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An Indoleamine System in Photoreceptor Cell Terminals of the Long-Evans Rat Retina

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Uptake of ^{3}H-serotonin is localized to the outer plexiform layer in Long-Evans rat retinas. Autoradiographic accumulation is seen only after *in vitro* incubation in the light, with retinas isolated from the underlying sclera. Potassium stimulates the release of ^{3}H-serotonin. In this species, amacrine cells do not accumulate these compounds; thus the outer plexiform layer appears to be the only site of uptake and release of this indoleamine.

The age-related loss of ^{3}H-serotonin accumulation in the outer plexiform layer of retinal dystrophic rats coincides temporally with the spontaneous degeneration of photoreceptor cells that occurs in this species. Electron-microscopic autoradiography of ^{3}H-serotonin accumulation further confirms that uptake is localized to rod and cone terminals in the outer plexiform layer. The specific accumulation of indoleamines into rod and cone terminals that is observed in the light but is absent in darkness suggests that indoles have an important physiological role in photoreceptors.

Serotonin (5-hydroxytryptamine, 5-HT) is present in the brain as a neurotransmitter (Bloom et al., 1972) and a precursor for melatonin in the pineal gland (Sitaram and Lees, 1978). Serotonin is also present in retina, although its levels are low (Thomas and Redburn, 1979; Osborne et al., 1982; Tornqvist et al., 1983). Synthesis, metabolism, and uptake systems for serotonin have been demonstrated in retinas of several species (Osborne, 1980). The retinal serotonin system has been previously localized to a specific set of amacrine cells in many species, although some important exceptions have been noted. For example, Tornqvist et al. (1983) report that guinea pig and albino rat retinas lack serotonin-accumulating amacrine cells. In this study we find that retinas from the pigmented Long-Evans rat are likewise devoid of serotonin-accumulating elements in the inner retina. In the outer retina, however, we find evidence for a serotonin system that has not been previously described. Our study includes an analysis of autoradiography and histofluorescence of indole compounds. On the basis of (1) the loss of the serotonin system coincident with degeneration of photoreceptor cells in the retinal dystrophic rat (the Noell line of Royal College of Surgeons rats) and (2) EM autoradiographic localization of ^{3}H-serotonin accumulation in rod and cone terminals, we suggest that the serotonin system is of photoreceptor origin. In addition, we demonstrate the regulation of serotonin by light, dark, and depolarizing levels of potassium. This raises the strong possibility that serotonin and/or related indoles play a physiologically important role in photoreceptors.

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Materials and Methods

Animals. Long-Evans rats, obtained from Charles River, Portage, MI, were bred and raised on a 14 hr light: 10 hr dark cycle. Retinal dystrophic (rdy) rats from the Noell line of the Royal College of Surgeons rats were bred and raised under a red safelight. The 20-d-old rats were housed in a litter with the mother. The 100-d-old rats were housed in a litter separate from the mother after 20–22 d of age. Since indole turnover (i.e., release and/or metabolism) appears to be activated by darkness, rats were kept in the dark 2 hr prior to decapitation in an attempt to deplete endogenous stores of serotonin.

Uptake of ^{3}H-serotonin. Animals were decapitated and rapidly enucleated in the light. Isolated retinas were incubated in oxygenated Krebs bicarbonate buffer containing 10 mM glucose. Portions of each retina were incubated in a 37°C water bath under ambient light or in total darkness in buffer containing 100 μCi/ml ^{3}H-5HT (1,2-^{3}H-(*n*)-hydroxytryptamine creatine sulfate; 26.7 Ci/mmol specific activity; New England Nuclear, Boston, MA), 100 μM ascorbic acid, and 10 μM pargyline. In selected samples, $CaCl_2$ was omitted and 10 mM $MgCl_2$ was added to the buffer. After 20 min, the tissue was prepared for analysis by thin-layer chromatography (TLC) or autoradiography.

Thin-layer chromatography. Retinas were homogenized in 50% ethanol and the particulate fraction removed by centrifugation. The soluble extracts were chromatographed on silica gel plates in butanol, acetic acid, and water (25:4:10). More than 80% of the recovered radioactivity comigrated with the ^{3}H-5HT standard.

Autoradiography. Isolated retinas or eyecups were removed and placed in 3% glutaraldehyde buffered with 0.05 M sodium cacodylate (pH 7.2) for 30 min at room temperature and subsequently refrigerated overnight. The tissue was rinsed with buffer and postfixed in 1% OsO_4 buffered with 0.05 M sodium cacodylate for 1½ hr at 0°C. After dehydration with a graded ethanol series, the tissue was embedded in Epon 812 epoxy resin.

Blocks were sectioned for both light and electron microscopy and autoradiography. Sections were coated with Kodak Nuclear Track Emulsion, type NTB-2, for light-microscopic autoradiography and Ilford L-4 emulsion for electron-microscopic autoradiography. Sections were incubated for 2–4 weeks for light-microscopic autoradiographs and for 2–4 months for electron-microscopic autoradiographs. Some sections were stained with toluidine blue for comparison with unstained sections.

Treatment with 5,7-dihydroxytryptamine (DHT). Long-Evans rats were anesthetized with an Acepromazine-ketamine-Rompun cocktail (0.7, 21.4, and 4.3 mg/kg, respectively) via an intramuscular injection in the

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hindlimb. Each animal also received an injection of 50 mg/kg, i.p., pargyline. Thirty minutes after the injections, the eyes were bathed with 1% lidocaine hydrochloride and a 27-gauge × 0.5 in. needle was used for intraocular injections. The injection volume was 50 μl and contained 50 μg DHT in 0.9% NaCl, with ascorbic acid (1 mg/ml) as an antioxidant. The first group of animals was killed 4 hr after the intraocular injection and retinas were prepared for fluorescence microscopy. Single injections of DHT were previously shown by Ehinger and Floren (1978) to cause indoleamine-accumulating neurons to appear fluorescent in rabbit retina. Thus, the first group of animals was used to determine whether there were any indoleamine-accumulating cells in normal rat retina. A second intraocular injection was given to the remaining animals on the following day. One week later, a third intraocular injection of DHT was given 4 hr before killing the remaining animals. Cells that accumulated the toxin would be allowed to degenerate during the week-long interval, and their loss would be obvious through the specific loss of cells with DHT-induced fluorescence.

Histofluorescence. Localization of catecholamines and indoleamines was visualized by histofluorescence using the method of de la Torre and Surgeon (1976). Animals were decapitated and quickly enucleated. The globes were hemisected and the vitreous gently removed. Eyecups were immediately placed in O.C.T. cryostat embedding medium (Lab-Tek, Miles Laboratories, Naperville, IL) and frozen on dry ice. Frozen 20 μm sections were cut in a cryostat set at −20°C. The sections were placed on clean glass slides and immediately dipped into fresh glyoxylic acid stain (pH 7.4). The slides were blotted, dried under a stream of cold air for 3 min, then heated in an 80°C oven for 5 min. Coverslips were mounted with mineral oil and the sections observed under a Zeiss Fluorescence Photomicroscope III.

