The Journal of Neuroscience, August 1987, 7(8): 2273–2284

Dopaminergic Mechanisms Underlying the Reduction of Electrical Coupling Between Horizontal Cells of the Turtle Retina Induced by *d*-Amphetamine, Bicuculline, and Veratridine

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Previous studies have shown that dopamine, bicuculline, or *d*-amphetamine reduce the electrical and dye-coupling between the axon terminals of the horizontal cells of the turtle retina (see Piccolino et al., 1984). In the present study we observed similar effects following the application of veratridine. The actions of all these drugs were prevented by dopamine antagonists acting on D1 receptors such as flupenthixol and SCH 23390. However, in contrast to dopamine, the actions of *d*-amphetamine, bicuculline, and veratridine were attenuated or abolished by pharmacological agents (such as 6-OH-dopamine, α-methyl-*p*-tyrosine, or reserpine) known to reduce the release of dopamine from dopaminergic neurons. Moreover, the actions of veratridine and bicuculline were prevented by tetrodotoxin, indicating that one or more neurons in the dopamine pathway are spike-generating.

We conclude that *d*-amphetamine, bicuculline, and veratridine reduce electrical coupling between the axon terminals of the turtle horizontal cells by promoting the release of endogenous dopamine from the dopaminergic amacrine cells previously identified (Witkovsky et al., 1984). Electron-microscopic observations revealed that 6-OH-dopamine selectively attacked this population of amacrine cells. No degenerating terminals were found adjacent to the horizontal cell axon terminals. On this basis, we postulate that dopamine reaches the horizontal cell by diffusion through the extracellular space.

Beginning with the first demonstration of the presence of dopaminergic neurons in the retina of mammals (Malmfors, 1963; Haggendal and Malmfors, 1965), a large body of experimental evidence has pointed to a neurotransmitter role for dopamine in the vertebrate retina (reviewed in Ehinger, 1983; see also Witkovsky et al., 1984; Oyster et al., 1985). Dopaminergic neurons have been found in the retinas of all vertebrates investigated. Moreover, specific dopamine receptors have been shown to exist in the vertebrate retina (Schorderet and Magistretti, 1983), together with mechanisms for the synthesis, release, and uptake of dopamine. These mechanisms appear to be modulated by light. Recent studies indicate a role for dopamine in ganglion cell receptive-field organization (Jensen and Daw, 1983) and in photomechanical movements (Dearry and Burnside, 1985; Pierce and Besharse, 1985). Of particular interest for the present study, dopamine also has been found to reduce the permeability of gap junctions between horizontal cells of turtle (Gerschenfeld et al., 1982; Piccolino et al., 1984) and fish (Teranishi et al., 1983, 1984) retinas. This effect is mediated by the activation of D1-type dopamine receptors coupled to an adenylate cyclase that is very probably located on the membrane of the horizontal cells (Van Buskirk and Dowling, 1981; Piccolino et al., 1984; Lasater and Dowling, 1985).

In order for dopamine to control the electrical coupling between horizontal cells under physiological circumstances, retinal dopaminergic neurons must have a functional output on horizontal cells. Anatomical and physiological evidence in support of such a mechanism exists in the fish. Teleost retinas contain dopaminergic interplexiform cells that establish direct synaptic contacts with horizontal cells (Dowling and Ehinger, 1975). However, in turtle retina the dopaminergic amacrine cells thus far identified do not contact horizontal cells (Witkovsky et al., 1984). On anatomical grounds, therefore, it is difficult to conceive how dopamine could act as a physiological modulator of horizontal cell electrical coupling in the turtle.

In view of these apparently contradictory findings, we decided to investigate, in turtle retina, whether and how dopaminergic neurons influence horizontal cell coupling. We studied the effects of various drugs thought to act upon dopaminergic cells or dopamine receptors, before and after treatment, with agents that destroyed or inactivated dopaminergic neurons. The toxic effects of one of these agents, 6-hydroxydopamine, were also examined by electron microscopy.

Materials and Methods

Animals. The experiments were carried out on the isolated and superfused eyecup preparation of the turtle *Pseudemys scripta elegans,* obtained from Carolina Biological Supply (Burlington, NC). The experimental procedures have been described in detail elsewhere (Piccolino et al., 1984). The composition of the bicarbonate Ringer's solution used to superfuse the preparation was (in millimolar concentrations): NaCl, 110; KCl, 2.6; $NaHCO_3$, 22; $MgCl_2$, 2; $CaCl_2$, 2; D-glucose, 10. This solution was bubbled continuously with a mixture of 95% O_2 and 5% CO_2, to a final pH of 7.4.

Electrophysiology. Intracellular recordings were made with glass mi-

Received Apr. 3, 1986; revised Feb. 4, 1987; accepted Feb. 6, 1987.

This research was supported by Consiglio Nazionale delle Ricerche (NATO Visiting Professor Program) (P.W. and M.P.), by NIH Grant EY03750 to P.W., and by Grant EY01842 and an unrestricted grant from Research to Prevent Blindness to the Department of Ophthalmology, New York University Medical Center. We wish to thank P. Taccini for preparing the illustrations and G. C. Cappagli for technical assistance.

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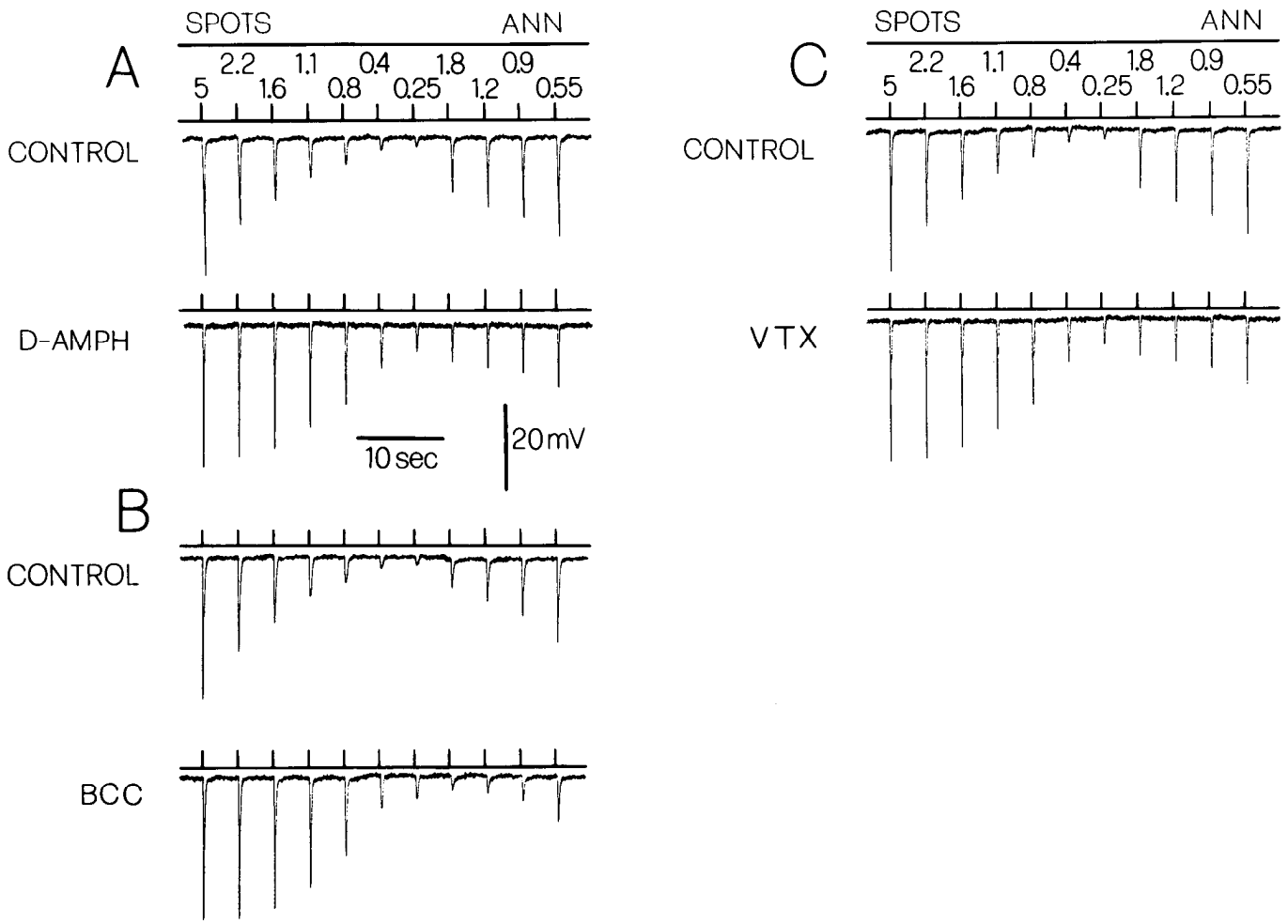

