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Dopamine decreases conductance of the electrical junctions between cultured retinal horizontal cells

(electrical synapses/interplexiform cells/cyclic AMP)

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ABSTRACT **Horizontal cells from the white perch were isolated by enzymatic treatment and trituration of the retina and were maintained in culture for 1–5 days. Overlapping pairs of horizontal cells were identified, and the two cells were recorded from simultaneously, using whole-cell patch clamp techniques. Electrical coupling between cells was determined by passing current pulses into one cell, the driver cell, while (*i*) recording voltage changes in the other, follower cell, or (*ii*) measuring current flow into the follower cell. Most cell pairs of the same morphological type were coupled electrically, with coupling coefficients often >0.9. Junctional resistance was typically found to be between 20 and 60 MΩ and junctional conductance was between 150 and 500 nS. After application of 1-μl pulses of dopamine (200 μM) to coupled pairs of cells, the coupling coefficient fell to ≈0.1, junctional resistance increased to 300–700 MΩ, and junctional conductance decreased to 15–30 nS. Recovery of coupling took, for most cell pairs tested, 8–15 min after dopamine application. The exogenous application of 8-bromo-cyclic AMP (0.5–1 mM) also caused uncoupling of horizontal cell pairs; however, neither isoprenaline nor L-glutamate altered coupling significantly.**

Horizontal cells are second-order neurons that mediate lateral inhibitory effects in the outer plexiform layer of the retina (1–3). In many species, adjacent horizontal cells are coupled electrically, which serves to extend the effective receptive field size of these neurons (4–7). In fish, for example, the receptive fields of horizontal cells typically measure from 200 μm to >2 mm in diameter (8–12), whereas the dendritic diameters of individual cell perikarya of horizontal cells range from 30 to 150 μm (11, 13).

In teleosts, two chemical synapses onto the horizontal cells have been identified. One, from the photoreceptors, appears to use L-glutamate, or a glutamate-like substance, and mediates the graded changes in horizontal cell membrane potential that occur in response to light and dark (13–15). The second synaptic input is from the interplexiform cells, neurons that sit among the amacrine cells and extend processes in both plexiform layers of the retina (16). The interplexiform cells use dopamine as their neurotransmitter and this monoamine has been shown to decrease the receptive field size of horizontal cells (17–19) and, hence, the effectiveness of these elements in mediating lateral inhibition (20). Dopamine has also been shown to decrease the diffusion of fluorescent dye between horizontal cells (18, 19), suggesting that dopamine decreases receptive field size by altering the electrical coupling between horizontal cells. A dopamine-sensitive adenylate cyclase has been localized to horizontal cells in carp (21), providing the hypothesis that cyclic AMP may be involved in modifying the electrical coupling between these cells (22). Experiments showing that analogs of cyclic AMP and forskolin, an activator of adenylate cyclase, mimic dopamine in restricting receptive field diameter and dye diffusion between horizontal cells provide evidence in favor of this hypothesis (18, 19).

In this report, we describe the properties of electrical coupling that occurs between horizontal cells isolated from the retina and maintained in culture for 1–5 days. When dopamine is applied to these cells, junctional conductance is reversibly decreased, resulting in a reduction of the coupling coefficient between cells by up to 90%. Application of 8-bromo-cyclic AMP, a membrane-permeable analog, also uncouples the horizontal cells. These experiments provide direct evidence that the dopaminergic input from interplexiform cells modifies horizontal cell activity by decreasing the conductance of the electrical junctions between these neurons.

MATERIALS AND METHODS

Cell Culture. White perch (*Roccus americana*), 4–6 inches long, were dark-adapted for 40–60 min. Under red light, the animals were decapitated, eyes enucleated and hemisected, and the retinas were dissected from the back of the eye. After incubation for 40 min in Leibowitz's (L-15) tissue culture medium containing 1.75 mg of papain per ml activated with L-cysteine, the retinas were washed in fresh L-15 medium and the cells were dispersed by trituration with Pasteur pipettes (15). The cells were maintained in L-15 medium in plastic tissue culture dishes for 1–5 days before use. Prior to recording, the medium was removed and replaced with oxygenated teleost Ringer's solution. Good recordings were obtained for 2–4 hr thereafter. Test agents were applied in Ringer's solution via pipettes (10–30 μm tip diameters) attached to a pressure ejection system (15). The pipettes were positioned to within 100 μm of the cell pairs and the Ringer's solution was expressed in 1–2 pulses of 1 μl or less in volume.