In an attempt to enhance serotonin-induced histofluorescence, animals were pretreated with colchicine to inhibit axoplasmic transport, with tryptophan to provide precursor, and with pargyline to inhibit metabolism, according to the method described by Rassmussen and Bunney (1982). Rats were anesthetized with chloral hydrate (400 mg/kg, i.p.). Ten microliters of saline containing 3 μg colchicine was injected into the right eye of each animal. Tryptophan methyl ester (100 mg/kg) and pargyline (100 mg/kg) were injected intraperitoneally, 22 hr later. After an additional 2 hr, retinas were removed and prepared for histofluorescence.

Unless otherwise noted, all chemicals were obtained from Sigma, St. Louis, MO.

^{3}H-serotonin release. Isolated retinas were incubated in 0.5 ml oxygenated Krebs bicarbonate buffer containing 10 mM glucose, 100 μM ascorbate, 10 μM pargyline, and 50 μCi ^{3}H-5-HT for 15 min at 37°C under a continuous stream of 95% O_2 and 5% CO_2. Retinas were then rinsed with buffer and placed in a 2 ml superfusion chamber connected to a continuous flow pump. All perfusion buffers contained 6 μM imipramine to inhibit reuptake. One milliliter of the perfusate was collected per minute. After a 10 min perfusion period in standard buffer, the potassium concentration was increased to 56.2 mM for 5 min. After a return to standard buffer for 12 min, the potassium concentration was increased a second time.

Results

Light-microscopic autoradiography of ^{3}H-serotonin accumulation

In vitro incubation of isolated Long-Evans rat retinas in control buffer for 20 min does not significantly alter normal morphology (Fig. 1, upper left). Cell bodies are characteristically arranged in 3 major layers. The outer nuclear layer (ONL) contains cell bodies of the photoreceptor cell. The inner nuclear layer (INL) contains cell bodies of horizontal cells, bipolar cells, Muller cells, and amacrine cells. The ganglion cell layer (GCL) contains cell bodies of displaced amacrine cells and ganglion cells. Two plexiform layers are clearly discernible, with the outer plexiform layer (OPL) represented by a narrow band containing synaptic endings of photoreceptor cells, horizontal cell neurites, and bipolar cell dendrites. The inner plexiform layer (IPL) is seen as a much broader band and contains synaptic contacts between amacrines, bipolars, and ganglion cells.

When micromolar concentrations of ^{3}H-serotonin are included during the 20 min incubation period, a specific accumulation pattern is observed. A thin band of label is specifically associated with the outer portion of the OPL (Fig. 1, upper right). Cell bodies of the photoreceptor cells also show light labeling in this section. There is a light and uniform distribution of grains throughout the inner half of the retina, with no evidence of specific accumulation of the ligand within any cells in the GCL or INL. The only exception is accumulation by elements associated with a blood vessel seen in the GCL. These observations confirm previous reports that amacrine cells of the rat retina do not accumulate serotonin (Tornqvist et al., 1983).

The specific labeling pattern in the OPL is seen only if the *in vitro* incubation occurs in the absence of underlying sclera. Autoradiograms of attached retina in an isolated eyecup after a 20 min *in vitro* incubation show no specific accumulation of serotonin in either inner or outer portions of the retina, with light uniform labeling throughout, except for labeling of vascular elements (Fig. 1, lower left). This observation suggests that removal of the sclera is necessary to allow adequate diffusion of molecules among all cell types of both inner and outer retina.

Regulation of ^{3}H-serotonin accumulation by light, dark, and magnesium

The specific uptake of ^{3}H-serotonin into the outer plexiform layer was found to be particularly sensitive to lighting conditions during the incubation period. When isolated retinas are incubated in ^{3}H-serotonin for 20 min in the dark and prepared for autoradiography, we do not observe the specific accumulation of label in the OPL that is normally seen after incubation in the light (Fig. 2, top). Under dark incubation conditions, only sparse, uniform labeling is seen throughout the retina, except for labeling of vascular elements in the inner retina. Incubated retinas that are subsequently homogenized and prepared for scintillation counting show a 50% inhibition of ^{3}H-serotonin accumulation in the dark (7213 cpm/mg protein/min ± 527 SEM; n = 3) as compared to in the light (13,906 cpm/mg protein/min ± 822 SEM; n = 3).

The lack of specific labeling could be due to either dark-induced inhibition of uptake or dark-induced loss of ^{3}H-serotonin through metabolism or calcium-dependent release. Addition of magnesium and removal of calcium to inhibit release during a dark incubation period results in only modest autoradiographic accumulation of ^{3}H-serotonin in the OPL (Fig. 2, center). Scintillation counting shows no difference after dark incubation in high-magnesium, low-calcium buffer (6475 cpm/mg protein/min ± 447 SEM; n = 3), as compared to dark controls (see above), presumably because the increase is so small compared to background accumulation. High-magnesium, low-calcium buffer had no effect on incubations in the light, as assessed by autoradiography (Fig. 2, bottom) or scintillation counting (11,575 cpm/mg protein/min ± 769 SEM; n = 3). Control values are listed above. Magnesium is a potent blocker of synaptic release and has little known effect on uptake (Douglas, 1968; Miledi, 1973; Levy et al., 1974); thus the modest magnesium enhancement of accumulation in the dark seen autoradiographically indicates that release of ^{3}H-serotonin during the dark incubation period can only account in small part for the lack of net accumulation under these conditions.

Histofluorescence

Treatment of neuronal tissues with glyoxylic acid results in the formation of blue fluorescent derivatives of catecholamines, such