Figure 1. Drug-induced modifications of the receptive field of H1ATs in untreated retinas. Intracellular recordings of the responses of H1ATs to spots and annuli (*ANN*) of light of fixed intensity and different sizes, obtained in control saline (*CONTROL*), are compared to the responses obtained from the same H1AT after the application of (*A*) *d*-amphetamine sulfate (50 μM; *D-AMPH*); (*B*) bicuculline methobromide (50 μM; *BCC*); and (*C*) veratridine (10 μM; *VTX*). In this and the following figures showing electrophysiological records, the presentation of the light stimuli (10 msec flashes) is monitored above the response tracings. Numbers near the stimulus trace indicate, in millimeters, the diameters of the light spots or the inner diameters of the annuli. The outer diameters of the annuli were always 5 mm. Light attenuated by 2.1 log units.

cropipettes filled with a solution containing either 3 M buffered potassium acetate and 0.2 M potassium chloride (resistance, 60–150 MΩ) or Lucifer yellow (30 gm/liter in 23 mM LiCl; resistance, 300–600 MΩ). The structures impaled were identified by the characteristics of their light responses and the identification was confirmed by intracellular injection of Lucifer yellow. Lucifer yellow was injected by applying 0.5 sec pulses of 5-nA-intensity hyperpolarizing current through the microelectrode at 0.5 Hz frequency for 5 min. After 10–30 min, the retina was stripped from the eyecup and fixed in a freshly prepared solution of formaldehyde at 40 gm/liter in 100 mM phosphate buffer (pH 7.2) for 10–12 hr at 4°C. Thereafter, it was dehydrated in graded alcohols, cleared in xylene, and whole-mounted in Entellan.

Optics. Light stimulation of the retina was provided by a conventional double-beam photostimulator that generated circular or annular light stimuli of different diameters. Neutral-density filters were used to attenuate light intensity. The flux density of the unattenuated light on the retina was about $1.5–3 \times 10^{-5}$ μW/μm².

Pharmacology. In some experiments the eye was injected on 2 successive d, 5–12 d before enucleation, with variable volumes of a saline solution (NaCl, 9 gm/liter) containing 6-OH-dopamine HCl (10 gm/liter), pargyline HCl (10 gm/liter), and buffered ascorbic acid (1 gm/liter). Control experiments were carried out by injecting the same volumes of saline, containing ascorbic acid but lacking both 6-OH-dopamine and pargyline. In other experiments the eye was treated with 30–300 μg of reserpine base on 2 successive d just before enucleation. Reserpine was first dissolved in a drop of acetic acid, then diluted in distilled water to the desired concentration so as to inject the desired dose in 20–50 μl of the solution. It is possible that a portion of the injected drug precipitated upon contact with the vitreous, since a precipitation cloud was seen in reserpine-injected eyes. In control experiments, the same volume of distilled water, containing acetic acid but no reserpine, was injected. In a third series of experiments, the eyecup preparation was incubated for long periods at 4°C in bicarbonate Ringer's containing 100 μm α-methyl-*para*-tyrosine (α-M-*p*-T as methyl ester HCl) and 1 mM sodium ascorbate. The same solution was used thereafter to superfuse the preparation at room temperature during the electrophysiological experiment. In control experiments the retina was incubated with Ringer's containing 1 mM sodium ascorbate but lacking α-M-*p*-T at the same temperature and for comparable times. In a few experiments (see Results), α-M-*p*-T was also injected in the eye *in vivo* (500 μg, dissolved in 10 mM sodium ascorbate-containing Ringer's.

Dopamine HCl, bicuculline methobromide, veratridine, 6-OH-dopamine HCl, α-methyl-*para*-tyrosine methyl ester HCl, and pargyline HCl were purchased from Sigma (St. Louis, MO). *d*-Amphetamine sulfate was a gift from colleagues. Flupenthixol was generously supplied by Lundbeck (Denmark), sulpiride by Ravizza (Italy), and SCH 23390 [(R)-(+)-8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1 H-3-benzapin-7-ol; hemimaleate] by Essex (Italy). Drugs were normally dissolved in the perfusion saline just before use. Sulpiride and SCH 23390 were first dissolved in diluted solutions of HCl, whereas veratridine was

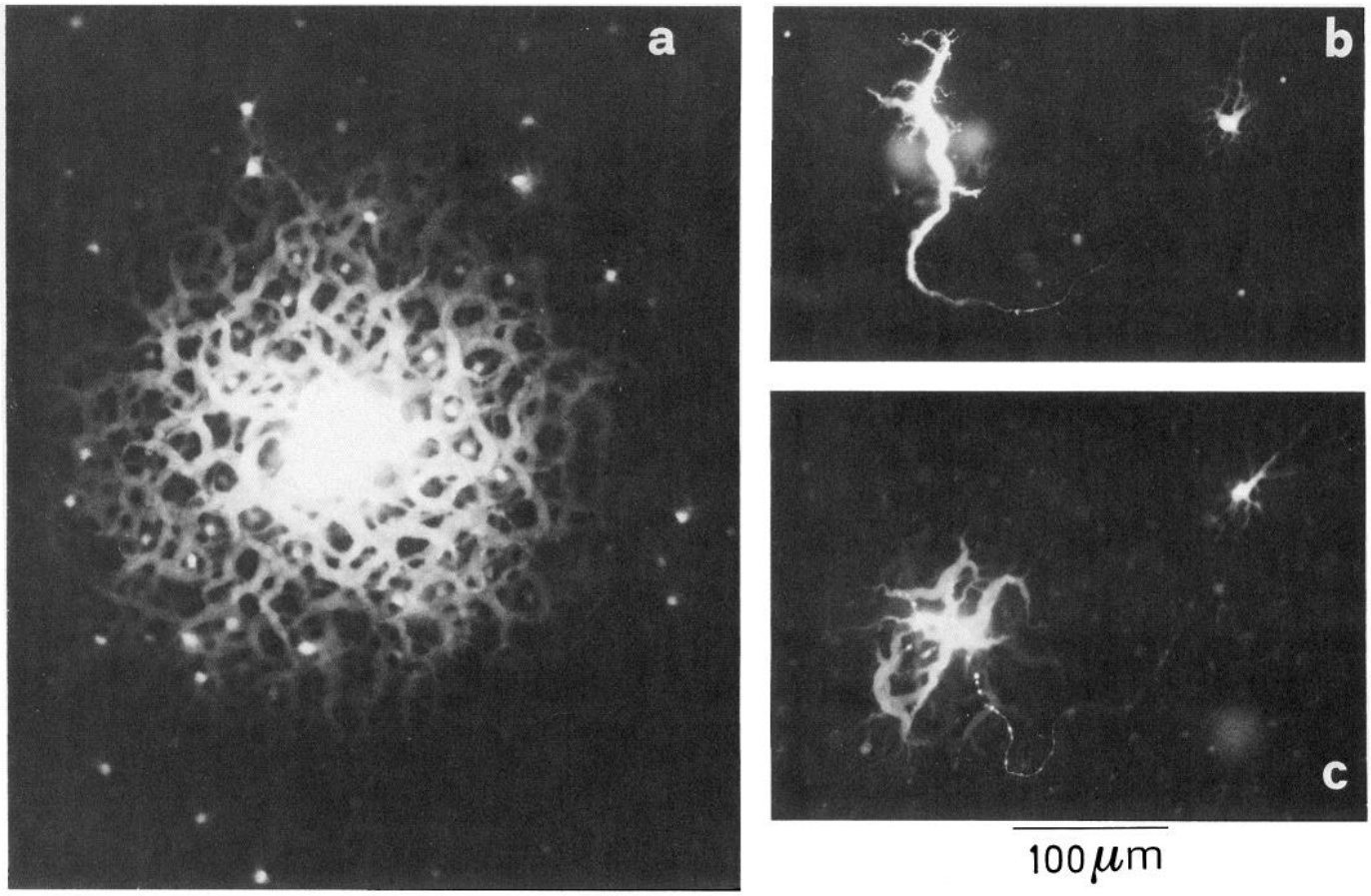

Figure 2. Diffusion of intracellularly injected Lucifer yellow in the H1AT network in control conditions (*a*), in the presence of 50 μM *d*-amphetamine (*b*) and of 10 μM veratridine (*c*). Notice in *a* the extensive spreading of the dye from the injected H1AT (the most fluorescent structure in the center of the microphotograph) to adjacent H1ATs. In contrast, the staining is restricted almost solely to the injected H1AT in *b* and *c*. Note also the backfilling of the perikarya, which is not reduced by drug treatment.

prepared as a stock solution of 10 mM in ethanol. Dopamine and *d*-amphetamine were protected from oxidation by adding 1 mM ascorbic acid (buffered to pH 7) to the saline.

