. S. & Kuhar, M. J. (1979) *Brain Res.* **179,** 255–270.
36. Unnerstall, J. R., Niehoff, D. L., Kuhar, M. J. & Palacios, J. M. (1982) *J. Neurosci. Methods* **6,** 59–73.
37. Zarbin, M. A., Wamsley, J. K., Palacios, J. M. & Kuhar, M. J. (1986) *Brain Res.* **374,** 75–92.
38. Daly, J. W. (1983) in *Physiology and Pharmacology of Adenosine Derivatives*, eds. Daly, J. W., Kuroda, Y., Phillis, J. W., Shmizu, H. & Ui, M. (Raven, New York), pp. 59–69.
39. Daly, J. W. (1985) in *Advances in Cyclic Nucleotide and Protein Phosphorylation*, eds. Cooper, D. M. F. & Seamon, K. B. (Raven, New York), pp. 29–46.
40. Bruns, R. F., Daly, J. W. & Snyder, S. H. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 5547–5551.
41. Berne, R. M., Rubio, R. & Curnish, R. R. (1974) *Circ. Res.* **35,** 262–271.
42. Kleihues, P., Kobayashi, K. & Hossman, K.-A. (1974) *J. Neurochem.* **23,** 417–425.
43. Wojcik, W. J. & Neff, N. H. (1982) *J. Neurochem.* **39,** 280–282.
44. Lee, K. S. & Reddington, M. (1986) *Neuroscience* **19,** 535–549.
45. Wamsley, J. K., Palacios, J. M. & Kuhar, M. J. (1981) *Neurosci. Lett.* **27,** 19–24.
46. Scott, T. G. (1967) *J. Comp. Neurol.* **129,** 97–113.
47. Barnes, E. M. & Thampy, K. G. (1982) *J. Neurochem.* **39,** 647–652.
48. Lewis, M. E., Patel, J., Moon Edley, S. & Marangos, P. J. (1981) *Eur. J. Pharmacol.* **73,** 109–110.
49. Geiger, J. D. (1986) *Brain Res.* **363,** 404–408.
50. Atweh, S. F., Murrin, L. C. & Kuhar, M. J. (1978) *Neuropharmacology* **17,** 65–71.
51. Stone, J. & Hoffman, K.-P. (1972) *Brain Res.* **43,** 610–616.
52. Hoffman, K.-P. (1973) *J. Neurophysiol.* **36,** 409–424.
53. Boycott, B. B. & Wassle, H. (1974) *J. Physiol. (London)* **240,** 397–419.
54. Geiger, J. D. & Nagy, J. I. (1984) *Brain Res. Bull.* **13,** 657–666.
55. Nagy, J. I., Geiger, J. D. & Daddona, P. E. (1985) *Neurosci. Lett.* **55,** 47–55.
56. Bisserbe, J. C., Patel, J. & Marangos, P. J. (1985) *J. Neurosci.* **5,** 544–550.
57. Nagy, J. I., LaBella, L. A., Buss, M. & Daddona, P. E. (1984) *Science* **224,** 166–168.
58. Heller, I. H. & McIlwain, H. (1973) *Brain Res.* **53,** 105–116.
59. Okada, Y. & Mitsuo, S. (1979) *Brain Res.* **160,** 368–371.