Patch Electrodes and Recording. The whole-cell tight seal patch clamp technique was used to record from cell pairs (23). Patch electrodes with relatively large tip diameters (i.e., with resistances of 5–8 MΩ or ≈1% of the input resistance of isolated horizontal cells) were fashioned according to standard procedures (23). Two clamp amplifiers were used, with 0.1 GΩ feedback resistors in the headstages. Series resistance compensation was used in the driver cell amplifier.

The usual protocol was to establish a seal on both cells of a pair, wait ≈30 sec, then break through the cell membrane under the electrode with suction. Coupling between cells was evaluated in two ways. In most experiments, one of the clamp amplifiers was maintained in a voltage-clamp mode, and the other was in a current-clamp mode. In these experiments, current pulses were passed into the voltage-clamped cell, termed the driver cell, to shift the membrane potential by a predetermined amount, while passive membrane poten-

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tial changes due to current flow across the junctional membrane were recorded in the current-clamped cell, termed the follower cell. In these experiments, the driver cell was held at −60 mV. This mode of recording allowed us to evaluate a coupling coefficient between pairs of cells. In other experiments, both cells were voltage-clamped at −60 mV, and trains of current pulses were passed into the driver cell. To maintain the follower cell at the holding potential, the voltage clamp on that cell was required to pass current pulses of opposite polarity into the cell, the magnitude of which was a measure of the current flowing across the junction. This procedure allowed us to determine junctional resistance and conductance.

In early experiments, we found that the anions used in the patch electrodes affected the electrical coupling between cells. When patch electrodes were filled with solutions containing F^- as the major anion, good coupling was observed, but the effects of dopamine were weak or inconsistent. On the other hand, if aspartate, gluconate, or Cl^- were the principal anions used in the patch electrodes, the coupling observed was usually weak. Therefore, for most experiments, a mixture of 40% KF/60% K-aspartate was used in the patch pipettes. Good coupling and consistent drug effects were observed with this combination. The composition of the pipette solution was as follows: 72 mM K-aspartate/48 mM KF/11 mM EGTA/1 mM $CaCl_2$/4 mM KCl/1 mM MgATP/10 mM Hepes buffer.

RESULTS

Four morphologically distinct types of horizontal cells are readily identified in cultures of the white perch retina. Types H1 and H2 appear similar to the cone-related luminosity-type horizontal cells observed in the pikeperch retina (11). Type H3 is likely to be a cone-related chromaticity-type of horizontal cell (11), while H4 may be a chromaticity-type cell or a rod-related horizontal cell. In perch retinal cell cultures of relatively high density, pairs of horizontal cells that have various degrees of process or perikaryal overlap are often seen (Fig. 1). Such pairs involve cells of the same type (Fig. 1 *a* and *b*) or cells of a different type (Fig. 1*c*). Occasionally, three or more overlapping cells are encountered (Fig. 1*d*). Most of our observations were on pairs of H2 or pairs of H3 cells.

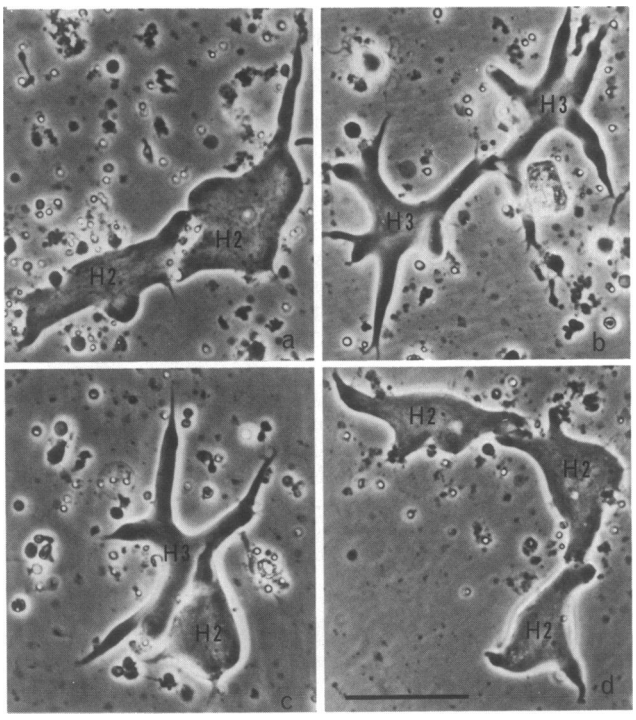

FIG. 1. Overlapping horizontal cells in culture. (*a*) Pair of H2 cells; (*b*) pair of H3 cells; (*c*) pair consisting of an H2 and an H3 cell; (*d*) three overlapping H2 cells. In short-term culture the H2 cells have only a few short blunt processes, while the H3 cells typically have 4–6 elongated processes. (Bar = 50 μm.)