Morphology. The eyecup was fixed in a freshly prepared mixture of 1% paraformaldehyde, 2% glutaraldehyde, 100 mM cacodylate buffer, pH 7.2, and 1.8 mM CaCl for 1 hr at 4°C. The tissue then was postfixed in 1% OsO_4 in cacodylate buffer for 1 hr at 4°C. Following a rinse in maleate buffer, pH 5.5, the eyecup was stained *en bloc* in filtered uranyl acetate, dehydrated in graded alcohols, and embedded in Spurrs resin. Sections were mounted on single-slot grids and viewed in a JEOL 100CX electron microscope.

Results

As in the previous investigation of dopamine action on the turtle horizontal cells (Piccolino et al., 1984), our electrophysiological experiments were restricted to the study of the axon terminals of the axon-bearing, or H1, horizontal cells (Leeper, 1978). H1 axon terminals (H1AT) have a resting potential in darkness of about −20 mV and respond to illumination of their receptive field with graded hyperpolarizations, whose amplitude increases with the retinal surface illuminated up to areas greatly exceeding their anatomical arborization (see Leeper and Copenhagen, 1983, and Piccolino and Witkovsky, 1984, for a review). This spatial summation is a functional expression of the electrical coupling between H1ATs, a coupling that is mediated by extensive gap junctions (Witkovsky et al., 1983). When electrical coupling is reduced, as occurs following the application of dopamine (Piccolino et al., 1984), the extent of the spatial summation area of the H1AT receptive field is narrowed. Accordingly, the changes in H1AT electrical coupling induced by pharmacological treatments were assessed by measuring H1AT receptive-field profiles before and after drug application. In addition, we obtained a direct estimate of the permeability of the H1AT network by the spread of the fluorescent dye Lucifer yellow. The dye was injected intracellularly through the recording electrode into an H1AT, from which it spread into neighboring elements of the horizontal cell network. Lucifer yellow is known to permeate gap junction channels, and it has been widely used in the study of electrical synapses (Stewart, 1978, 1981). The 2 methods provide complementary information on the functional state of the electrical synapses in the H1AT network (Piccolino et al., 1984). We investigated the action of 3 drugs: *d*-amphetamine, bicuculline, and veratridine. We first report the results of experiments carried out in normal, "untreated" retinas, and next the results from retinas treated with different pharmacological agents presumed to interfere with the functioning of the dopaminergic system.

Results in normal retinas.

d-Amphetamine, bicuculline, and veratridine all reduced the receptive-field profile of H1ATs in untreated retinas, as illustrated in Figure 1. The experiments with *d*-amphetamine and

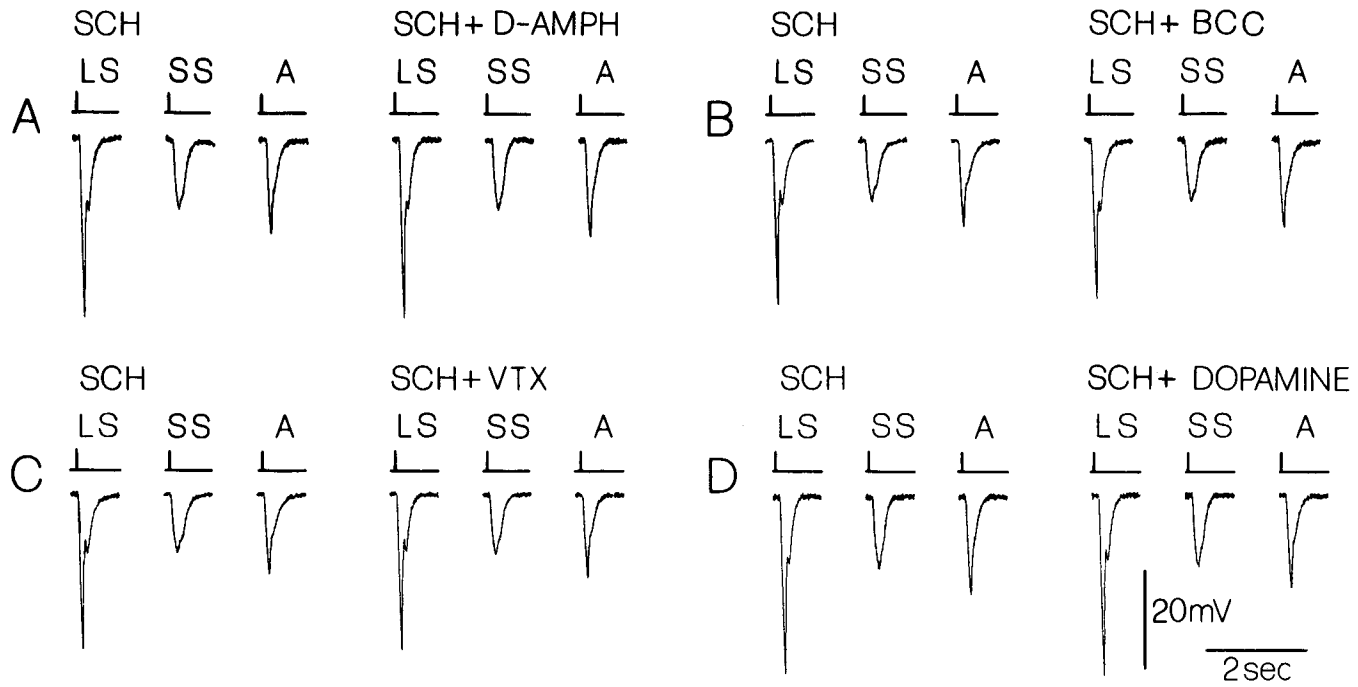

Figure 3. Drug-induced effects on the light responses of H1ATs recorded in retinas superfused with SCH 23390 (1 μM). In this and the following figures, each panel illustrates the response induced by a large spot (*LS*), a small spot (*SS*), and an annulus (*A*) prior to and following the application of the drug tested. *A*, *d*-Amphetamine, 80 μM (*D-AMPH*). *B*, Bicuculline, 70 μM (*BCC*). *C*, Veratridine, 10 μM (*VTX*). *D*, Dopamine, 20 μM. Each retina had been superfused for at least 50 min with SCH 23390 before applying the test drug. Stimulus diameters (in mm): large spot, 5.0; small spot, 1.6; annulus, 0.9. Light attenuated by 2.1 log units.

bicuculline confirm previous observations (Piccolino et al., 1982, 1984) and are presented to allow for a comparison with the results obtained in "treated" retinas. Thus, we will describe in some detail only the action of veratridine (Fig. 1*C*). The retina was stimulated by fixed-intensity, white light stimuli of different spatial configurations but centered on the H1AT receptive fields. They included spots of diameters varying from 5 to 0.25 mm, or annuli of a large, fixed outer diameter (5 mm) and variable inner diameter, decreasing from 1.8 to 0.25 mm. In control conditions, the response elicited by small light spots was small compared to that elicited by a large annulus. The application of veratridine (10 μM) resulted in a clear-cut increase of the amplitudes of the small spot responses and in a decrease of the responses to annuli, whereas the amplitude of the response elicited by the largest spot was not modified appreciably. Moreover, veratridine did not significantly alter the H1AT membrane potential in darkness. These effects, which were observed in more than 40 experiments, indicate that the summation area of the H1AT receptive field narrowed following the application of veratridine. We found, moreover, that the H1AT receptive-field changes induced by veratridine were the functional expression of a reduced permeability of the electrical junctions joining these structures. As illustrated in Figure 2*c*, veratridine strongly reduced the spreading of intracellularly injected Lucifer yellow in the H1AT network. In control conditions, the dye illuminated an intricate syncytium of H1 axon terminals (Fig. 2*a*), whereas in the presence of veratridine the staining was limited almost solely to the injected axon terminal together with the parent cell body. Veratridine was found to be effective at concentrations as low as 1–2 μM, but in most experiments we applied 10 μM in order to obtain more reproducible effects. At this concentration, the receptive-field modifications appeared 6–10 min after drug application and were complete in about 20–25 min. Application of the vehicle used to dissolve veratridine (0.02–0.1% ethanol) did not modify H1AT response properties. *d*-Amphetamine (Fig. 2*b*) and bicuculline (not illustrated; see Piccolino et al., 1982) also strongly reduced the spread of Lucifer yellow in the H1AT network.

