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Modulation of Cone Horizontal Cell Activity in the Teleost Fish Retina. II. Role of Interplexiform Cells and Dopamine in Regulating Light Responsiveness

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Following the destruction of the terminals of the dopaminergic interplexiform cells by intraocular injections of 6-hydroxydopamine (6-OHDA), cone horizontal cells exhibited high light responsiveness in prolonged darkness and their responses to moderate and bright full-field flashes were as large as 60 mV. Furthermore, the light responsiveness of these cells in the 6-OHDA-treated retinas was not enhanced by background illumination.

The application of dopamine (50 μM) by superfusion to 6-OHDA-treated retinas resulted in a decrease in light responsiveness and changes in response waveform of the cone horizontal cells. Twenty minutes following dopamine application the responses of the cone horizontal cells closely resembled the response of cells recorded in prolonged dark-adapted retinas. Dopamine caused similar changes in cone horizontal cells recorded in light-exposed retinas, but had no obvious effects on rod horizontal cells.

The selective dopamine D1 receptor antagonist, Sch 23390, enhanced cone horizontal cell responsiveness when applied to prolonged dark-adapted retinas, mimicking background illumination. The light responsiveness of cone horizontal cells recorded after application of Sch 23390 was less than that for cells in retinas that had been exposed to background lights, but light responsiveness could not be further enhanced by background illumination. Another dopamine antagonist, (+)-butaclamol, was found to have effects similar to Sch 23390 on cone horizontal cells, but (−)-butaclamol, the inactive enantiomer, did not enhance the light responsiveness of these cells.

The results suggest that the dopaminergic interplexiform cells play a crucial role in the regulation of cone horizontal cell responsiveness by prolonged darkness and background illumination. These cells may release dopamine tonically in the dark, which suppresses cone horizontal cell responsiveness. Background illumination may decrease dopamine release and liberate cone horizontal cells from the suppression.

In the teleost retina, cone horizontal cells receive 2 known synaptic inputs (Dowling and Ehinger, 1975, 1978; Dowling et al., 1976); one is from cones and the other from interplexiform cells. The cones appear to use L-glutamate or a glutamate-like substance as their neurotransmitter (Ishida and Fain, 1981; Lasater and Dowling, 1982), whereas the interplexiform cells are dopaminergic (Ehinger and Falck, 1969; Ehinger et al., 1969; Dowling and Ehinger, 1978). The dopaminergic interplexiform cells provide mainly a centrifugal pathway in the retina (Dowling and Ehinger, 1978). That is, they receive their input in the inner plexiform layer from amacrine cells and centrifugal fibers, whereas most of their output is in the outer plexiform layer, onto the cone horizontal cells. The dopaminergic interplexiform cells make some synapses with bipolar cell dendrites in the outer plexiform layer, but they have never been observed to make junctions with photoreceptors or with rod horizontal cells. Thus, the dopaminergic interplexiform cells appear to be concerned principally with cone pathways in the teleost retina.

In fish the role of the dopaminergic interplexiform cell in visual information processing has been studied by analyzing the action of dopamine on horizontal cells and other retinal neurons (Hedden and Dowling, 1978; Negishi and Drujan, 1978; Teranishi et al., 1983, 1984; Hida et al., 1984; Lasater and Dowling, 1985). Dopamine has been shown to exert multiple effects on cone horizontal cells. It reduces the responsiveness of these cells to full-field light stimuli (Hedden and Dowling, 1978; Negishi and Drujan, 1978; Mangel and Dowling, 1985, 1987) and also alters the receptive field size of the cells by decreasing the conductance of the electrical junctions between the cells (Teranishi et al., 1983, 1984; Hankins and Ruddock, 1984; Lasater and Dowling, 1985). In addition, dopamine decreases both the dark-release and glutamate-induced release of GABA from horizontal cells (Yazulla and Kleinschmidt, 1982; Yazulla, 1985).

Dopamine appears to exert its effects on the cone horizontal cells by raising intracellular levels of cyclic AMP. Dopamine by itself has no effects on the membrane potential or membrane resistance of isolated cone horizontal cells (Lasater and Dowling, 1985; Knapp and Dowling, 1987). On the other hand, dopamine potently activates adenylate cyclase in the teleost retina (Dowling and Watling, 1981) and promotes the accumulation of cyclic AMP in horizontal cells (Van Buskirk and Dowling, 1981). The actions of dopamine on horizontal cells in both fish and turtle retinas can be mimicked by cyclic AMP, cyclic AMP analogs,

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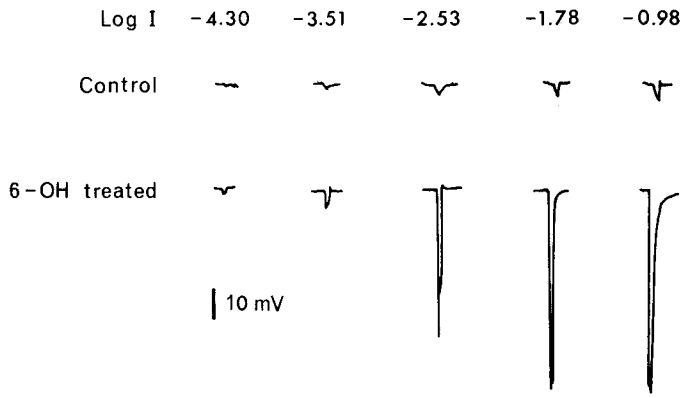

Figure 1. Responses of L-type cone horizontal cells to full-field flashes recorded in an untreated retina (*upper row*) and in a 6-OHDA-treated retina (*lower row*) maintained in prolonged (>2 hr) darkness. The treated retina was dissected from a fish that had received intraocular injections of 10 μl 6-OHDA on 2 consecutive days 1 week before the recording experiment. The responses recorded from the 6-OHDA-treated retina were much larger in size and had faster-rising phases and on-transients. Intensities of the light flashes, indicated above the response traces, are in relative log units. Full-field, 500 msec flashes of white light were used in all experiments.

and cyclic AMP-promoting agents (Piccolino et al., 1984; Teranishi et al., 1984; Lasater and Dowling, 1985; Mangel and Dowling, 1987).

Overall, the actions of dopamine on the horizontal cells appear to diminish the effectiveness of these neurons in mediating synaptic interactions. Decreasing light responsiveness, shrinking receptive field size and depressing neurotransmitter release are all effective ways of lessening the influences of horizontal cells. Since horizontal cells are known to mediate lateral inhibitory effects in the outer plexiform layer and to form the antagonistic surround responses of cones, bipolar cells, and certain ganglion cells (Werblin and Dowling, 1969; Naka, 1972; Naka and Witkovsky, 1972; Mangel and Miller, 1987), a decrease in receptive field surround responses is expected following dopamine application to the retina. Such changes in the receptive fields of bipolar cells and cones have been observed in the carp retina (Hedden and Dowling, 1978).