Properties of Horizontal Cell Coupling. Fig. 2 (*Left*) shows the results of an experiment with a pair of H2 cells in which small current pulses were applied to the voltage-clamped driver cell that shifted the membrane potential by 10 mV. A potential change of nearly 10 mV occurred in the follower cell, indicating that the two cells were tightly coupled. Reversing the polarity of the current pulses did not alter the amplitude of the potential shifts observed in the follower cell. In other experiments, in which both cells were voltage-clamped, the driver and follower cells were alternately interchanged. Coupling was generally observed to be symmetrical, although on one or two occasions the pulse recorded in one cell was somewhat larger than the pulse recorded in the other cell, perhaps because of different nonjunctional conductances in the two cells.

Fig. 2 (*Right*) shows a larger current pulse injected into the driver cell that shifted the holding potential by 20 mV. A potential of 19.0 mV was recorded in the follower cell. The coupling coefficient, defined as the voltage recorded in the follower cell divided by the voltage shift occurring in the driver cell (24), was 0.95 for this pair of cells, a typical result for tightly coupled cell pairs. In a sample of 89 overlapping pairs of cells of the same type (53 type H2, 24 type H3, 9 type H1, and 3 type H4), 93% (83 cells) showed evidence of coupling and of these, 71% (63 cells) had coupling coefficients of 0.6 or better. On the other hand, of 16 pairs of overlapping cells of different type (see Fig. 1*c*), 81% (13 cells) showed no evidence of coupling, and of the 3 that showed coupling, it was very weak. In one instance, a coupling coefficient was determined for 3 overlapping H2 cells (Fig. 1*d*). The electrodes were positioned on the 2 end cells, and current pulses were passed through the 3 cells. The coupling coefficient in this instance was found to be 0.9.

To determine the resistance of the junctions between well-coupled cells (i.e., with coupling coefficients of >0.9), pulses were applied to the current-clamped cell in four experiments, and the amount of current passing into the voltage-clamped cell was measured. The resistances of these junctions were determined to be 42, 26, 35, and 45 MΩ. Typical input resistances of single isolated perch horizontal cells determined by whole-cell patch clamping ranged between 500 and 1000 MΩ. Thus, the resistance of the junctions was <10% of the input resistance of the cells, consistent with coupling ratios of >0.9 for these pairs of horizontal cells.

Effects of Dopamine on Horizontal Cell Coupling. The application of short pulses (1 sec) of Ringer's solution contain-

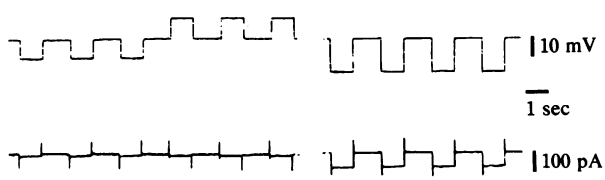

FIG. 2. Coupling between a pair of cultured H2 cells. Lower traces show current records from the voltage-clamped driver cell, while upper records show voltage records from the current-clamped follower cell. Current pulses were applied to the driver cell to shift the membrane potential by 10 mV (*Left*) or 20 mV (*Right*). Since the cells were tightly coupled, the shifts in potential in the follower cell were close to that induced by the current pulses in the driver cell. The brief deflections at the beginning and end of the current pulses represent capacitative transients in this and in the following figures.