Results in "treated" retinas

Effect of dopamine antagonists. Previous studies showed that the action of dopamine on the electrical coupling between H1ATs is mediated by the activation of D1-type dopamine receptors (Piccolino et al., 1984; Piccolino and Witkovsky, 1985). It was found that dopamine effects were prevented by dopamine antagonists acting on both D1 and D2 receptors (such as flupenthixol and haloperidol), and by the selective D1 antagonist SCH 23390, but not by the selective D2 antagonist L-sulpiride (see Creese et al., 1982, and Stoof and Kebabian, 1985, for recent reviews on the pharmacology of dopamine receptors). If the effects of *d*-amphetamine, bicuculline, and veratridine on H1ATs are mediated through a release of endogenous dopamine, then one should find that these dopamine-like effects would be blocked by dopamine antagonists acting on D1-receptors. The results of our work support this hypothesis. First, we found that the application of flupenthixol (10–50 μM) prevented the modification of H1AT coupling induced by *d*-amphetamine, bicuculline, and veratridine (not illustrated). Second, we found that *d*-amphetamine, bicuculline, and veratridine were unable to modify H1AT receptive fields in retinas treated with 1 μM SCH 23390; in this case, 20 μM dopamine was also without effect (Fig. 3). Flupenthixol and SCH 23390 did not, by themselves, induce any significant modification of the H1AT receptive field (not illustrated). We also found that the effects of *d*-amphetamine, bicuculline,

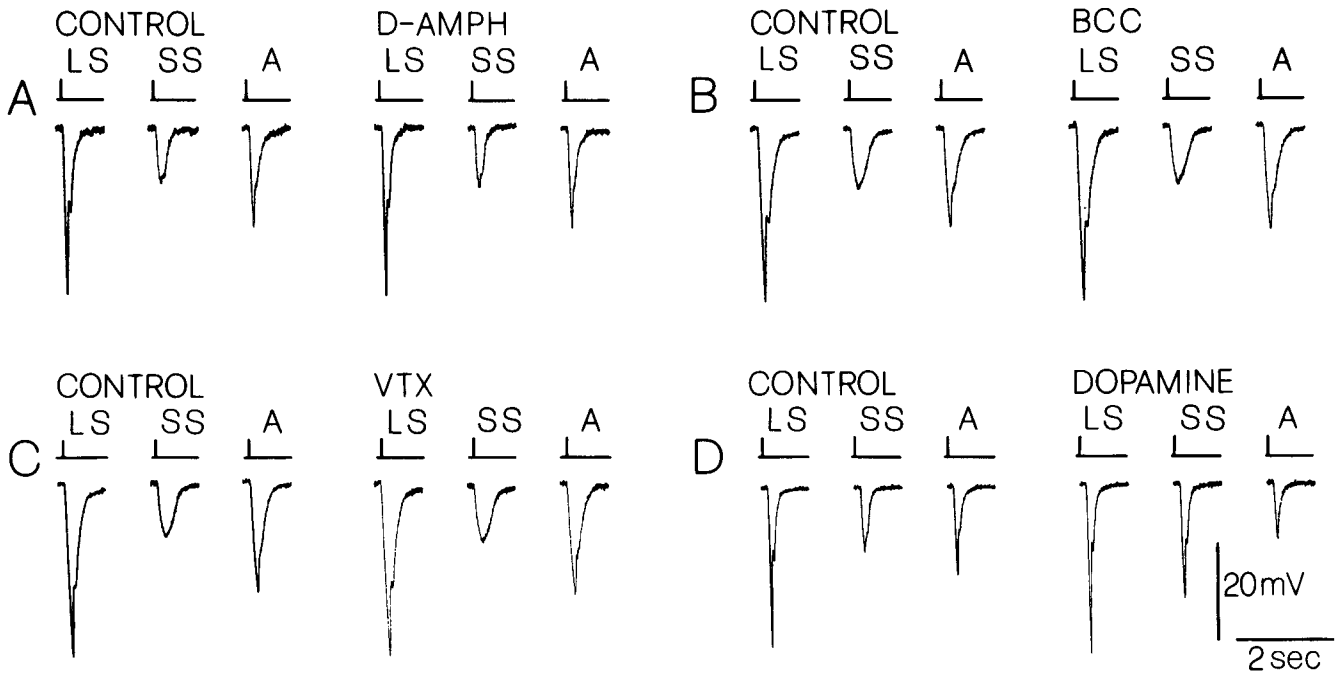

Figure 4. Drug-induced effects on the light responses of H1ATs recorded in retinas treated with 2 intravitreal injections of 6-OH-dopamine (40 μg) for 7–10 d (see Materials and Methods). *A, d*-Amphetamine, 100 μM (*D-AMPH*). *B,* Bicuculline, 50 μM (*BCC*). *C,* Veratridine, 10 μM (*VTX*). *D,* Dopamine, 10 μM. Diameter of the light stimuli (in mm): large spot, 5; small spot, 1.6; annulus, 0.9. Light attenuated by 1.8 log units.

or veratridine were not blocked by application to the retina of the D2 antagonist L-sulpiride in concentrations up to 100 μM (not illustrated), thus confirming that the dopamine receptors involved in the H1AT modifications were of the D1 type.

Treatment with 6-OH-dopamine. To test whether the effects of *d*-amphetamine, bicuculline, and veratridine actually involve the release of endogenous dopamine, we investigated the action of these drugs in retinas treated with diverse agents that interfere with the ability of dopaminergic neurons to release their transmitters.

In a first series of experiments we treated retinas with 6-OH-dopamine, a neurotoxin that preferentially attacks dopaminergic cells (Sachs and Jonsson, 1975); Figure 4 illustrates typical results obtained. For clarity, only the responses to a large spot, a small spot, and an annulus are presented in this and the following figures, both in control conditions and in the presence of the drug tested. *d*-Amphetamine, bicuculline, and veratridine were completely ineffective in modifying that H1AT receptive field in 6-OH-dopamine-treated retinas, whereas dopamine had its usual action, inducing a large increase of the small spot response and a decrease of the annular response. Similar results were observed in 12 retinas treated with 6-OH-dopamine in doses between 20 and 50 μg, 7–12 d before enucleation. In other retinas, treated with smaller doses or for shorter periods, the effects of *d*-amphetamine, bicuculline, and veratridine were reduced but not abolished. In control experiments in which the eye was treated with saline containing only ascorbic acid, the effects of *d*-amphetamine, bicuculline, and veratridine were similar to those observed in untreated retinas. The efficacy of dopamine in reducing H1AT coupling in 6-OH-dopamine-treated retinas clearly indicates that 6-OH-dopamine did not interfere with the action of dopamine at the postsynaptic level.

Morphological effects of 6-OH-dopamine treatment. Electron microscope observations of 6-OH-dopamine-treated retinas revealed degenerative changes in a subclass of retinal amacrine cells. These changes were dose- and time-dependent and appeared to be correlated with the functional deficits revealed by electrophysiological experiments. That is, they were well developed in retinas treated with double injections of 20–40 μg 6-OH-dopamine 11–13 d prior to experimentation; these same retinas failed to show any effect of veratridine, bicuculline, or *d*-amphetamine. The morphological alterations affected only presumed amacrine cell perikarya and numerous processes in the inner plexiform layer (Figs. 5, 7, 8). No modifications were observed in cells or processes of the distal retina (Figs. 5, 6*a*). In this regard, we were particularly interested in testing the possibility that at least some dopaminergic neurons were interplexiform cells. If this were the case, degenerating profiles might be expected within the distal portions of the inner nuclear layer and the outer plexiform layer, where the distal extension of the interplexiform cell arborizes (Dowling et al., 1976). As Figures 5 and 6*a* illustrate, however, these regions of the turtle retina appeared normal. A degenerating perikaryon located at the junction of the inner nuclear and inner plexiform layer is shown in Figure 5, which portrays an advanced stage of cellular necrosis in which the cytoplasm stains darkly and cellular organelles are severely disrupted. It is noteworthy that the surrounding tissue of the inner nuclear layer appears normal, whereas the inner plexiform layer contains a minority of swollen processes. A portion of a less severely damaged amacrine cell perikaryon is illustrated in Figure 6*b*. Here the cell cytoplasm is not so electron-dense, but mitochondria are swollen and their cristae disoriented. A lysosomal organelle is present (arrow) and cisternae appear swollen. In general, each drug-treated retina contained a spectrum of affected perikarya, ranging in severity from a few swollen mitochondria to the sort of severely distorted profile seen in Figure 5. The inner plexiform layer of a similarly treated retina contained numerous swollen processes; one of these is