Furthermore, it has long been known that the antagonistic surround responses of ganglion cells decrease substantially or disappear completely in prolonged darkness (Barlow et al., 1957; Donner and Reuter, 1965; Enroth-Cugell and Lennie, 1975; Masland and Ames, 1976). In accord with these observations, Mangel and Dowling (1985, 1987) found that, in prolonged darkness, the responsiveness and receptive field size of cone horizontal cells in the carp retina decreased. They also showed that the application of dopamine to the retina mimics the effects of prolonged darkness. These findings suggest that the interplexiform cells and dopamine may be involved in the regulation of cone horizontal cell responsiveness by prolonged darkness and background illumination. In this paper we examine the role of the interplexiform cells and dopamine in mediating the effects of prolonged darkness and background illumination on cone horizontal cell responsiveness in the white perch retina.

Materials and Methods

Details of the preparation, photostimulator and intracellular recording techniques were given in the preceding paper (Yang et al., 1988). Test drugs were added to the superfusion medium and applied to the retina by means of a 4-way stopcock and manifold system (Mangel and Dowling, 1987). Changes from normal Ringer's solution to test drug solutions could be made routinely without disturbing intracellular recordings from horizontal cells.

Dopamine solutions were protected from oxidation by adding 0.1 mM ascorbic acid to the superfusion medium. Ringer's containing only ascorbate had no effects on horizontal cells. Sch 23390 [(+)-R-*-Chloro-2, 3,4,5-1H-3benzazepine-7-ol], a selective dopamine D1 receptor antagonist (Schering Pharmaceuticals), and (+)-butaclamol and (−)-butaclamol (Research Biochemicals Inc.) were first dissolved in methanol and then diluted in the Ringer's. All drug solutions were prepared immediately before the start of an experiment.

Intraocular injection of 6-OHDA. Injections of 10 μl 6-OHDA (Sigma, 2 mg/ml in 0.9% NaCl, with 1 mg/ml ascorbate added as an antioxidant) were made into both eyes of a white perch on 2 consecutive days, 1 week before the recording experiments. This treatment destroys the dopaminergic terminals and depletes dopamine from the retina (Ehinger and Nordenfelt, 1977; Dowling and Ehinger, 1978; Negishi et al., 1981; Watling et al., 1982; Cohen and Dowling, 1983). In a few instances, the dopamine content of the white perch retina was examined after 6-OH dopamine treatment. In most cases, dopamine levels were less than 5% of control levels (assays kindly carried out by Dr. R. Kropf).

Results

Effects of 6-OHDA treatment on cone horizontal cell responsiveness

Cone horizontal cells recorded in retinas maintained in prolonged darkness and treated with 6-OHDA exhibited very large responses. Indeed, responses to even moderate intensity light flashes occasionally exceeded 60 mV. Responses to light flashes of increasing intensity from a prolonged dark-adapted, 6-OHDA-treated retina of an L-type cone horizontal cell are illustrated in the lower row of Figure 1, while responses to the same intensity flashes recorded from a cell in an untreated prolonged dark-adapted retina are shown in the upper row. Except near threshold, the light-evoked responses were much larger in amplitude in the cell from the 6-OHDA retina. Furthermore, the responses were faster and, when elicited with moderate intensity flashes, were characterized by conspicuous on-transients. The features of responses recorded from cells from 6-OHDA-treated retinas contrast sharply with the small, slow responses observed in untreated retinas.

The differences in response amplitudes of cone horizontal cells from 6-OHDA-treated and untreated (control) retinas maintained in prolonged darkness are further illustrated in Figure 2, which shows V–Log I curves recorded under these 2 conditions. In the treated retinas ($n = 6$), the V–Log I curve was dramatically steeper than that obtained from cells in untreated retinas ($n = 8$), and it is similar to that of cells recorded in retinas exposed to background illumination (see Fig. 3 of the preceding paper). Moreover, in the 6-OHDA-treated retinas, responses of cone horizontal cells could not be further enhanced by background illumination. Figure 3 shows this result obtained from an L-type cone horizontal cell in a 6-OHDA-treated retina. A V–Log I curve was determined first in prolonged darkness and then following a 5 min presentation of dim background light (Log $I = -2.53$). The V–Log I curve following background illumination was displaced slightly (0.2 log unit) to the right on the intensity axis, and no enhancement of response amplitudes was observed. Presentation of a moderate (Log $I = -1.78$) background light further displaced the data to the right on the intensity axis (data not shown), and again no enhancement of response amplitudes was observed.

In 6-OHDA-treated retinas, both C-type and rod horizontal cells were often recorded. The C-type cells acted exactly like L-type cells. They exhibited large response amplitudes when