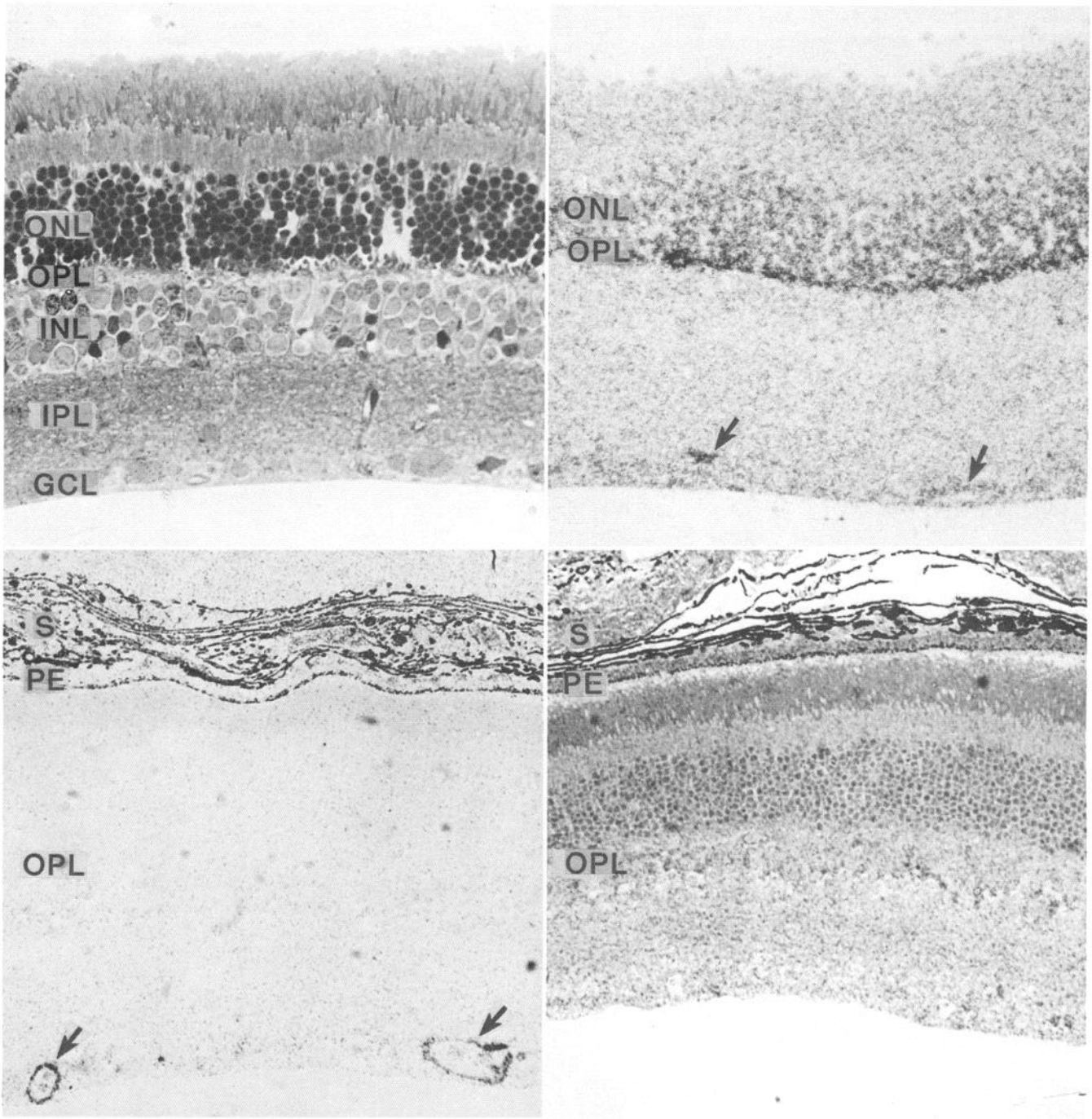

Figure 1. Light microscopic autoradiographs of ^{3}H-serotonin accumulation in retinas of Long-Evans rat. *Top left,* Isolated retina incubated in control buffer for 20 min shows normal morphology. *ONL,* Outer nuclear layer; *OPL,* outer plexiform layer; *INL,* inner nuclear layer; *IPL,* inner plexiform layer; *GCL,* ganglion cell layer. ×375. *Top right,* Isolated retina incubated in ^{3}H-serotonin shows specific accumulation of label in the *OPL,* and perhaps in the *ONL. Arrows* denote labeled vascular elements. Unstained. ×375. *Bottom left,* Attached retina (eyecup preparation) incubated in ^{3}H-serotonin shows no specific neuronal accumulation. *Dark pigment granules* are observed in the pigment epithelium. Note labeling of blood vessels (*arrows*). Unstained. ×275. *Bottom right,* Stained autoradiograph of attached retina for comparison with photo at left. ×275.

as dopamine, and yellow fluorophores of indoleamines, such as serotonin (de la Torre and Surgeon, 1976). As has previously been reported (Ehinger, 1966), treatment of rat retinas with glyoxylic acid produces blue fluorescence, characteristic of dopamine, in a single diffuse band in the IPL (Fig. 3). Fluorescence appears in a punctate fashion and is presumed to be associated with cell processes or varicosities from amacrine cells. Fluorescent cell bodies are sparsely distributed within the INL. A band of endogenous histofluorescence is also observed in the outer retina, in a location corresponding to the OPL. In comparison to the histofluorescence in the inner retina, the fluorescence in the outer retina is very faint and difficult to visualize under these conditions. It is yellow in color, which is characteristic of an indoleamine derivative, and appears to be more uniform rather than punctate in its distribution. Prominent fluorescent patterns are also observed in association with the sclera. The histoflu-