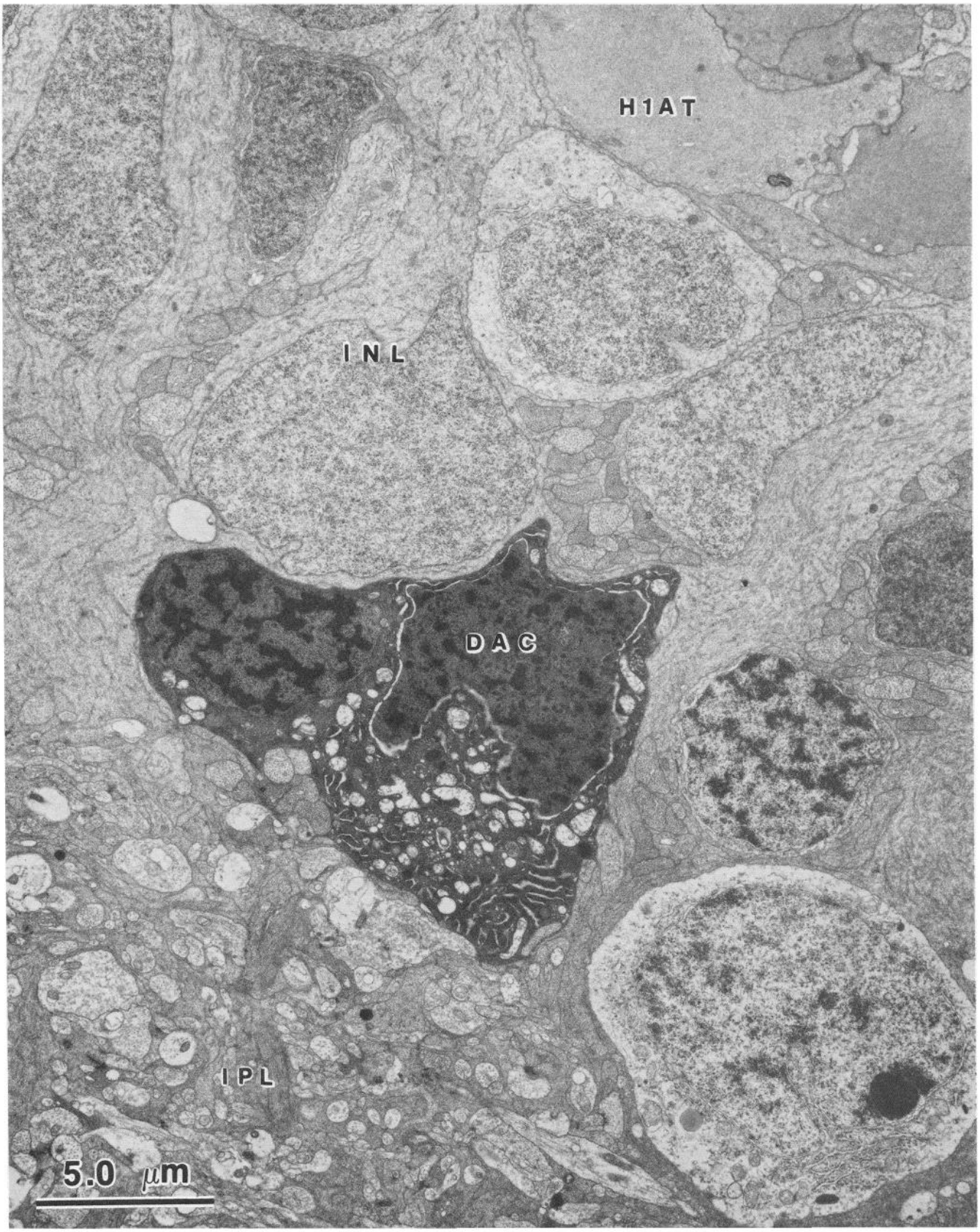

Figure 5. Low-magnification survey picture of a retina treated with 2 injections of 40 μg 6-OH-dopamine followed by 12 d of survival. A presumed dopaminergic amacrine cell (*DAC*) shows marked darkening of the perikaryal cytoplasm and massive disruption of cytoplasmic organelles. Many processes in the inner plexiform layer (*IPL*) appear swollen. In contrast, other presumed amacrine and bipolar cells in the inner nuclear layer (*INL*) appear normal. A horizontal cell axon terminal (*H1AT*) is seen at upper right. The 5 μm *marker bar* indicates that the minimum diffusion distance between the DAC and H1AT is about 10 μm.

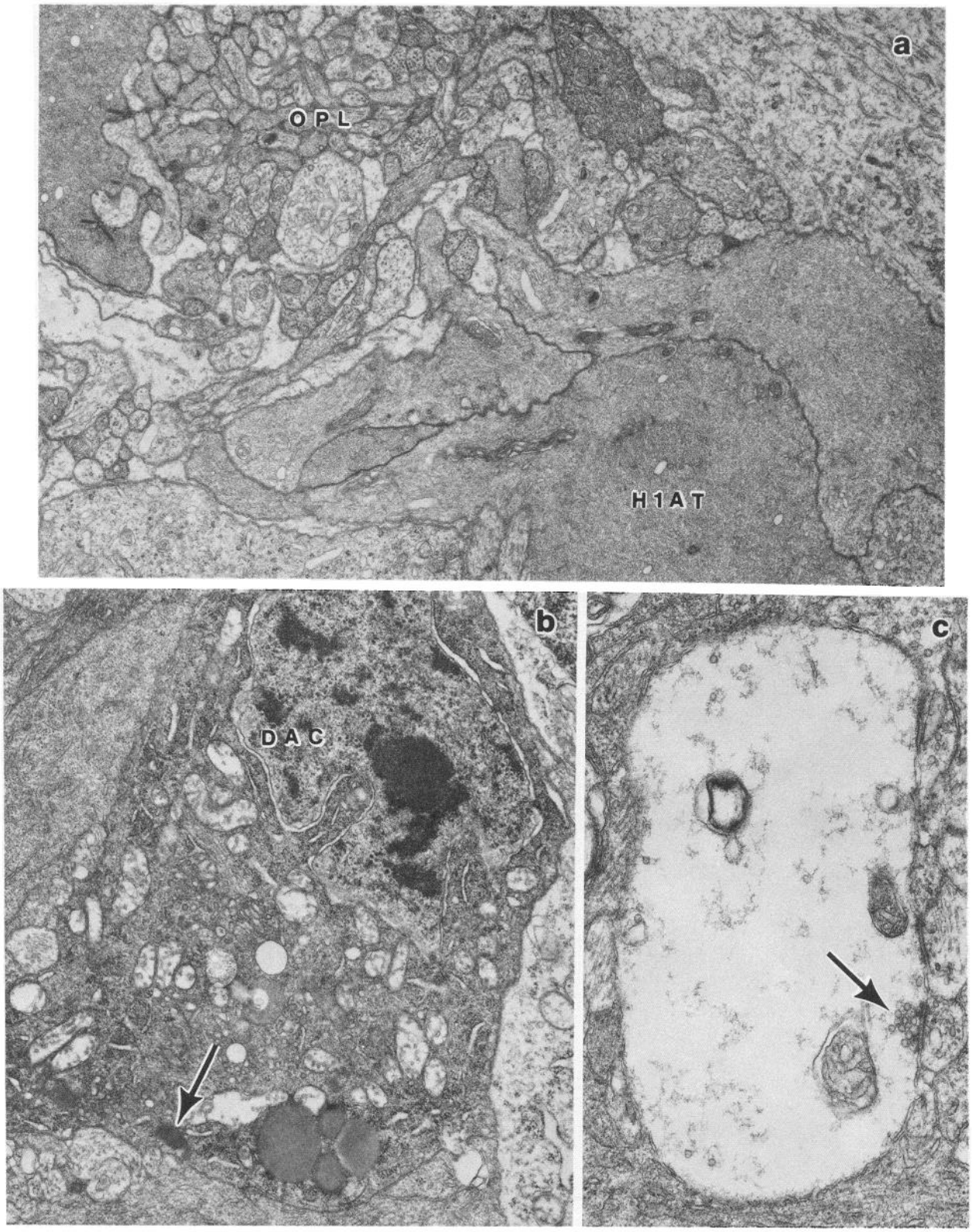

Figure 6. *a,* A low-magnification survey picture of the distal inner nuclear layer, in which the horizontal cell axon terminals (*H1AT*) and the outer plexiform layer (*OPL*) are found. Different retina but same 6-OH-dopamine treatment as for Figure 5. No degenerating cells or processes were seen in these retinal regions. ×13,200. *b,* Same retina as in *a.* A presumed dopaminergic amacrine cell (*DAC*) showing moderate disorganization of cytoplasmic organelles and a slight darkening of the cytoplasm. A lysosomal body is indicated by an *arrow.* ×16,000. *c,* Same retina as in *a.* A markedly swollen process in the inner plexiform layer surrounded by tissue of normal appearance. The affected process is identified as belonging to an amacrine cell because it is the presynaptic component of a conventional synapse (*arrow*). ×35,000.