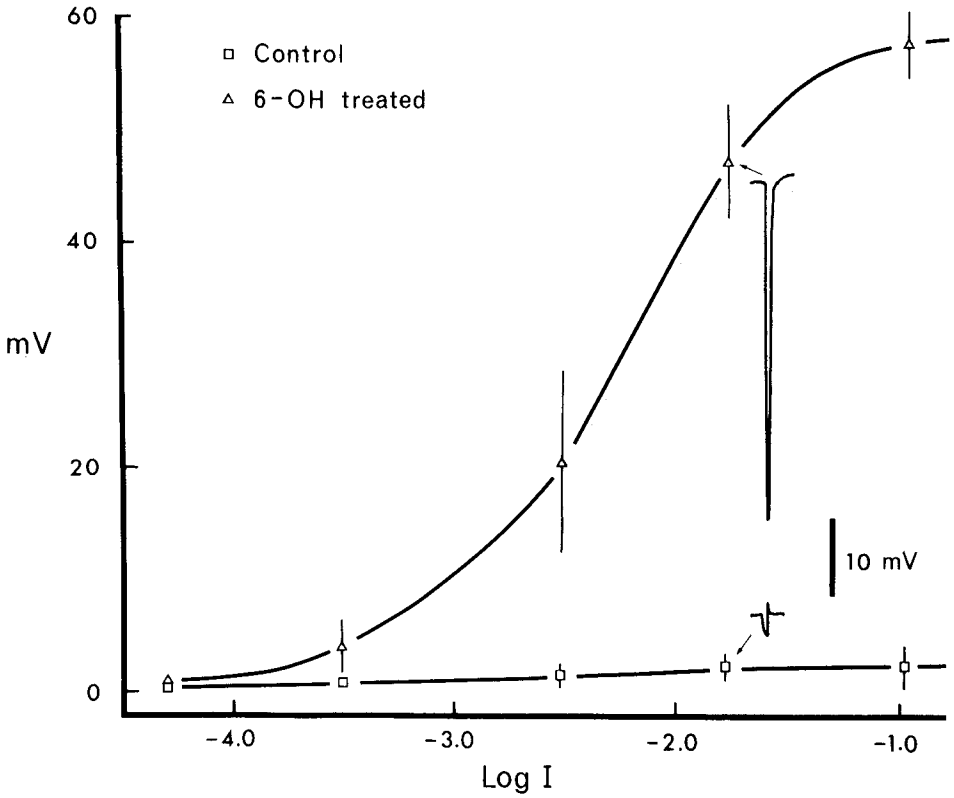

Figure 2. Averaged *V*–Log *I* curves of L-type cone horizontal cells in untreated (*squares*) and 6-OHDA-treated (*triangles*) retinas in prolonged darkness. Each data point represents the mean ±SD. The average *V*–Log *I* curve in the treated retinas ($n = 6$) was dramatically steeper than the average curve determined in untreated (control) retinas ($n = 8$). Also illustrated are representative responses to one flash intensity (Log $I = -1.78$) in a treated and untreated retina.

recorded in retinas maintained in prolonged darkness, and background illumination did not further sensitize the cell's response. Rod horizontal cells, on the other hand, were unaffected by 6-OHDA treatment of the retina.

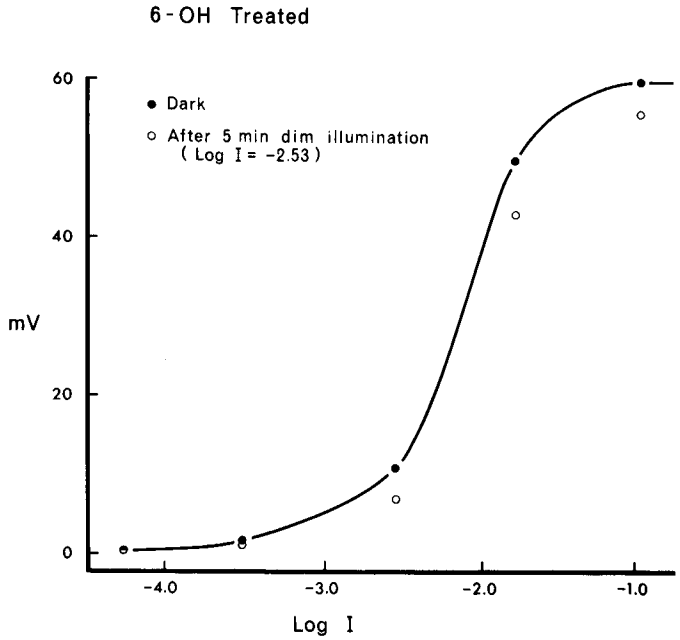

Figure 3. Lack of effect of background illumination on an L-type cone horizontal cell recorded in a 6-OHDA-treated retina. A *V*–Log *I* curve was first determined in prolonged (>2 hr) darkness (*filled circles*). Following 5 min of dim background light (Log $I = -2.53$), a second *V*–Log *I* curve was determined (*open circles*), which was slightly displaced to the right on the intensity axis but otherwise similar to the initial *V*–*Log I curve.*

Effects of dopamine on cone horizontal cell responsiveness

We next examined the effects of dopamine application on the response properties of cone horizontal cells in retinas treated with 6-OHDA ($n = 5$). A representative result obtained in an L-type cone horizontal cell is shown in Figure 4, where changes in responses to full-field test flashes (Log $I = -2.53$), recorded at different times after dopamine application, are illustrated. In this case, dopamine (50 μM) was applied to a 6-OHDA-treated retina that had been maintained in prolonged darkness; similar results were observed in 6-OHDA retinas exposed to background light. In the experiment shown in Figure 4, Ringer's containing dopamine was applied for 18 min. Following the onset of dopamine superfusion, the response to the full-field test flash began to decrease within about the first 2 min. Thereafter, the response diminished steadily so that after about 13 min the

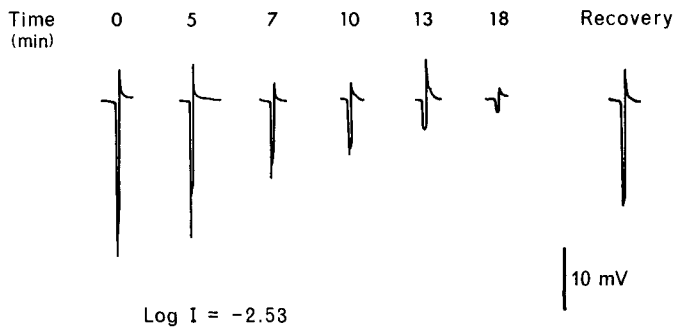

Figure 4. Effects of dopamine application (50 μM) on the responses of an L-type cone horizontal cell recorded from a 6-OHDA-treated retina. Over time the responses to the test flash (Log $I = -2.53$) decreased in size, became slower, and showed noticeable changes in waveform. At *right* is a response recorded from another horizontal cell impaled 65 *min after dopamine application ceased.*