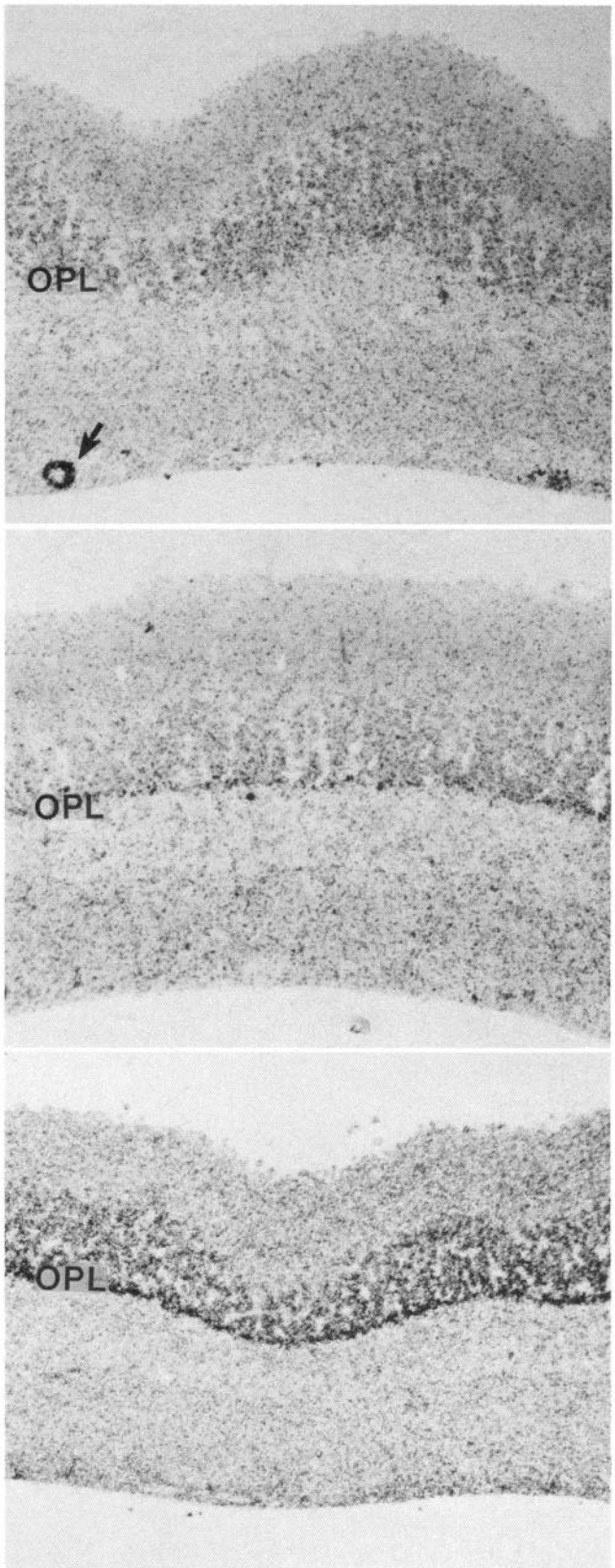

Figure 2. Effect on light, dark, and magnesium on ^{3}H-serotonin accumulation. *Top,* Isolated retina incubated in ^{3}H-serotonin for 20 min in the dark shows no specific neuronal accumulation. *Arrow* denotes labeled blood vessel similar to that seen under light conditions. Unstained. ×275. *Center,* Isolated retina incubated in ^{3}H-serotonin for 20 min in the dark in buffer with calcium chloride omitted and 10 mM magnesium chloride added. There is a very modest accumulation of ^{3}H-serotonin in the *OPL* under these conditions. Unstained. ×275. *Bottom,* Isolated retina incubated in ^{3}H-serotonin for 20 min in the light in low-calcium, high-magnesium buffer. Accumulation pattern is similar to that seen in control buffer in the light. ×275.

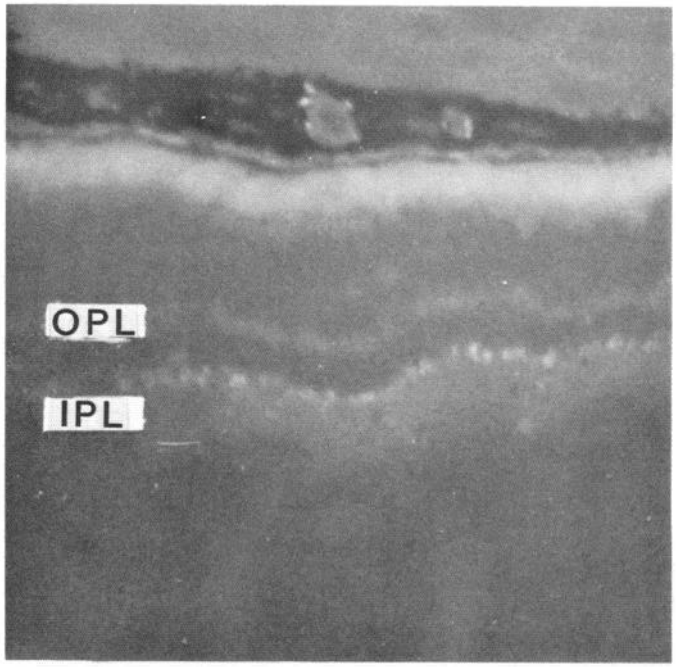

Figure 3. Endogenous histofluorescence of rat retina after glyoxylic acid treatment. Dopamine histofluorescence appears in the *IPL*; indoleamine histofluorescence appears in the *OPL*. Prominent fluorescent patterns are also present in the sclera. ×275.

orescence of the OPL remains faint even after the application of a variety of *in vivo* enhancement techniques that have been shown to enhance serotonin histofluorescence in brain (Rassmussen and Bunney, 1982). These include pretreatment of the animal *in vivo* with colchicine, pargyline, and tryptophan.

DHT is a specific neurotoxin for serotonin-accumulating neurons and has thus been widely used as a specific marker for serotonergic neurons in various areas of the CNS (Lachenmayer and Nobin, 1973; Baumgarten and Bjorklund, 1976), including retina (Ehinger and Floren, 1978). We have previously shown that intraocular injections of DHT caused necrosis of serotonin-accumulating amacrine cells in the rabbit retina (Mitchell and Redburn, 1985). Under these same conditions, however, we observe no specific neurotoxic effects of this compound in retinas from Long-Evans rat (Fig. 4, top and center). Photoreceptor cell nuclei appear normal. There is no evidence of swelling in either the IPL or OPL; no significant necrosis is observed in either the INL or the GCL.

DHT is an intracellular toxin and therefore must be accumulated intracellularly in sufficient quantities to express neurotoxic effects (Lachenmayer and Nobin, 1973). DHT is accumulated via the serotonin-uptake system and thus expresses its effects primarily in serotonin-accumulating neurons. The lack of neurotoxic effects in the Long-Evans rat retina may suggest that the DHT is not accumulated at sufficient concentrations to produce a neurotoxic effect. In order to test this hypothesis, we