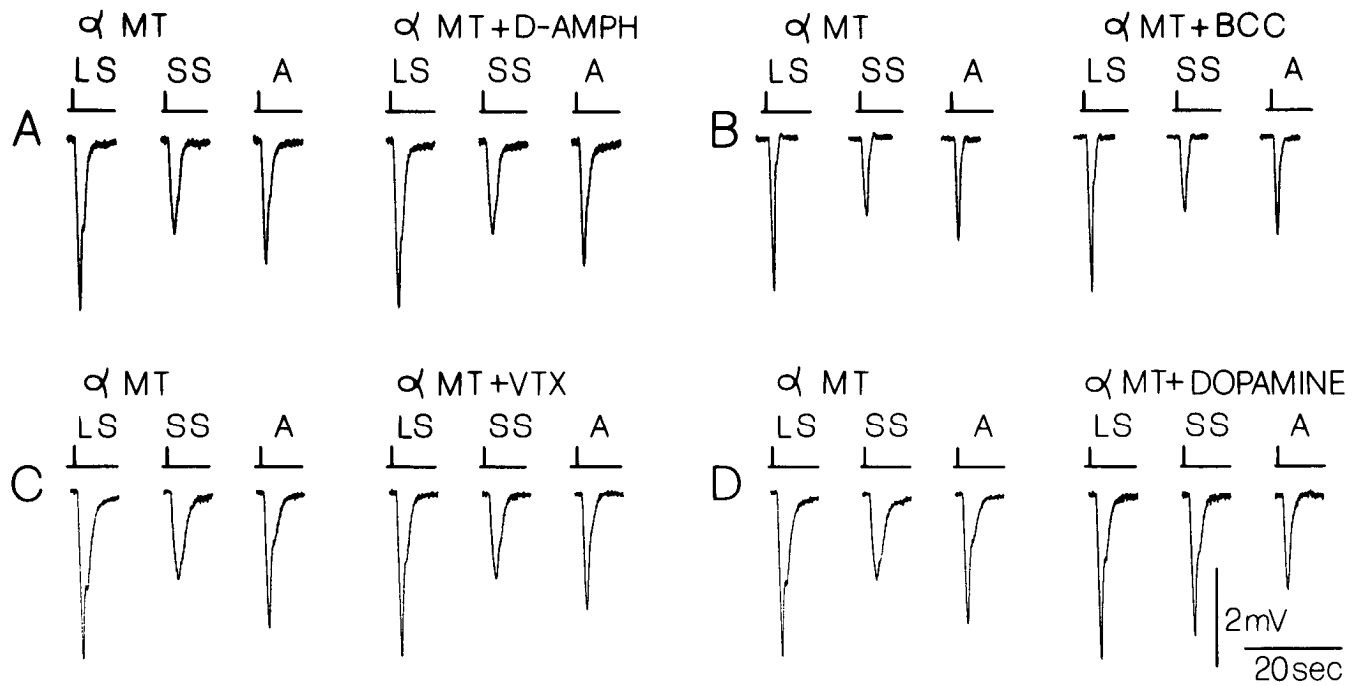

Figure 7. Drug-induced effects on the light responses of H1ATs recorded in retinas treated with α-M-*p*-T (100 μM) *in vitro* for more than 12 hr (see Materials and Methods). *A*, *d*-Amphetamine, 100 μM (*D-AMPH*). *B*, Bicuculline, 50 μM (*BCC*). *C*, Veratridine, 10 μM (*VTX*). *D*, Dopamine, 10 μM. Diameter of the light stimuli (in mm): large spot, 5.0; small spot, 1.6; annulus, 0.9. Light attenuated by 2.1 log units.

illustrated in Figure 6*c*. It is very probable that this process belongs to an amacrine cell, because it retains a small cluster of paramembranous agranular vesicles adjacent to a region of membrane specialization. This configuration is characteristic of so-called conventional synapses, for which only amacrine cells are the presynaptic component in the inner plexiform layer.

*Treatment with α-methyl-*para*-tyrosine.* α-Methyl-*p*-tyrosine, which blocks tyrosine hydroxylase, the rate-limiting enzyme in catecholamine synthesis (Spector et al., 1965; Weissman et al., 1966) is also known to interfere with the ability of catecholaminergic neurons to release their transmitter. α-M-*p*-T treatment leads to a depletion of catecholamine stores derived mainly from

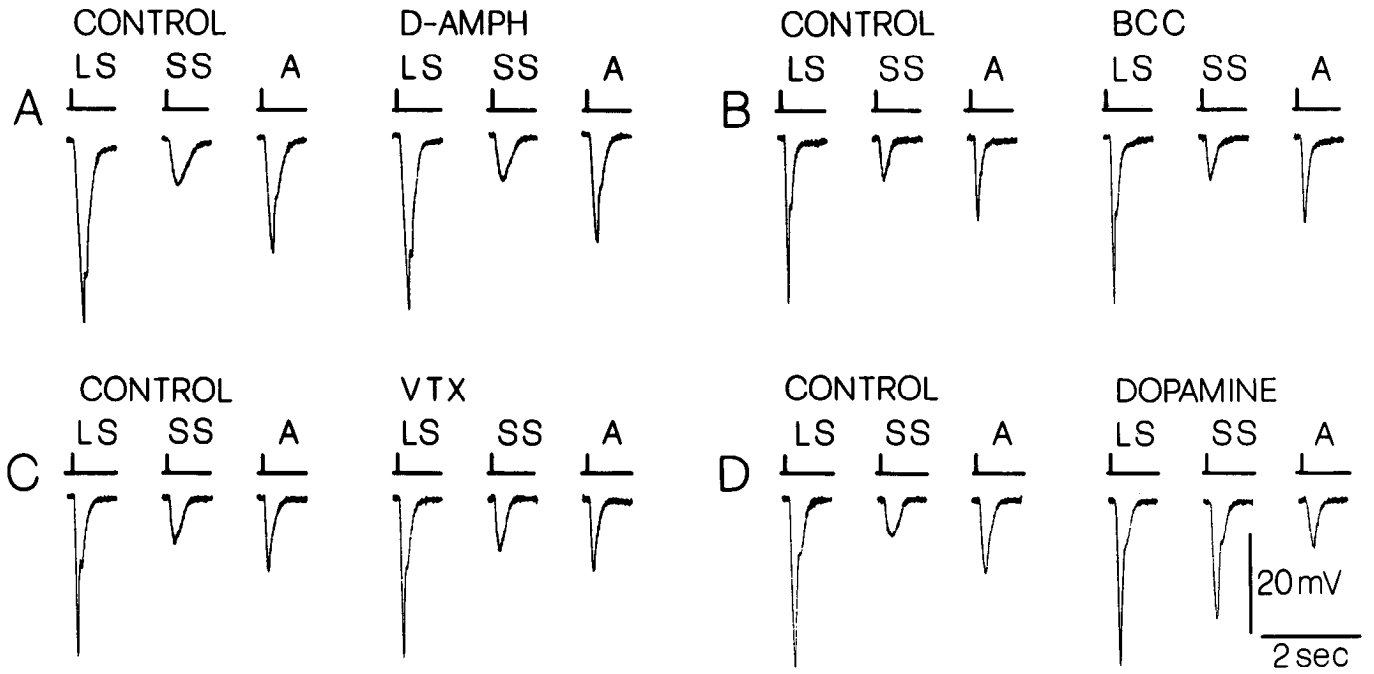

Figure 8. Drug-induced effects on the light responses of H1ATs recorded in retinas treated *in vivo* with reserpine (300 μg; 2 intravitreal injections; see Materials and Methods). *A*, Effect of 80 μM *d*-amphetamine (*D-AMPH*). *B*, Effect of 50 μM bicuculline (*BCC*). *C*, Effect of 10 μM veratridine (*VTX*). *D*, Effect of 10 μM dopamine. Diameter of the light stimuli (in mm): large spot, 5.0; small spot, 1.1; annulus, 1.2. Light attenuated by 1.8 log units in *A* and 2.1 log units in *B–D*.