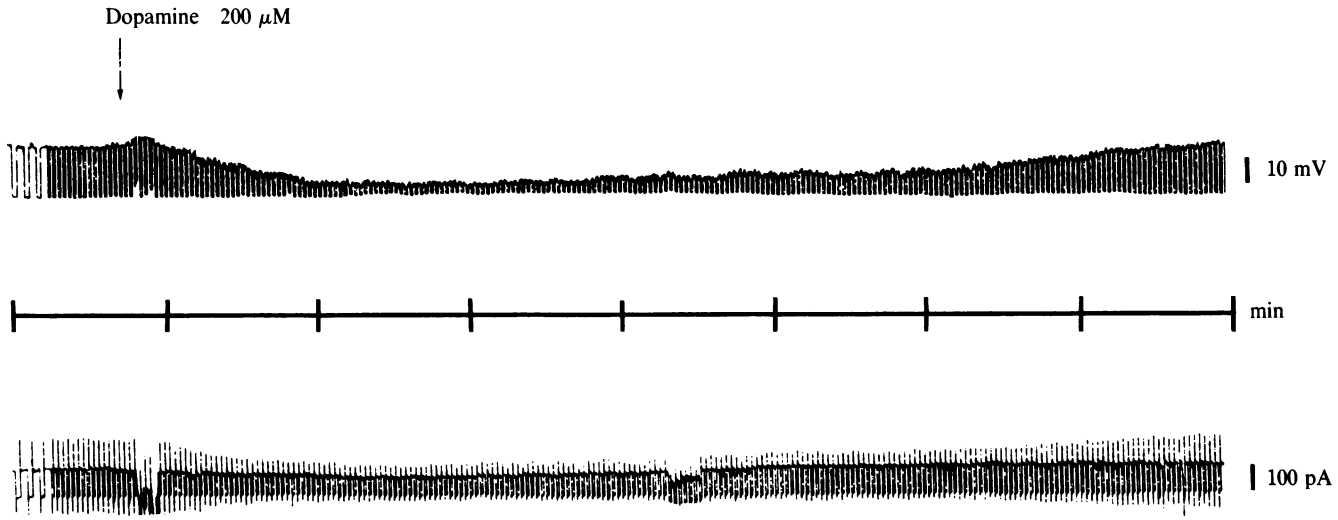

FIG. 3. Effects of dopamine on cell coupling. Dopamine was applied to a pair of H2 cells (arrow). Coupling began to decrease after ≈15 sec, as shown by the smaller voltage changes observed in the follower cell (upper trace). As uncoupling proceeded, the membrane potential of the follower cell hyperpolarized from −60 mV, the holding potential of the driver cell, to approximately −78 mV. After ≈2 min, the cells were maximally uncoupled. The coupling recovered over the next 5–6 min. A movement artifact is seen in both traces at the time of dopamine application.

ing dopamine to coupled cells significantly altered the strength of coupling (Fig. 3). Typically, a change in coupling was observed within 10–30 sec after application of the drug, with peak effects occurring after 2–4 min, and recovery required an additional 5–10 min. After the application of Ringer's solution containing relatively high concentrations of dopamine (100–200 μM), the coupling coefficient decreased by up to ≈90% (i.e., to ≈0.10). As the coupling decreased, the driver cell was no longer able to maintain the follower cell at the holding potential (−60 mV) and the membrane potential of the follower cell hyperpolarized to approximately −80 mV, the usual resting potential of horizontal cells in culture. During recovery, the coupling coefficient increased to its former value, and the resting potential of the follower cell depolarized to the holding potential of the driver cell (−60 mV).

Another effect of the uncoupling action of dopamine is seen in the current record of the driver cell (Fig. 3). As uncoupling occurred, the current required to shift the membrane potential −20 mV decreased, reflecting the increased input resistance of the cell. When pulses of Ringer's solution containing lower concentrations of dopamine were applied (25 or 50 μM), the effects on coupling were correspondingly decreased, suggesting that the uncoupling response to dopamine was graded. In all, we observed uncoupling effects of dopamine on 18 pairs of cells; of these 12 pairs were of type H2 cells, 5 pairs were of type H3 cells and 1 pair was of type H1 cells. Dopamine was not applied to any of the 3 pairs of H4 cells recorded from in this study.

On a few occasions, dopamine was applied to a pair of cells when both were voltage-clamped and the potential of one of the cells shifted by 20 mV with current pulses. Fig. 4 shows such an experiment on the pair of H3 cells shown in Fig. 1*b*. With the uncoupling of the cells in response to dopamine, the current pulses required to depolarize the driver cell by 20 mV decreased sharply in magnitude, reflecting the increase in junctional resistance between the cells. The current pulses passed into the follower cell also decreased, reflecting the decreased conductance of the junctional membrane. Initially, the current required to depolarize the driver cell by 20 mV was 240 pA (lower trace). The current passing into the follower cell from the driver cell was 230 pA (upper trace), indicating a junctional resistance of about 80 MΩ or a junctional conductance of 120 nS; 4 min after dopamine application, current passing into the follower cell was 30 pA, showing that the resistance had increased to 660 MΩ, while junctional conductance had decreased to ≈15 nS. With time,