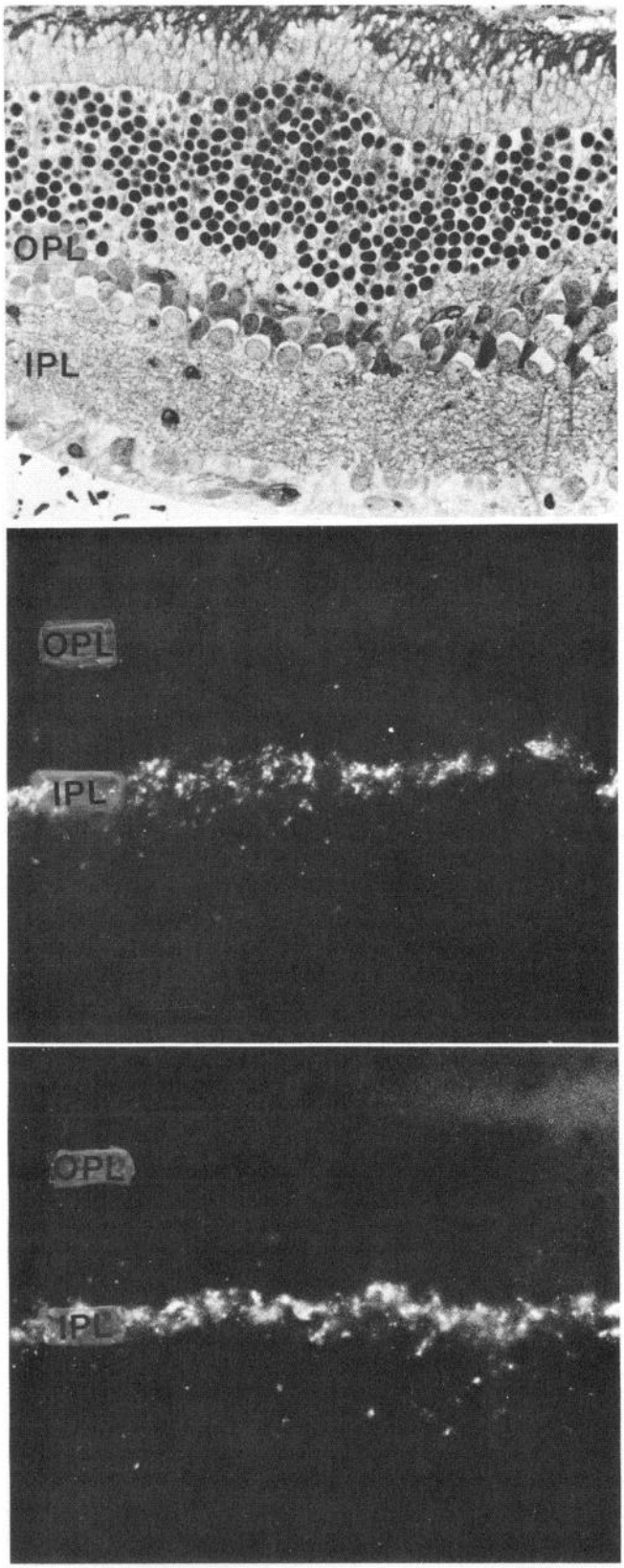

Figure 4. Morphology and histofluorescence of DHT-treated retinas. *Top,* Retinal morphology is not significantly altered after intraocular injection of DHT. (See control tissue in Fig. 1, *top left,* for comparison.) *Center,* In control tissue a bright band of histofluorescence, corresponding to dopamine fluorescence, is seen in the *IPL.* (The histofluorescence in the *OPL* shown in Fig. 3 cannot be appreciated under these photographic conditions.) *Bottom,* Four hours after intraocular injection of the DHT, no change in fluorescence patterns is observed. All magnifications ×450.

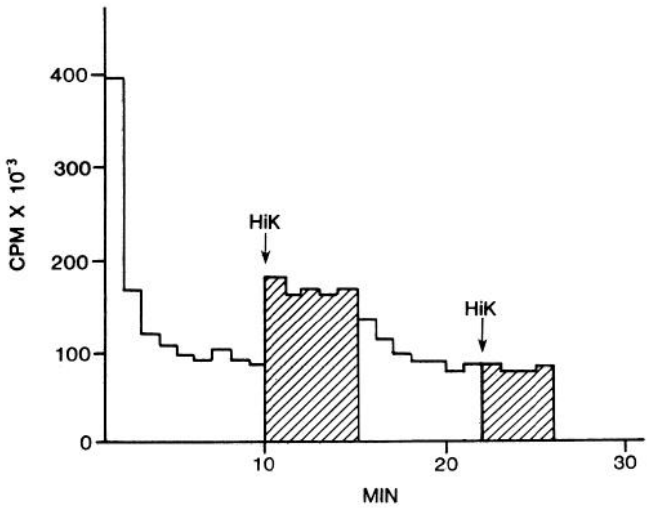

Figure 5. Release of ^{3}H-serotonin from isolated, intact rat retina. After preincubation in ^{3}H-5-HT, retinas were perfused with buffer containing 6.2 mM potassium (*open bars*) for 10 min. Addition of depolarizing levels of potassium (56.2 mM final concentration) for 5 min caused an increase in release rates (*striped bars*). After return to original buffer, a second application of 56.2 mM potassium did not stimulate release. Values shown are from a representative experiment; $n = 3$.

examined histofluorescent patterns of rat retina 4 hr after intraocular injection of DHT. Since DHT forms a fluorescent derivative when treated with glyoxylic acid, uptake of DHT can be visualized by histofluorescence (Ehinger and Floren, 1978). Under these conditions, we observed no significant change in the fluorescence associated either with inner or outer plexiform layers (Fig. 4, bottom). These data indicate that DHT is not accumulated by cells of the rat retina in amounts sufficient to elicit neurotoxic effects or to reach the limits of detection by histofluorescence techniques.

^{3}H-serotonin release

Isolated retinas were incubated in ^{3}H-serotonin for 20 min, rinsed, and placed in a superfusion chamber in the light. The perfusion buffer contained imipramine to inhibit reuptake. Perfusates were collected at the rate of 1 ml per min and counted. A stable, basal efflux rate can be established within 10 min (Fig. 5). Addition of depolarizing levels of potassium (56.2 mM final concentration) approximately doubles the release rate during a 5 min exposure period. A return to control buffer (6.2 mM potassium) lowers the release rate to prestimulation levels. A second exposure to high levels of potassium fails to elicit a significant increase in release rates.

^{3}H-serotonin accumulation in dystrophic rats

To determine whether or not photoreceptor cell terminals are responsible for serotonin accumulation in the OPL, we analyzed serotonin-uptake patterns in retinas from the rat rdy, which exhibit spontaneous retinal degeneration. In these animals, photoreceptor cells slowly degenerate, sparing the other cell types associated with the OPL (Noell, 1965; Cotter and Noell, 1985). Retinas appear relatively normal at 20 d of age, with only subtle

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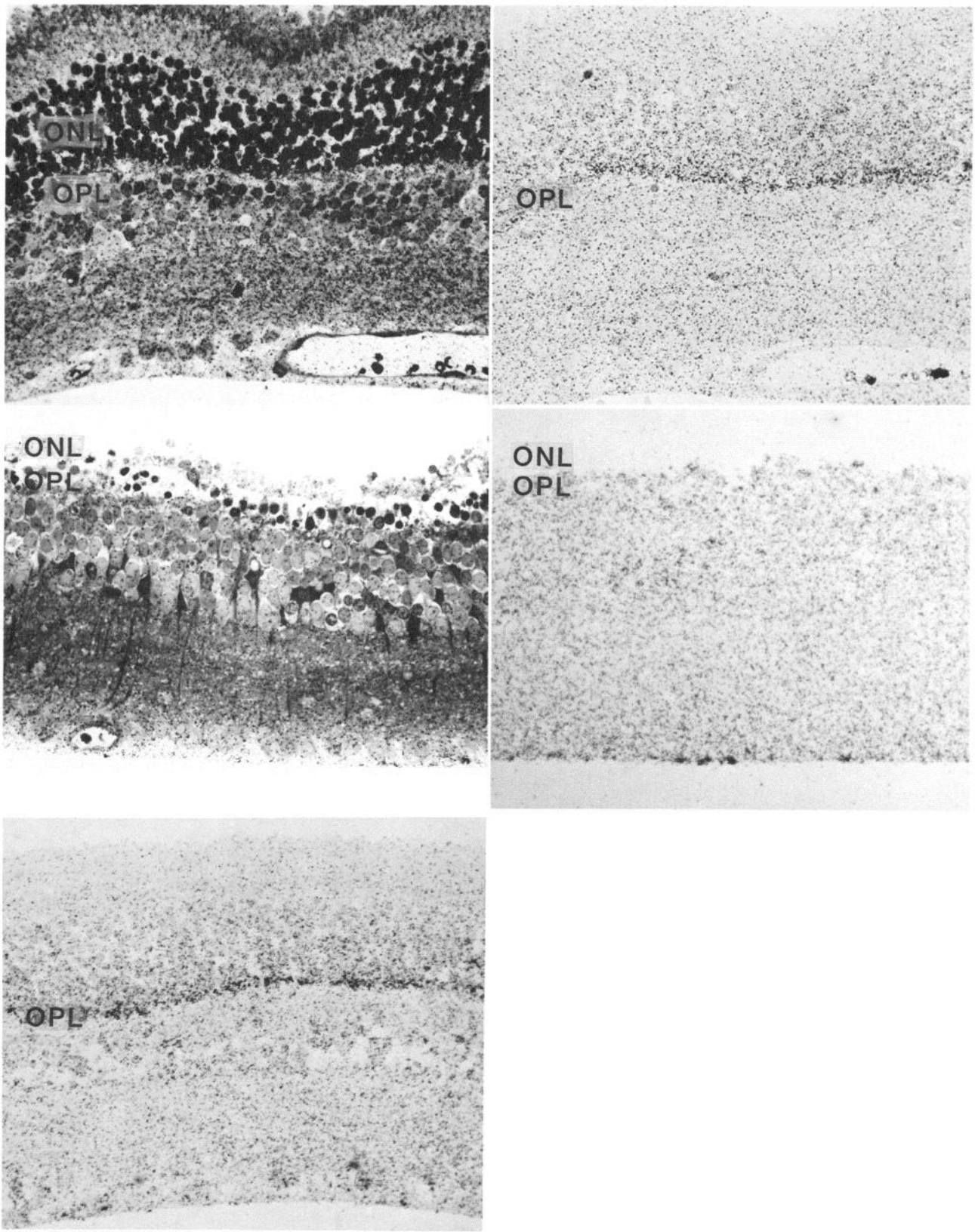