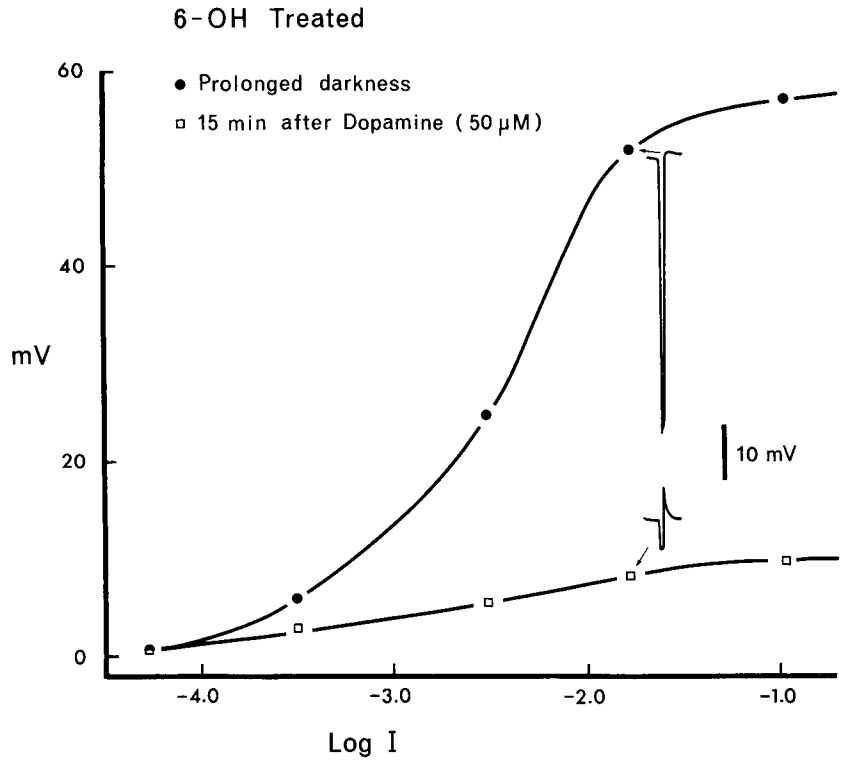

Figure 5. *V*–Log *I* curves of the cell, whose responses are shown in Figure 4, in prolonged darkness (*filled circles*) and after 15 min of dopamine (50 μM) application (*open squares*). The curve recorded after dopamine was extremely flat, resembling that of cells recorded in untreated prolonged dark-adapted retinas. Also illustrated are responses to one intensity flash (Log $I = -1.78$) recorded before and after dopamine application.

response to the test flash had decreased from 26 to 5.5 mV. Noticeable changes in response waveform accompanied the decrease in response size: the rising phase of the response became slower, the on-transient disappeared, and the depolarizing off-rebound became more prominent. By 18 min of dopamine superfusion the response further decreased to 3 mV, and the off-rebound was reduced in size so that the responses were quite similar in waveform to responses obtained in prolonged dark-adapted, untreated retinas. These characteristic changes caused by dopamine were observed in all 6 cells studied. The time course of the changes varied somewhat from cell to cell, but they were all completed within 30 min of the onset of dopamine application.

Following superfusion of a retina for 15–20 min with Ringer's containing dopamine, full recovery of horizontal cell responses to initial control levels was not observed, but partial recovery could often be followed for up to 1 hr after the retina was washed with normal Ringer's. On the right side of Figure 4 is illustrated the response of a cell impaled 65 min after the termination of dopamine superfusion. The response was larger than those recorded earlier, and it showed a faster time course.

Figure 5 shows the changes in *V*–Log *I* curves observed in the cell, whose responses are illustrated in Figure 4, before and after superfusion of the retina for 15 min with Ringer's containing dopamine. After dopamine, the responses to full-field flashes were depressed at all stimulus intensities so that the *V*–Log *I* curve was extremely flat and similar to that of cells recorded in untreated, prolonged dark-adapted retinas (compare with Fig. 3 of the preceding paper).

During dopamine application the membrane potential of the cone horizontal cells also showed characteristic changes. Figure 6 shows these changes for the cell whose responses are shown in Figures 4 and 5. Initially, the cell slightly depolarized; then, the membrane potential appeared to stabilize. However, after about 8 min of dopamine superfusion, the membrane potential rapidly hyperpolarized to about −43 mV and then remained at that level. This sequence of membrane potential changes was not always observed. In other retinas, only the initial depolarization was observed, and the membrane potential remained slightly depolarized for the duration of the dopamine application. In all retinas, recovery of membrane potential following the termination of dopamine application occurred over time. Figure 6 shows that the membrane potential of a cell impaled 45 min after the termination of dopamine application was similar to initial resting levels (open squares).

We also investigated the effects of dopamine application on cone horizontal cells in untreated, light-exposed retinas ($n = 5$). For these experiments we used cone horizontal cells that had been significantly enhanced in responsiveness following the presentation of a moderate (Log $I = -1.78$) background light. As noted in the previous paper (Yang et al., 1988), such cells remained in a stable, highly responsive state for more than 2 hr following light exposure. Unless otherwise specified, all experiments testing the effects of dopamine on non-6-OHDA-treated retinas were performed under these conditions (termed controls).

Figure 7 shows responses of an L-type cone horizontal cell from such a retina to full-field test flashes (Log $I = -2.53$) recorded at different times during dopamine (50 μM) superfusion. The effects of dopamine on the response of the cell to the full-field test flashes were qualitatively similar to those observed in 6-OHDA-treated retinas; that is, response amplitudes decreased with time, the responses became slower, and the on-transients disappeared. For the cell shown in Figure 7, the response to the test flash decreased in amplitude from 16 to 3 mV in the first 15 min, and by 18 min the responses recorded were quite similar in size and waveform to those obtained in prolonged dark-adapted retinas. Again, although the time course

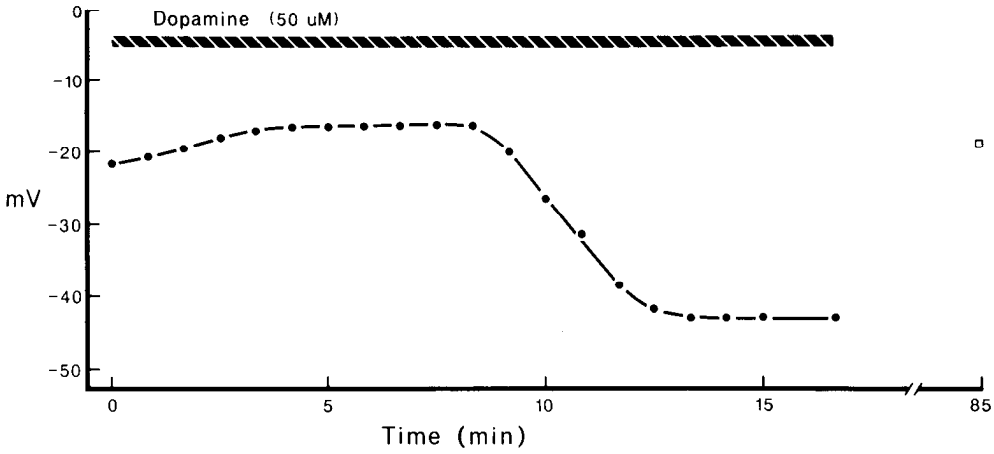

Figure 6. Effects of dopamine application (50 μM) on the membrane potential of an L-type cone horizontal cell recorded from a 6-OHDA-treated retina. Following dopamine application, the cell slightly depolarized in the first few minutes and the membrane potential then leveled off. The cell began to hyperpolarize after about 8 min, and within the next 5 min, the membrane potential reached −43 mV. The *open square* on the right side of the figure indicates the membrane potential of a cell impaled 65 min after dopamine application ceased.

of the changes varied from cell to cell, similar effects were observed in all 10 cells studied.