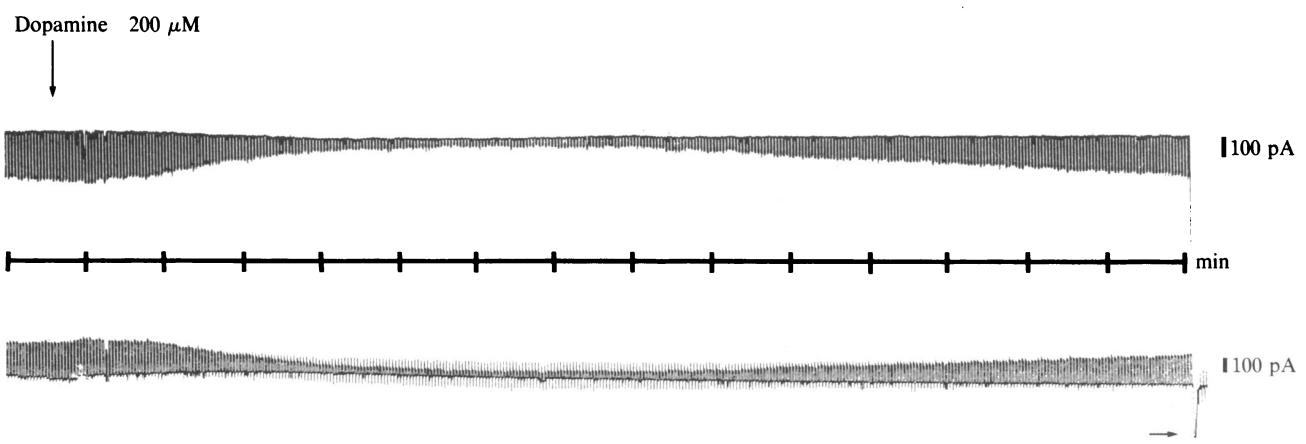

FIG. 4. Effects of dopamine on a pair of H3 cells. In this case, both cells were voltage-clamped and the membrane potential of the driver cell (lower trace) shifted in pulses of +20 mV. Four minutes after dopamine application, the cells were maximally uncoupled. Coupling recovered slowly thereafter. Arrow on the right of the driver cell current trace indicates the point at which the follower cell burst open and died. This resulted in a transient influx of ≈300 pA of current into the driver cell.

coupling began to recover and after ≈14 min the junctional current and conductance had increased to ≈180 pA and 90 nS, respectively. In other experiments using more tightly coupled cell pairs, we found initial junctional conductances to range between 150 and 500 nS and, after dopamine application, the junctional conductances fell to 15–30 nS.

Toward the end of this experiment, the follower cell burst open and died, for reasons that were not clear. This resulted in a large influx of current (≈300 pA) into the driver cell (arrow). The junctional membrane sealed over within 2–3 sec (25), as shown by the return of the current trace to baseline. It was then possible to determine the nonjunctional resistance of the cell. Less than 20 pA was now required to shift the membrane potential 20 mV, indicating an input resistance of the cell of at least 1 GΩ.

Effects of Other Agents on Horizontal Cell Coupling. Isoprenaline, the specific β-adrenoreceptor agent, along with dopamine, has been shown to stimulate the accumulation of cyclic AMP in perch retinas (26). However, isoprenaline, unlike dopamine, does not stimulate cyclic AMP accumulation in horizontal cell fractions (unpublished observation). We observed no effects of isoprenaline (200 μM) on horizontal cell coupling (six experiments). On the other hand, we found that 8-bromo-cyclic AMP at concentrations of 0.5–1 mM rapidly uncoupled horizontal cells in culture (five experiments). However, recovery following the application of 8-bromo-cyclic AMP was very slow, if at all, and frequently, for unknown reasons, the membrane potential of the follower cell became very unstable and fluctuated after application of this agent.

In three experiments, L-glutamate was applied to pairs of coupled horizontal cells. Fig. 5 shows one example. L-Glutamate (100 μM) induced a large transient inward current flow (≈300 pA) into the cells, as shown in the record of the voltage-clamp driver cell (lower trace). The inward current caused a transient membrane potential depolarization in the current-clamped follower cell, probably because the voltage clamp on the driver cell was inadequate to maintain the holding potential on the two cells. However, the coupling between the cells was affected very little by L-glutamate application; i.e., the coupling coefficient decreased transiently only from ≈0.9 to 0.8 and then rapidly recovered.