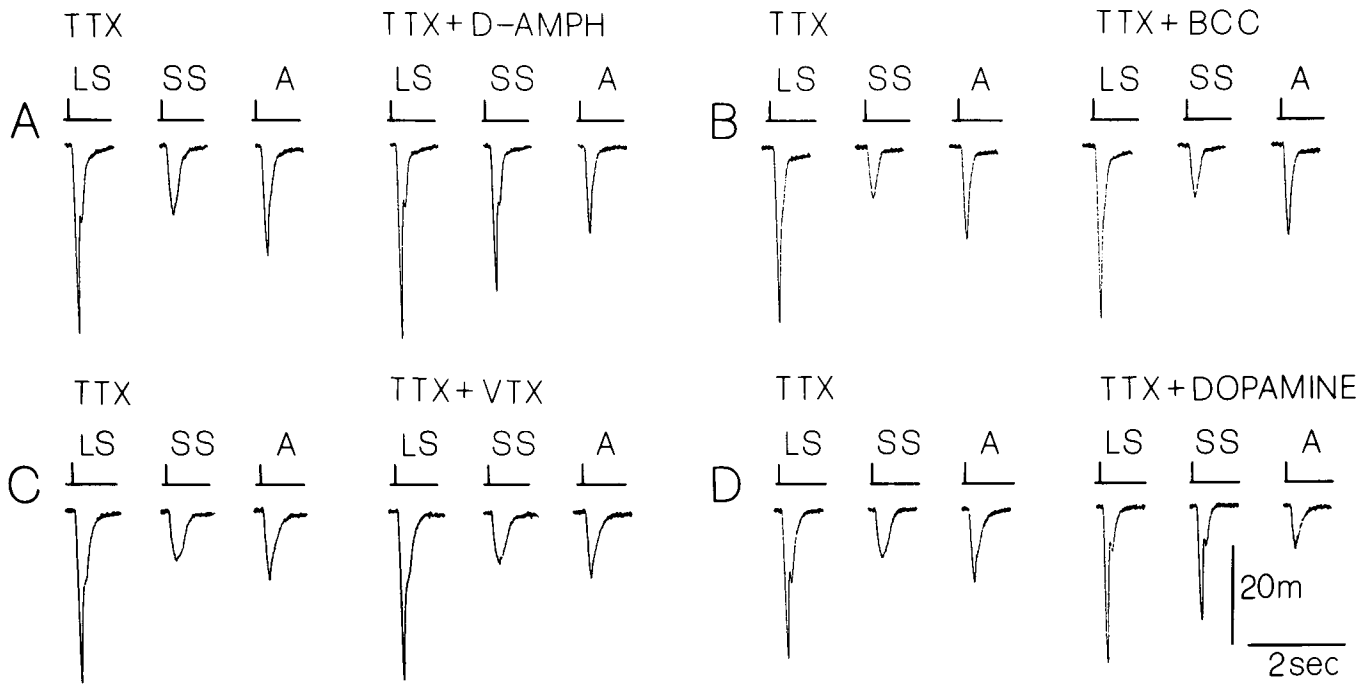

Figure 9. Drug-induced effects on the light responses of H1ATs recorded in retinas perfused with 1 μM TTX. *A*, *d*-Amphetamine, 120 μM (*D-AMPH*). *B*, Bicuculline, 100 μM (*BCC*). *C*, Veratridine, 10 μM (*VTX*). *D*, Dopamine, 10 μM. The retina was perfused with TTX for at least 40 min before applying the test drug. Diameter of the light stimuli (in mm): large spot, 5.0; small spot, 1.6 in *A*, 1.1 in *B–D*; annulus, 0.9. Light attenuated by 2.1 log units.

the pool of newly synthesized transmitter. This pool is preferentially mobilized by physiological stimuli, as well as by *d*-amphetamine (Dingell et al., 1967; Besson et al., 1969; Svensson, 1970; Glowinski et al., 1979). In most experiments, retinas were treated with α-M-*p*-T in a prolonged incubation (12–24 hr) at 4°C in the presence of 100 μM concentration of the drug in saline. As illustrated in Figure 7, *A* and *B*, *d*-amphetamine, and bicuculline were completely ineffective in modifying the H1AT receptive field in α-M-*p*-T-treated retinas, whereas dopamine still had a strong uncoupling effect. Thus, as with the results obtained in 6-OH-dopamine-treated retinas, the failure of *d*-amphetamine and bicuculline to reduce H1AT coupling in α-M-*p*-T-treated retinas could not be accounted for by an interference with dopamine action at the postsynaptic level. As for the action of veratridine in α-M-*p*-T-treated retinas, the results varied with different experiments. In some cases, as in Figure 7*C*, veratridine effects were strongly reduced in α-M-*p*-T-treated retinas, while in other preparations that had received a similar treatment, veratridine was almost as effective as in untreated retinas. We found also a similar variability of veratridine action in retinas treated *in vivo* with α-M-*p*-T (500 μg were injected intravitreally 13, 7, and 1 hr before the animal was killed). We have no explanation for the erratic nature of these results, but it is evident, on the whole, that veratridine effects were more resistant to α-M-*p*-T treatment than were the effects of *d*-amphetamine and bicuculline. On the basis of the evidence available from other systems, a plausible hypothesis is that veratridine effects involved a release of dopamine from storage pools known to be relatively insensitive to a block of dopamine synthesis (see Shore, 1972; Glowinski et al., 1979).

Treatment with reserpine. To explore the above possibility, we carried out experiments on retinas treated *in vivo* with reserpine, the rauwolfia alkaloid known to deplete monoaminergic neurons of their storage pools of catecholamines (see Slotkin, 1974). Reserpine has already been shown to reduce dopamine content in the retina (Da Prada, 1977). Reserpine was injected intravitreally on the 2 successive days just prior to the experiment, in doses ranging between 30 and 300 μg (see Materials and Methods). Typical results of such experiments are illustrated in Figure 8. Following treatment with reserpine, *d*-amphetamine and bicuculline failed to modify H1AT receptive fields, veratridine had a very small effect, whereas dopamine still exerted a strong uncoupling action. The block of the effects of *d*-amphetamine and bicuculline was also observed with the smallest doses of reserpine used (30–50 μg), whereas in order to reduce the effects of veratridine, it was usually necessary to inject higher doses (100–300 μg). Treatment of the eye with the vehicle used to dissolve reserpine (diluted acetic acid) had no effect per se.

With regard to veratridine, it was found repeatedly that the small residual effect observed in reserpine-treated retinas had a very delayed onset (25–40 min, compared to a 6–10 min delay in control experiments). We speculated that this delayed effect could be due to the release of dopamine synthesized *ex novo* in the presence of veratridine, since veratridine is a powerful activator of dopamine synthesis (Horwitz and Perlman, 1984; El Mestikawy et al., 1985). With this possibility in mind, we combined *in vivo* reserpine treatment with α-M-*p*-T treatment *in vitro*. Under these conditions (not illustrated), veratridine failed to reduce H1AT coupling. Thus, the results obtained in reserpine-treated retinas also suggest that the reduction of H1AT coupling induced by *d*-amphetamine, bicuculline, or veratridine involves an increased release of endogenous dopamine.

Treatment with tetrodotoxin. The dopamine-like action of veratridine observed in untreated preparations led us to explore whether the process responsible for dopamine release could involve the activity of spike-generating neurons. We perfused the

retina with saline containing 1 μM TTX, the blocker of voltage-dependent sodium channels (Naharashi, 1974), and then studied the effects of various drugs on the H1AT receptive field. TTX by itself was without any significant effect on the H1AT receptive field, nor did it modify H1AT membrane potential (not illustrated). However, TTX completely blocked the effects of bicuculline and veratridine (Fig. 9, *B* and *C*), although it did not interfere with the action of *d*-amphetamine and dopamine (Fig. 9, *A, D*). The persistence of dopamine and *d*-amphetamine actions in TTX-treated retinas was expected, since dopamine action is very probably exerted directly on horizontal cells (Piccolino et al., 1984; Lasater and Dowling, 1985) and, on the other hand, *d*-amphetamine is known to exert its releasing effect in a voltage-independent way (see Raiteri and Levi, 1978, and Trendelenburg, 1979). Also expected was the lack of effect of veratridine under these conditions, since TTX is known to block the action of veratridine on electrically excitable cells (Ulbricht, 1969). Somewhat unexpected and interesting was the TTX-induced block of the bicuculline effect, which suggests that the action of bicuculline on H1ATs involves the activation of spiking neurons. This possibility is discussed below.