Figure 6. ^{3}H-serotonin accumulation in retinas of rdy rat. *Top left,* Morphology of 20-d-old rdy rat is similar to that of Long-Evans control rats. ×450. *Top right,* Accumulation of ^{3}H-serotonin in the *OPL* of the 20-d-old rdy rat is similar to that in the Long-Evans control rat. Unstained. ×450. *Center left,* Retina from 100-d-old rdy rat shows loss of inner and outer segments as well as of most cell bodies in the *INL.* The inner retina appears relatively normal. ×500. *Center right,* Retina from 100-d rdy rat does not specifically accumulate label. ×500. *Bottom,* Retina from age-matched (100-d-old) Long-Evans control rat displays uptake similar to that of young adult controls. ×225.

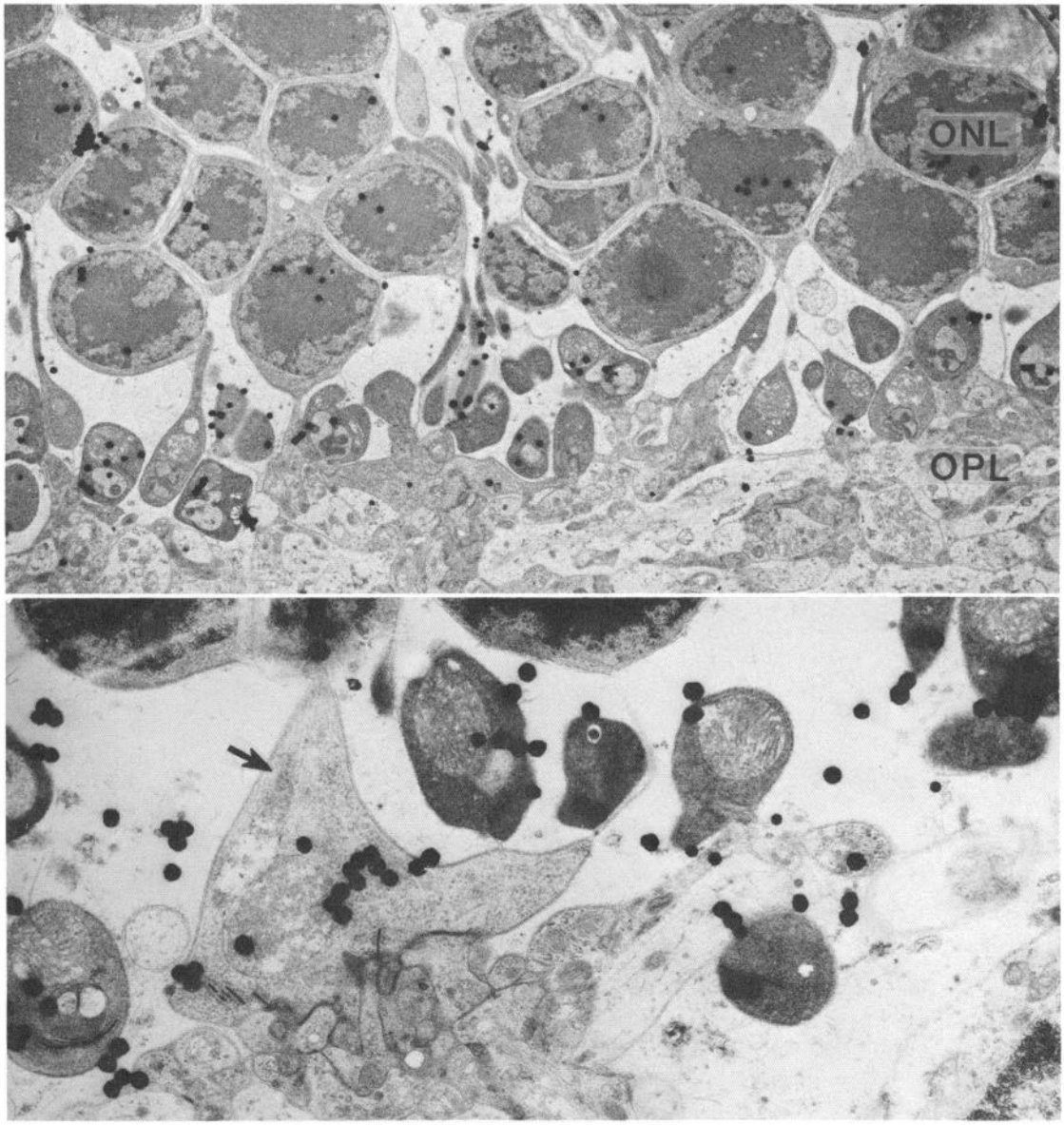

Figure 7. EM autoradiography of ^{3}H-serotonin accumulation. *Top,* Individual grains appear in close proximity to virtually every photoreceptor terminal. Other elements of the *OPL* are not labeled. Grains are also present in photoreceptor cell bodies. ×4000. *Bottom,* Cone terminals (*arrow*), although few in number, are also labeled. ×8000.

signs of degeneration in rod photoreceptor cells. By 100 d of age, the majority of rod photoreceptor cells have undergone degeneration and only a few cone cell remnants remain in the ONL. Horizontal and bipolar cell processes in the OPL appear to be intact at 100 d of age.

Accumulation of ^{3}H-serotonin by retinas of 20-d-old rdy rat retinas (Fig. 6, upper left and right) was virtually indistinguishable from that seen in control retinas of Long-Evans rats (Fig. 1, upper right). Specific accumulation of label is observed in a band corresponding to the location of the OPL. In contrast, retinas from 100-d-old rdy rats do not show accumulation of ^{3}H-serotonin in the OPL (Fig. 6, center, left and right). The loss of serotonin uptake in the OPL in the 100-d-old rdy rat retina is not a simple function of age. Age-matched control retinas from 100-d-old Long-Evans control rats show accumulation of ^{3}H-serotonin in the OPL similar to that seen in the 20-d-old control rat (Fig. 6, bottom). Thus, the loss of ^{3}H-serotonin accumulation appears to result specifically from the loss of photoreceptor cells and their terminals, which suggests that these elements are indeed responsible for serotonin accumulation.