In Figure 8, *V*–Log *I* curves recorded before and after prolonged (20 min) dopamine application to the retina are shown for the cell whose responses are illustrated in Figure 7. Following dopamine application, the *V*–Log *I* curve was quite flat, and the light evoked responses only slightly increased in size (by 4 mV) over a range of 2.55 log units. The *V*–Log *I* curve was similar to that obtained from cells in retinas maintained in prolonged darkness.

As was the case for cells in 6-OHDA-treated retinas, changes in membrane potential were typically observed during dopamine application. The cells almost always depolarized somewhat in the first 10 min of dopamine superfusion, but thereafter, the membrane potential either stabilized or, in some cases, hyperpolarized by 20–25 mV.

Effects of dopamine antagonists on cone horizontal cell responsiveness

The finding that dopamine application mimics the effect of a prolonged period of darkness on the responsiveness of cone horizontal cells suggests that dopamine is tonically released in the retina in darkness and suppresses cone horizontal cells. If this is so, antagonists of dopamine should increase the light responsiveness of cone horizontal cells when applied to prolonged dark-adapted retinas. Figure 9 shows the effects of Sch 23390 (10 μM), a selective and potent D1 dopamine receptor antagonist (Iorio et al., 1983), on an L-type cone horizontal cell recorded from a retina maintained in prolonged darkness. During drug application, the cell gradually hyperpolarized by 18 mV, and the membrane potential became stable after 10–12 min. This hyperpolarization was accompanied by a substantial increase in response amplitudes. At 10 min after Sch 23390 application, the response amplitude to a full-field flash of Log $I = -0.98$ had increased from 5 to 22 mV. Furthermore, the light responses were faster after drug application. Longer applications of Sch 23390 did not cause further increases in response amplitudes; rather, the responses tended to decrease slightly in size. These effects of Sch 23390 were observed in 7 of 9 cells studied, and a 10 min application of Sch 23390 increased the maximum response amplitudes of these cells to 20–30 mV. Two cells were unchanged in response amplitudes following Sch 23390 application.

V–Log *I* curves recorded from another cone horizontal cell in prolonged darkness before and following a 10 min application of Ringer's containing Sch 23390 are shown in Figure 10. The *V*–Log *I* curve recorded after drug application was considerably steeper than before drug application; the response to a dim flash

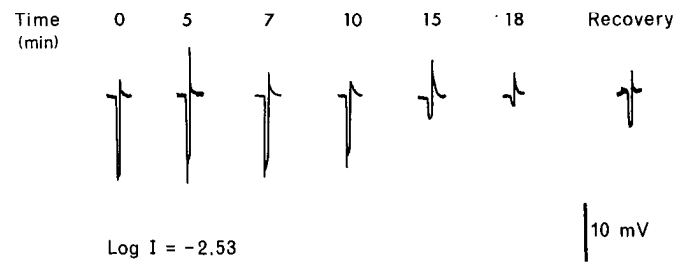

Figure 7. Effects of dopamine application (50 μM) on the responses of an L-type cone horizontal cell recorded in a light-sensitized untreated retina. Over time, responses decreased in amplitude and became slower. On the *right* is shown the response of another cell impaled 77 min after the retina was washed with normal Ringer's.

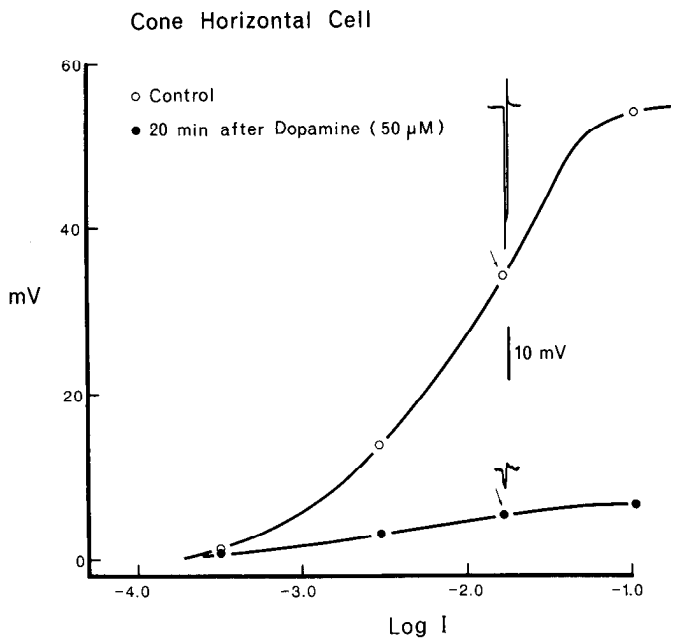

Figure 8. *V*–Log *I* curves of the cell whose responses are shown in Figure 7 before and after 20 min of dopamine application (50 μM). Following dopamine, the *V*–Log *I* curve was much flatter.

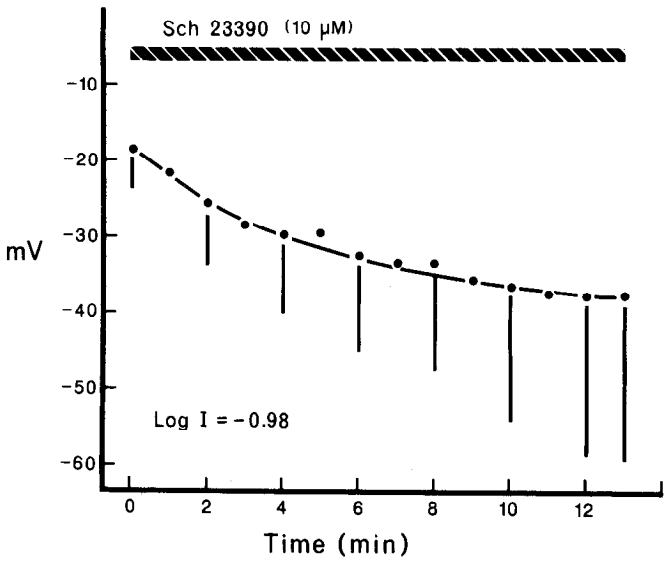

Figure 9. Effects of Sch 23390 (10 μM) on an L-type cone horizontal cell recorded in prolonged (>2 hr) darkness. Membrane potentials are indicated by the *filled circles*, while the *vertical bars* represent response amplitudes to bright test flashes (Log $I = -0.98$). Following Sch 23390 application, the cell gradually hyperpolarized and the responses increased in size.