Discussion

Mechanism of action of bicuculline, d-*amphetamine, and veratridine on the dopamine system of the turtle retina*

The main conclusion to be drawn from the results of the present study is that dopaminergic neurons of the turtle retina affect electrical coupling between horizontal cell axon terminals. This conclusion derives from a number of pharmacological manipulations, each of which is thought to modify the rate at which endogenous dopamine is released. We studied the actions of 3 different drugs—*d*-amphetamine, bicuculline, and veratridine—all of which were found to mimic dopamine effects, although they are known not to have a direct stimulant action on dopamine receptors. *d*-Amphetamine is well known for its transmitter-releasing action on catecholaminergic neurons (see Moore, 1978; Raiteri and Levi, 1978; Trendelenburg, 1979). Bicuculline has been shown to promote dopamine release in the fish retina (O'Connor et al., 1984, 1985). Veratridine is a general releaser of chemical transmitters from the synaptic terminals of spike-generating neurons (Blaustein, 1975; Mulder et al., 1975; Ross and Kelder, 1976). Thus, the dopamine-like action of each of these drugs on turtle H1ATs probably is mediated by an increased release of endogenous dopamine, although in our work we have not directly measured this release.

The actions of *d*-amphetamine, bicuculline, and veratridine were blocked by dopamine antagonists acting on D1 receptors, the receptors involved in dopamine action on H1ATs. Moreover, the dopamine-like action of all 3 drugs was found to depend critically on the integrity of the retinal dopaminergic system. Treatment with 6-OH-dopamine, α-M-*p*-T, or reserpine reduced or abolished the effects of *d*-amphetamine, bicuculline, and veratridine. These same treatments did not interfere with the uncoupling action of dopamine on the H1AT network, thus excluding an interference at the postsynaptic level of dopamine action. The most obvious interpretation of these findings is, therefore, that *d*-amphetamine, bicuculline, and veratridine all modify H1AT coupling indirectly. Similar results have been obtained in teleost retina (Negishi et al., 1983).

Against this conclusion it might be argued that the effects of the 3 agents investigated were due to the release of other transmitters and, in particular, other transmitter amines, rather than dopamine. Veratridine, for example, releases synaptic transmitters from all spike-generating neurons. *d*-Amphetamine is also capable of releasing serotonin and adrenergic amines, and treatment with 6-OH-dopamine, α-M-*p*-T, or reserpine might also have interfered with serotoninergic and adrenergic transmission. This hypothesis, however, does not account for the blocking action of dopamine antagonists on the effect of *d*-amphetamine, bicuculline, and veratridine. Serotonin is, moreover, excluded for several reasons: (1) α-M-*p*-T is known not to interfere with serotoninergic transmission; (2) 6-OH-dopamine treatment completely prevented the actions of *d*-amphetamine, bicuculline, or veratridine at doses that did not affect serotoninergic neurons of turtle retina (Witkovsky et al., 1987); and (3) exogenous serotonin does not modify H1AT coupling (M. Piccolino and P. Witkovsky, unpublished observations). Although a primary action of other neurotransmitters in the H1AT-coupling reduction induced by *d*-amphetamine, bicuculline, and veratridine is excluded on these grounds, it is still possible that other transmitters could modulate the release of dopamine. In fact, it is not crucial for our working hypothesis that *d*-amphetamine, bicuculline, and veratridine act directly on dopaminergic neurons, only that, whatever the primary target of their action, these drugs reduce the electrical coupling between H1ATs by increasing the release of endogenous dopamine.

The functional link between dopaminergic amacrines and horizontal cells

The results of the morphological component of the present study support the conclusion that the dopaminergic cell of the turtle retina is an amacrine cell, not an interplexiform cell. That is, the pattern of degeneration induced by 6-OH-dopamine treatment affected presumed amacrine perikarya and their processes in the inner plexiform layer, but did not include cell processes in the outer retina, where a putative interplexiform cell would be expected to arborize (Dowling et al., 1976). This conclusion agrees with that of an earlier study in which the dopaminergic neurons of turtle retina were characterized as amacrine cells by immunocytochemical, fluorescence, and autoradiographic methods (Witkovsky et al., 1984). Therefore, a direct contact between dopaminergic neurons and horizontal cells in turtle retina appears to be excluded on morphological grounds. It follows that either dopamine acts on a hypothetical neuron, which in turn synapses upon the H1AT, or dopamine diffuses from the amacrine cell to the H1AT, a distance of about 10 μm (cf. Fig. 5). There are no data in support of the first hypothesis, and the available evidence, although indirect, favors the second alternative. Piccolino et al. (1984) found that the uncoupling action of dopamine upon H1ATs persisted after treatment of the retina with cobalt, which blocked calcium-dependent synaptic transmission. This suggests that turtle horizontal cells have a receptor for dopamine on their membranes. In other parts of the nervous system, e.g., frontoparietal cortex (Descarries et al., 1977), ependymal plexuses (Chan-Palay, 1976), and the autonomic nervous system (Gabella, 1976), there is electron-microscopic evidence that catecholamines are released from nerve terminals at distances of up to several microns from their target. Recent studies of cone retinomotor movements, moreover, have demonstrated that photoreceptors have a dopamine receptor (Dearry and Burnside, 1985). Whatever the source of this dopamine, it does not reach the photoreceptor via direct synapse. The evidence cited thus favors the possibility that, in turtle retina, dopamine diffuses to the H1AT membrane from a distant

source, thus acting through an unconventional mechanism that could be referred to as local hormone action or "nonsynaptic modulation" (see Vizi, 1981). Speaking hypothetically, a similar mechanism might be functionally advantageous when a small number of neurons must control a great number of target cells distributed over relatively large areas. In fact, retinal horizontal cells form a continuous layer in the distal retina and their number greatly exceeds that of dopaminergic neurons, which represent only a very small percentage of retinal cells (see Ehinger, 1983). It is of interest that a recent study of serotoninergic amacrines in rabbit retina (Vaney, 1986) showed them as creating a widespread plexus in the inner plexiform layer, which was postulated to be a substrate for diffuse release of transmitter.

Inner retinal circuitry underlying dopamine function

Our results shed some light on the functional characteristics of the retinal dopaminergic neurons. The dopamine-like action of bicuculline and the blocking effect of TTX on the action of bicuculline and veratridine could be interpreted as indicating that the dopaminergic amacrine cell produces action potentials. Evidence that TTX affects retinal amacrine cell function in mudpuppy retina was provided by Miller and Dacheux (1976). They showed that large and small spikes, which they supposed would reflect somatic and dendritic spikes, respectively, of amacrine cells were both abolished by submicromolar doses of TTX, although light-evoked EPSPs remained. Comparable results were obtained for so-called transient amacrine cells of the carp retina by Murakami and Shimoda (1977).

Although many amacrines are spike-producing (Kaneko, 1970), surveys of inner retinal neurons have also revealed amacrines that do not fire spikes (Naka and Ohtsuka, 1975; Marchiafava and Weiler, 1982). Thus, TTX could have acted either by blocking spike firing in the dopaminergic amacrine itself and/or in a hypothetical neuron that makes synaptic contact with the dopaminergic amacrine. Support for the first hypothesis is suggested by results in rat retina (Morgan and Kamp, 1980; Marshburn and Iuvone, 1981). In that system, the rate of dopamine synthesis, which normally reflects the electrical activity of dopaminergic neurons, is reduced by GABA agonists and increased by GABA antagonists. The actions of GABA agonists, moreover, were also observed on isolated cells in culture, indicating that the dopaminergic neuron has a GABA receptor. If this also applied to the turtle retina, then TTX could have blocked bicuculline effects only by acting on the dopaminergic neuron itself. A plausible site for GABA–dopamine interactions in the turtle retina would thus appear to be at the level of amacrine cells; however, a direct demonstration of this neuronal connection remains to be accomplished.

Tonic release of transmitter

In turtle retina, GABA antagonists have a strong uncoupling effect on H1ATs, whereas exogenous GABA has only a modest "coupling" effect (Piccolino et al., 1982). These observations suggest that there may be a tonic release of GABA that nearly saturates GABA receptors and thus keeps the rate of dopamine release low. On these grounds, it is easy to explain why dopamine antagonists by themselves have a minimal effect on H1AT coupling, whereas they effectively block the action of exogenous dopamine.

In conclusion, we believe that our study, by showing the existence in the turtle of a functional link between dopaminergic and horizontal cells, indicates a physiological role for dopamine in retinal signal transmission, one aspect of which is a neurotransmitter-operated control of gap junction permeability between nerve cells. Such a control is a novel form of modulation of cellular communication, which extends the functional capabilities of the nervous system by combining, in a network of interacting elements, the advantages of both electrical and chemical transmission.

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