Electron-microscopic autoradiography of ^{3}H-serotonin accumulation in the outer plexiform layer

EM autoradiography further confirms the uptake of ^{3}H-serotonin specifically by terminals of photoreceptor cells. In retinas incubated for 20 min in ^{3}H-serotonin and processed for EM autoradiography, grains are observed in close association with photoreceptor cell terminals of both rods and cones (Fig. 7 in Table 1). There is relatively little labeling associated with the remaining elements of the OPL. Grains are also associated with photoreceptor cell bodies, although their density is significantly lower (Table 1). Grain density in the sparse population of cone terminals observed appeared to be equivalent to that seen in rod terminals.

Table 1. Distribution of ^{3}H-serotonin autoradiographic grains in photoreceptor terminals and cell bodies

Cellular compartment	Average no. of grains in each compartment (mean ± SEM)	Density (grains/μ^2)	Unlabeled (%)	Number counted
Photoreceptor terminals	3.5 ± 0.5	0.75 ± 0.1	23.4	47
Photoreceptor cell bodies	3.6 ± 0.6	0.16 ± 0.2	22.6	53
Inner portion of the OPL		0.05 ± 0.01		

Discussion

Autoradiographic localization of an indoleamine system in rat retina

In this study, we provide evidence for the presence of an indoleamine system within rod and cone terminals in rat retina. The localization of the indoleamine system is based primarily on the ability of rod and cone terminals to accumulate and release ^{3}H-serotonin. At the light-microscopic level, autoradiographic localization of ^{3}H-serotonin is associated specifically with the outer portion of the outer plexiform layer. With pargyline and ascorbate included in the incubation buffer to inhibit metabolism and oxidation, the tritium label remains as authentic ^{3}H-serotonin when the retina is maintained in the light. This is not the case when the retina is exposed to darkness, as will be discussed below.

Electron-microscopic autoradiography demonstrates that specific accumulation of ^{3}H-serotonin in the outer plexiform layer is associated with the terminal region of rods and cones. There is no specific accumulation in inner or outer segments. Accumulation by photoreceptor cell bodies is only slightly enhanced compared to that seen in the remaining retina. Other elements of the outer plexiform layer, including horizontal and bipolar cells neurites, do not appear to accumulate serotonin. Although this represents the most likely interpretation of our autoradiographs, other interpretations might be considered. Since both bipolar and horizontal cell neurites form deep invaginations within rod and cone terminals, it is possible that the labeled compound is associated with either of these postsynaptic elements and, as a result, produces an autoradiographic labeling pattern coincident with photoreceptor terminals. Although no statistical analyses were made of the intraterminal distribution of grains, our subjective interpretation is that the grains do not appear to be associated with postsynaptic elements in the triad. In addition, grains were rarely seen in the inner portion of the outer plexiform layer occupied by neurites of horizontal and bipolar cells, which are postsynaptic to photoreceptors. These observations, although not conclusive, do suggest that the labeling observed is associated with the presynaptic element rather than the postsynaptic element of the photoreceptor cell synapse.

Further support for this interpretation is provided by studies that demonstrate a loss of serotonin accumulation in the outer plexiform layer coincident with the degeneration of photoreceptor cells in the rdy rat retina. At 20 d of age, retinas begin to show signs of degeneration in rods, which appear to be the initial target of this degenerative disease (LaVail et al., 1974; Kaitz, 1976; Cicerone et al., 1979; Noell et al., 1981). ^{3}H-serotonin uptake is normal at this stage, which suggests that the loss of serotonin uptake is not a major feature of the onset of the disease.

At an advanced stage of dystrophy (100 d), rod cells are virtually absent and there is no detectable accumulation of ^{3}H-serotonin in the outer plexiform layer. A small population of partially degenerative cones is present. The loss of serotonin accumulation in the outer plexiform layer thus coincides with the disappearance of the degenerated rod cells and the partial degeneration of cone cells. These data, taken with EM autoradiographic observations, support the notion that photoreceptor cells are indeed the site of serotonin accumulation in the outer plexiform layer.

Animals at this stage of degeneration do retain minimal visual capabilities, suggesting that some degree of visual information processing is occurring via the remaining cone terminals (LaVail et al., 1974; Noell et al., 1981). The lack of specific accumulation of serotonin by cone terminals in late-stage rdy retinas suggests that either cones do not normally take up serotonin or their accumulation of serotonin at this degenerative stage is below the level of autoradiographic detection. Our EM autoradiographic results in control Long-Evans rats, which show specific accumulation by cone terminals, suggest that the latter possibility is more likely.

Many vertebrate retinas demonstrate specific accumulation of serotonin by a subset of amacrine cell bodies and processes within specific sublamina of the inner plexiform layer (Ehinger and Floren, 1980; Osborne, 1982; Mitchell and Redburn, 1985). However, ^{3}H-serotonin is not accumulated by any element in the inner retina of the rat. In particular, there are no amacrine cells that are specifically labeled by ^{3}H-serotonin. The autoradiographic grains of ^{3}H-serotonin observed in the inner retina are uniformly distributed and are significantly reduced compared to labeling in the outer plexiform layer. These data confirm previous reports (Ehinger and Floren, 1979, 1980) that the rat retina does not possess ^{3}H-serotonin-accumulating amacrine cells. Thus serotonergic properties in these retinas can be attributed solely to photoreceptor cell terminals.

The retina of the squirrel monkey provides yet another variation in the distribution of serotonin-accumulating cells. It has recently been reported that horizontal cells accumulate serotonin in this species (Floren and Hendrickson, 1984). Our results in Long-Evans rat retinas did not demonstrate any specific accumulation of serotonin by horizontal cells. Both the EM autoradiography and the experiments with the rdy rat essentially rule out the possibility that the labeling we observed in the outer plexiform layer is associated with any element other than the photoreceptor terminal.

Characteristics of indoleamine accumulation

Accumulation of serotonin within photoreceptor terminals has not previously been reported for retinas of any species. This apparent discrepancy between our findings and previous reports by others may in fact be related to a number of characteristics of the serotonin-uptake system in the outer plexiform layer that differ significantly from the serotonin-uptake system observed in amacrine cells. One of the more important technical considerations is the fact that specific accumulation is observed only when the isolated retina is incubated *in vitro* in buffer containing the label. Intraocular injections or *in vitro* incubations of eyecup

preparations with retina attached do not allow free diffusion of the label through both retinal surfaces. We assume that the attached retinal preparations used by other investigators precluded the distribution of serotonin to the outer portions of the retina, which would be required for uptake by photoreceptor terminals. On the other hand, attached retinal preparations are sufficient for labeling of serotonin-accumulating amacrine cells.