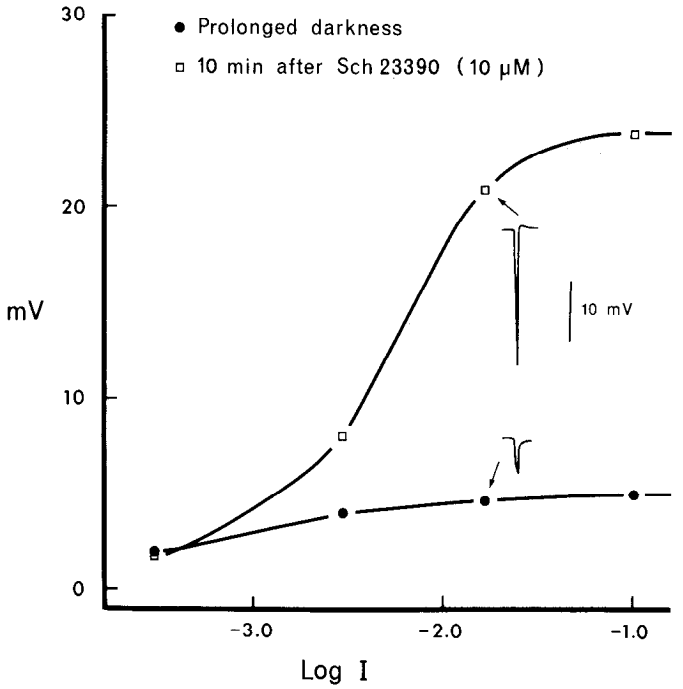

Figure 10. *V*-Log *I* curves of the cell whose responses are shown in Figure 11 before and after 10 min of Sch 23390 application. Compared with the curve recorded in prolonged darkness (*filled circles*), the curve recorded after drug application (*squares*) was considerably steeper. Note also that the responses sped up after drug application.

(Log $I = 3.53$) was hardly changed, but the response to a relatively bright flash (Log $I = -0.98$) was increased from 5 to 24 mV. Although the *V*-Log *I* curves recorded following Sch 23390 application were consistently less steep than those obtained from light-exposed retinas, they were affected very little, if at all, by the presentation of moderate background illumination (Log $I = -1.78$). In other words, the responsiveness of these cells was no longer affected by background illumination.

We also examined the effects of (+)-butaclamol, another antagonist of dopamine (Iversen, 1975), and obtained similar results. Figure 11*A* shows the changes in both membrane potential and response amplitude following (+)-butaclamol (25 μM) application to a cone horizontal cell recorded in a retina kept in prolonged darkness. In response to the drug the cell slightly hyperpolarized, and the response to a bright test flash (Log $I = -0.98$) increased significantly reaching an amplitude of about 25 mV in 10 min. On the other hand, (−)-butaclamol, an inactive enantiomer, did not show similar effects. As shown in Figure 11*B*, following 14 min application of (−)-butaclamol (25 μM), neither membrane potential nor response amplitudes changed significantly. Similar results to those shown in Figure 11 were obtained in another cell. In 2 other cases, (−)-butaclamol slightly depolarized the cells and a simultaneously recorded electroretinogram decreased greatly in amplitude. In no case, following (−)-butaclamol application, did response amplitudes of the horizontal cells grow.

As control for nonspecific effects of the dopamine antagonists on horizontal cell responses, (−)-butaclamol (25 μM) was applied in 2 cases to light-sensitized cells. In both cases, (−)-butaclamol depolarized the cells by 5 mV in the first 10 min of drug application and maximum response amplitudes decreased by 15–20%.

Discussion

In the preceding paper (Yang et al., 1988), it was shown that the light responsiveness of cone horizontal cells in the white perch retina was strongly suppressed in prolonged darkness and dramatically enhanced by background lights. Here, we have demonstrated that cone horizontal cells from retinas maintained in prolonged darkness, but treated with 6-OHDA, are highly responsive to illumination and behave like cells recorded in light-sensitized retinas. Furthermore, application of dopamine to 6-OHDA-treated or light-sensitized retinas strongly suppresses the responsiveness of cone horizontal cells, mimicking the effects of prolonged darkness. Conversely, Sch 23390, a specific D1 dopamine receptor antagonist (Iorio et al., 1983) and (+)-butaclamol, another dopamine antagonist (Iversen, 1975), enhance the responsiveness of cone horizontal cells in prolonged darkness, mimicking the effect of background illumination. These results collectively suggest that the interplexiform cells and dopamine play a crucial role in the regulation of the light responsivenes of cone horizontal cells by prolonged darkness and background illumination.

The results reported here largely confirm carlier studies on the effects of dopamine on the light responsiveness of cone horizontal cells in the carp and goldfish retinas (Hedden and Dowling, 1978; Teranishi et al., 1983, 1984; Mangel and Dowling, 1985, 1987), but in the white perch retina, the effects of dopamine, like those of prolonged darkness, are considerably more dramatic. In carp, dopamine and prolonged darkness reduce maximum response amplitudes to full-field flashes only by about 40% (Mangel and Dowling, 1985, 1987). In white perch retinas, on the other hand, dopamine and prolonged darkness reduced maximum response amplitudes from 50–60 mV down to 4–6 mV, a 10-fold change. These comparative findings also strengthen the notion that dopamine is involved in the suppression of cone horizontal cell responses by prolonged darkness. That is, although prolonged darkness affects the horizontal cells somewhat differently in the 2 species, dopamine mimics these effects in each case.

Dopamine also induces changes in response waveform of cone horizontal cell responses similar to those induced by prolonged darkness. That is, when the retina was kept in the dark for a prolonged period (>30 min), the responses of the cells ceased to show the on-transients and the time-to-peak of the response was noticeably lengthened (Yang et al., 1988). Similarly, after dopamine application to the retina the responses eventually became slower and the on-transient disappeared. We noted, however, that, in the first several minutes following dopamine application, the rising phase of response was unchanged, but the on-transient often became more conspicuous. Subsequently, the on-transients disappeared and responses became slower and appeared similar in response waveform to those recorded from cells in retinas maintained in prolonged darkness. In carp, Mangel and Dowling (1987) observed that dopamine and prolonged darkness enhanced the on-transients in the L-type horizontal cells. This may reflect the fact that dopamine and prolonged darkness do not depress the responses of the cone horizontal cells in carp to nearly the same extent as they do in the white perch. In other words, the enhanced on-transients observed after dopamine treatment in the carp may correspond to the enhanced on-transients seen in white perch in the first few minutes following dopamine application.