One important characteristic of the serotonin-uptake system in photoreceptor terminals is the high degree of sensitivity to lighting conditions during the incubation period. Exposure of the rat retina to darkness during *in vitro* incubation with the label blocks the specific accumulation of serotonin in photoreceptor cell terminals. In contrast, lighting conditions have no effect on the low, uniform distribution of autoradiographic grains observed over the remaining portions of the retina. Even under the most favorable lighting conditions used, however, we were unable to enhance serotonin accumulation in photoreceptor terminals to the level previously observed by autoradiography or histofluorescence in amacrine cells and their processes in other species, such as rabbit (Mitchell and Redburn, 1985). The uptake by photoreceptor terminals simply appears to be less robust than that observed in amacrine cells. Whether this property is a result of low transport affinity, low number of transport sites, smaller pool size, or higher turnover rate cannot be determined on the basis of these experiments.

Localization of indoleamine histofluorescence

A very pale yellow band of endogenous fluorescence, indicative of indoleamines, is observed in the outer plexiform layer in untreated retinas. We were unsuccessful in our attempts to enhance this fluorescence by treatment of the animal and/or isolated retina with tryptophan, serotonin, pargyline, ascorbate, colchicine, or varying lighting conditions. Serotonin accumulation, clearly demonstrated by autoradiography, is not observed by histofluorescence. These findings are in agreement with the widely recognized observation that histofluorescence is a less sensitive method for detecting indoleamines than is autoradiography. For example, uptake of serotonin into amacrine cells of cow and pig retina is seen only with autoradiography, not with histofluorescence (Ehinger et al., 1982; Osborne et al., 1982). Nevertheless, the endogenous fluorescence in the rat retina outer plexiform layer does not provide convincing evidence for an endogenous indoleamine pool in photoreceptor terminals. Until there is a clear demonstration of the localization of an endogenous indoleamine, we cannot be certain as to the exact nature of the indole compound that functions as the endogenous ligand for the indoleamine-accumulating system in photoreceptor terminals.

Uptake of the serotonin analog DHT is not detectable in any cell of the rat retina. DHT is a neurotoxin specifically accumulated by serotonergic neurons in the CNS, including serotonin-accumulating amacrine cells in retinas of other species (Lachenmayer and Nobin, 1973; Baumgarten and Bjorklund, 1976; Ehinger and Floren, 1978). This analog also produces a fluorescent derivative when exposed to glyoxylic acid. As previously reported (Mitchell and Redburn, 1985), the uptake of DHT by serotonergic amacrine cells in the rabbit retina after intraocular injection can be monitored by histofluorescence after short exposure (hours) and by cell necrosis and cell death after longer exposure (days). Intraocular injections of DHT equivalent to those used in rabbit had no toxic effect on cells in the outer retina of the rat. Likewise, we were unable to detect any DHT-induced fluorescence. Thus, the absence of neurotoxic effects may be due to lack of sufficient accumulation of DHT.

The fate of accumulated serotonin in photoreceptor terminals

There is no specific accumulation of serotonin in retinas exposed to darkness. Since the photoreceptor cell depolarizes and releases its neurotransmitter in response to darkness (for a review, see Rodieck, 1973), the lack of observed accumulation may result from a dark-stimulated release of serotonin. In support of this notion, our data demonstrate that in the light, depolarizing levels of potassium stimulate the release of ^{3}H-serotonin as measured by scintillation counting. On the basis of these observations, it is tempting to speculate that photoreceptors may use an indoleamine compound as a transmitter. However, recent evidence describing a melatonin system in retina may provide alternative interpretations.

Several reports over the past 2 decades have documented the presence of melatonin in retinas from various species (Quay, 1965; Cardinali and Rosner, 1971a; Bubenik et al., 1974). As in the pineal gland, melatonin synthesis in retina is known to increase in the dark and decrease in the light (Pang et al., 1977; Bubenik et al., 1978). The increase in both retinal and pineal melatonin synthesis results from dark activation of *N*-acetyl transferase (NAT), the rate-limiting enzyme for melatonin synthesis (Binkley et al., 1979, 1980; Iuvone and Besharse, 1983). Melatonin synthesis in the pineal gland has been localized to pinealocytes (Bubenik et al., 1974). Since pinealocytes in the pineal gland and the photoreceptors in the retina are analogous in many ways, photoreceptors are implicated as the source of retinal melatonin. Hydroxy indole-*O*-methyl transferase (HIOMT), one of the non-rate-limiting enzymes for melatonin synthesis, has been localized to photoreceptor terminals (Cardinali and Rosner, 1971b; Wiechmann et al., 1985). In a preliminary report, we showed that the ^{3}H-serotonin accumulated in rat retina in the light is rapidly converted to melatonin when the retina is subsequently exposed to darkness (Redburn and Mitchell, 1986). Therefore the decrease in ^{3}H-serotonin accumulation seen in darkness may be due, at least in part, to dark-stimulated activation of the NAT enzyme, which converts serotonin to melatonin.

Newly synthesized melatonin may be released by diffusion from photoreceptors (Yu and Chow, 1982), as is the case for pinealocyes (Cardinali, 1981). Melatonin is highly lipophilic (Lerner et al., 1959) and its release is not considered to be depolarization- or calcium-dependent, in contrast to most classical neurotransmitter release systems. Rather, its release (diffusion) rate is directly linked to its synthesis rate, as regulated by NAT (Cardinali, 1981). Serotonin is presumably released from pinealocytes via stimulation–secretion coupling, and it has neurotransmitter–neurohormone effects on other pineal cells and other parts of the brain (Quay, 1963, 1965; Koella, 1969; Owman, 1971). The indole system in the photoreceptor cell may mimic that of the pinealocyte in releasing both melatonin and serotonin under varying conditions and for different physiological purposes.

It is not possible to determine at this point whether any indole compound functions as the primary neurotransmitter released from photoreceptor cells, which subsequently stimulates and inhibits horizontal and bipolar cells. Primarily on the basis of electrophysiological and pharmacological experiments, the major photoreceptor neurotransmitter is currently thought to be an amino acid, such as glutamate or aspartate. (For a review,

see Miller and Slaughter, 1985.) However, it should be noted that electrophysiological analyses of serotonin receptors in retina have previously focused on the effects of indoles on elements of the inner plexiform layer (amacrine and ganglion cells) rather than on the outer plexiform layer (Straschill, 1968; Ames and Pollen, 1969; Straschill and Perwein, 1969; Thier and Wassle, 1984). Another complicating factor is that all of the reported studies have used species that contain indoleamine-accumulating amacrine cells. A more thorough assessment of the possible role of the indole system in photoreceptors would be greatly aided by an in-depth electrophysiological analysis of indoles in the outer plexiform layer of a species such as rat, which does not possess indoleamine-accumulating amacrine cells. Nonetheless, the available data do provide strong biochemical and morphological evidence for an indoleamine system in photoreceptors. Indoleamine uptake, synthesis, and release processes are localized in photoreceptor cells and appear to be regulated by light and dark. This regulation argues in favor of a physiologically important role for this indoleamine system.

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