With regard to changes in membrane potential induced in cone horizontal cells by dopamine and prolonged darkness, a significant discrepancy was occasionally noted. Although both dopamine and prolonged darkness caused the cells to depolarize initially, following prolonged dopamine application cone horizontal cells sometimes hyperpolarized to between −40 and −50 mV (Fig. 6). This level of hyperpolarization was never observed in cells recorded in prolonged dark-adapted retinas: the membrane potential of cone horizontal cells was always at a much more depolarized level after prolonged darkness (Yang et al., 1988). The reasons for this difference remain to be explained. A possible explanation is that the late hyperpolarization of cone horizontal cells is caused by a nonspecific effect of dopamine.

A detailed examination of the effect of prolonged dopamine application on the membrane potential of light-sensitized horizontal cells in the white perch retina has recently been completed by O. Umino in our laboratory. In a sample of 63 cells, he observed initial small depolarizations in 85% of the cells, initial small hyperpolarizations in 10% of the cells, and no significant membrane potential change in 3 cells (~5%). Late hyperpolarization of the cell membrane, similar to that observed here in a number of experiments (i.e., Fig. 6), was observed in only about 10% of the cells ($n = 6$) during prolonged dopamine superfusion. He also observed a few cases of hyperpolarization of the cell membrane during prolonged superfusion of the retina with control Ringer's, providing evidence that this phenomenon is not strictly related to dopamine application.

The effect of dopamine on rod horizontal cells was not extensively examined, but preliminary experiments showed that neither responsiveness nor response waveform of these cells was altered following 5 min of dopamine application. In addition, though dramatic changes in response properties of cone horizontal cells occurred in retinas treated with 6-OHDA, no changes could be detected in rod horizontal cells. In these treated retinas the rod cells showed response properties similar to those in untreated retinas. In goldfish retinas also, dopamine was not found to have effects on rod horizontal cells (Hedden and Dowling, 1978). These findings, in conjunction with the morphological observation that dopaminergic interplexiform cells in fish do not contact rod horizontal cells (Dowling and Ehinger, 1978),

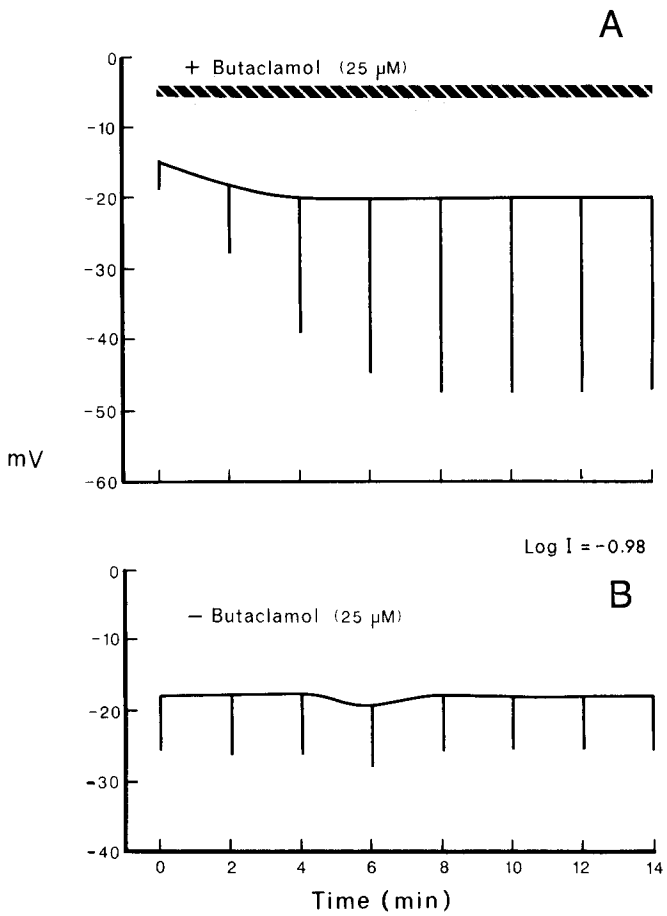

Figure 11. Effects of (+)-butaclamol (*A*), a dopamine antagonist, and (−)-butaclamol (*B*), an inactive enantiomer, on cone horizontal cells. The cells were recorded in prolonged darkness from 2 different retinas. In both *A* and *B*, changes in membrane potential are represented by the *continuous line*, while the *vertical bars* indicate amplitudes of the responses to test flashes (Log $I = -0.98$). Following (+)-butaclamol (25 μM), the cell hyperpolarized somewhat and responses increased significantly in size. In contrast, following (−)-butaclamol (25 μM), neither membrane potential nor response amplitude changed significantly.

suggest that the effects of dopamine in the teleost retina are limited to the cone horizontal cells and perhaps to cone pathways.

The fact that dopamine application mimics the action of prolonged darkness on the response properties of cone horizontal cells suggests that the suppression of light responsiveness in these cells results from the release of dopamine in the dark by the interplexiform cells. This suggestion is supported particularly by the experiments that examined the effects of dopamine receptor antagonists on the responsiveness of the cone horizontal cells. Application of Sch 23390 to the prolonged dark-adapted retina significantly increased light responsiveness of cone horizontal cells, similar to the effect of background lights. Moreover, both changes in membrane potential and response waveform occurring after Sch 23390 application were qualitatively similar to those caused by background lights. That is, the cells hyperpolarized and the light-evoked responses speeded up. Although Sch 23390 application did not increase the light responsiveness of cone horizontal cells to a level that it attained following background illumination, subsequent presentation of background lights to the cells failed to enhance their responsiveness further. This suggests that Sch 23390 not only antag-

onizes the effects of dopamine, which leads an increase of light responsiveness of cone horizontal cells, but exerts other effects that hinder further increase of the responsiveness of the cells. In support of this notion, we have found that Sch 23390, when applied to retinas that had been exposed to background lights, hyperpolarized the cone horizontal cells somewhat and decreased their light responsiveness. Such nonspecific effects of this agent may account for the large hyperpolarization of the cell membrane noted during superfusion of prolonged dark-adapted retinas with the drug (i.e., Fig. 9).

The conclusion that the increase of light responsiveness by Sch 23390 in prolonged dark-exposed retinas was caused by blockade of dopamine receptors was strengthened by the finding that (+)-butaclamol, another dopamine antagonist, had similar effects on cone horizontal cells. However, (−)-butaclamol, an inactive enantiomer, did not increase the light responsiveness of cone horizontal cells in prolonged dark-adapted retinas; indeed, in several cells (−)-butaclamol depolarized horizontal cells and decreased light-evoked responses, suggesting that this agent also has some nonspecific, deleterious effects on retinal function.

The results of experiments on the response properties of cone horizontal cells following the intraocular injection of 6-OHDA clearly demonstrate a crucial role of the dopaminergic interplexiform cells in the regulation of cone horizontal cell responsiveness by darkness and illumination. In eyes treated with 6-OHDA, cone horizontal cells were highly responsive even in prolonged darkness. Often the light responsiveness of these cells in prolonged darkness was greater than that observed in untreated retinas that had been exposed to background lights; furthermore, the light responsiveness was not increased by presentation of background lights. Responses of these cells in prolonged darkness exhibited fast rising phases and conspicuous on-transients, whereas in untreated retinas, responses of cone horizontal cells were characterized by these features only when they had been exposed to background lights. Since intraocular injections of 6-OHDA have been shown to destroy dopaminergic interplexiform cells in the teleost fish, as well as to decrease dopamine levels (Dowling and Ehinger, 1978; Negishi et al., 1981; Watling et al., 1982), these results provide additional evidence that the suppression of the light responsiveness of cone horizontal cells in prolonged darkness is due to the effects of dopamine released by the interplexiform cells in the dark.

In many parts of the brain dopamine has been shown to activate the enzyme adenylate cyclase and to cause substantial increases in intracellular cyclic AMP levels (Kebabian et al., 1972; Iversen, 1975; Daly, 1977). It has been previously shown that horizontal cells possess dopamine receptors linked to adenylate cyclase (Van Buskirk and Dowling, 1982) and that dopamine application to isolated horizontal cells stimulates the accumulation of cyclic AMP in a dose-related fashion. Recently, substantial evidence has been accumulated that dopamine exerts its effect on the receptive fields of horizontal cells via cyclic AMP (Teranishi et al., 1983, 1984; Piccolino et al., 1984; Lasater and Dowling, 1985). The question of whether dopamine suppresses the light responsiveness of cone horizontal cells by inducing accumulation of cyclic AMP remains to be answered, although this seems likely. For example, forskolin, a diterpene compound that stimulates adenylate cyclase without activating dopamine receptors (Seamon et al., 1981), has been shown to alter cone horizontal cell receptive field size in carp (Mangel and Dowling, 1987), and in 2 experiments with the white perch retina, we observed response amplitude suppression induced by forskolin. However, the time course of these effects and the changes in response waveform caused by forskolin were not exactly like those produced by dopamine.

It was recently found that dopamine and cyclic AMP enhance ionic conductances gated by kainate, an agonist of the photoreceptor transmitter, and by L-glutamate, the presumed photoreceptor transmitter, in isolated and cultured white perch cone horizontal cells (Knapp and Dowling, 1987). These findings provide a possible explanation for the suppression of the light responsiveness of cone horizontal cells in prolonged darkness. That is, if dopamine is released in the dark by the interplexiform cells and enhances the potency of the photoreceptor transmitter, the decrease in transmitter release caused by light would be rendered less effective, resulting in a decrease of the amplitude of cone horizontal cell responses to light. This mechanism would work if light modulates the dark release of transmitter from the receptor and does not turn it off completely regardless of stimulus intensity.

An augmentation by dopamine and cyclic AMP of cone horizontal cell sensitivity to the photoreceptor transmitter could also explain the depolarization of horizontal cells in prolonged darkness and the hyperpolarization of the cells during background illumination. That is, under most conditions, photoreceptors are thought to release transmitter that tonically depolarizes horizontal cells. If dopamine is released in the retina during prolonged darkness, this would facilitate the action of the photoreceptor transmitter on the horizontal cells, thereby depolarizing them. After background illumination, when dopamine release from the interplexiform cells is suppressed, the sensitivity of the horizontal cells to the photoreceptor transmitter would be decreased and the cells would hyperpolarize.

Another possible mechanism that might contribute to the suppression of light responsiveness of cone horizontal cells relates to anatomical changes that occur in prolonged dark-adapted retinas. It has been known for some years that significant changes in morphology occur in the outer plexiform layer of the carp when the animals are kept in the dark for long periods. Finger-like extensions of the cone horizontal cell processes, termed spinules, are abundant in the light-adapted retina but disappear after a prolonged period of dark adaptation (Raynauld et al., 1979; Wagner, 1986; De Juan and Dowling, 1987). This change in morphology may make synaptic transmission between photoreceptors and horizontal cells less effective and could be responsible for the suppression of cone horizontal cell responsiveness following prolonged darkness. Indeed, some evidence has been presented that feedback from L-type horizontal cells to cones is less effective after prolonged dark adaptation, perhaps reflecting these anatomical changes (Weiler and Wagner, 1984).

It was previously reported that the release of dopamine from cells in the retina is mediated by flickering light (Kramer, 1971; Hedden and Dowling, 1978; Bauer et al., 1980; Dowling and Watling, 1981; Reading, 1983). Furthermore, recent studies on the retinomotor movements in teleost fish indicated that dopamine-induced retinomotor movements in cones, rods, and pigment epithelium are similar to those observed to occur in the light (Dearry and Burnside, 1985, 1986), suggesting that dopamine is released in the fish retina in the light. On the other hand, results presented here suggest dopamine is released from interplexiform cells in the dark. Resolution of these apparently conflicting observations has not been achieved. In support of the idea that dopamine is released in the dark is the recent finding that cyclic AMP levels are elevated in dark-adapted

retinas (O'Connor et al., 1986). Furthermore, it has recently been reported that, in the cat and pigeon retinas, dopamine is released at the offset of light during flickering light stimulation (Hamasaki et al., 1986). As yet, no study of the release of endogenous dopamine in fish retinas following light exposure or during prolonged darkness has been reported.

Although exogenously applied dopamine mimics to a large extent the effects of prolonged darkness on cone horizontal cell responsiveness, the effects are not always identical. Beside the late hyperpolarization of membrane potential noted above, suppression of cone horizontal cell responsiveness by dopamine was often not as strong as that occurring after prolonged darkness. Similarly, antagonists of dopamine only partially mimic the effects of background illumination on cone horizontal cell responsiveness in prolonged darkness. On the other hand, when the interplexiform cells were destroyed by intraocular injections of 6-OHDA, the cone horizontal cells seemed to be completely liberated from the suppression caused by prolonged darkness. These observations raise the interesting possibility that the dopaminergic interplexiform cells may have effects on cone horizontal cells other than those mediated by dopamine. It may be that these interplexiform cells release neuroactive substances in addition to dopamine. In support of this idea, the colocalization of neuroactive substances in neurons has been demonstrated both in the retina and elsewhere in the brain (see, for example, Burnstock, 1976; Cuello, 1982; Lundberg and Hökfelt, 1983; Osborne, 1983; Chan-Palay and Palay, 1984; Lam et al., 1985, 1986; Li et al., 1986).

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