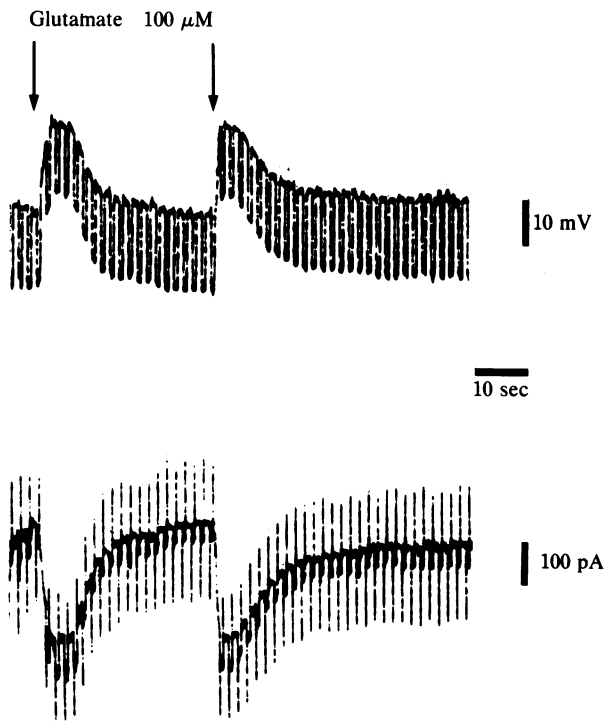

FIG. 5. Effects of L-glutamate on horizontal cell coupling. L-Glutamate induced inward current flow into the driver cell of ≈300 pA (lower trace). Because of the large inward flow of current induced by L-glutamate, the voltage clamp on the driver cell was unable to maintain the holding potential on the follower cell. Thus, the follower cell depolarized by ≈15 mV (upper trace). Note, however, that the coupling ratio between the cells changed little as a result of the L-glutamate application.

Finally, in two culture dishes, haloperidol was added to the Ringer's solution to give a final concentration of 50 μM. Subsequent application of dopamine (100 μM) to pairs of cells in these dishes had no effects on their coupling, although dopamine had clear-cut effects on cells from these dishes before the haloperidol was added.

DISCUSSION

The present experiments demonstrate that electrical coupling occurs between horizontal cells in culture. Furthermore, it appears that the coupling observed in cultural horizontal cells is similar to that seen in the intact retina. That is, in the intact retina only cells of the same morphological type are coupled (6, 7), and the coupling observed is usually very strong (5, 7). We have observed that the coupling coefficient of many pairs of isolated cells of the same type is 0.9 or greater. This is consistent with findings in the intact retina, which have shown that the receptive field diameter of horizontal cells is some 10 to 100 times larger than the diameter of the cell's dendritic field, and that fluorescent dye injected into a single horizontal cell in a dopamine-deprived retina rapidly diffuses into 10 or more adjacent cells (18). An interesting question is whether the coupling between cells in culture is established in culture or whether it is maintained during the isolation procedure from coupling established *in vivo*.

We have also found that pairs of coupled horizontal cells in culture are responsive to dopamine at concentrations (25–200 μM) known to activate adenylate cyclase in horizontal cells (21). Earlier experiments had shown that such concentrations of dopamine alter neither membrane potential nor membrane resistance of single isolated horizontal cells of the carp (15, 27), and we have confirmed this result for single isolated horizontal cells of the white perch. On pairs of isolated and coupled perch horizontal cells, on the other hand, dopamine acts to uncouple the cells. Concentrations of dopamine that maximally activate adenylate cyclase (100–200 μM) in horizontal cells decrease coupling by up to 90%; that is, the coupling coefficient may decrease to 0.1 or less. These observations also appear consonant with findings made on the intact fish retina, where it has been shown that after the application of dopamine to the retina, dye injected into a single horizontal cell is confined to the injected cell and does not diffuse (18).

Other evidence of the dramatic effect of dopamine on the coupling between horizontal cells is shown by the finding that after dopamine application, the membrane voltages of a coupled pair of cells become independent. That is, as shown in Fig. 3, a voltage-clamped driver cell can no longer maintain the follower cell at the holding potential.

Evidence that cyclic AMP is involved in the uncoupling of the horizontal cells after dopamine application is provided by our finding that 8-bromo-cyclic AMP acts as a powerful uncoupling agent. This, too, is consistent with results obtained with intact retinas where it has been shown that cyclic AMP analogs as well as forskolin, an activator of adenylate cyclase, decrease receptive field size of horizontal cells as well as dye diffusion between horizontal cells (18, 19).

It would appear that the effects of dopamine on horizontal cell coupling are specific, as shown by two experiments. First, isoprenaline, which activates adenylate cyclase in the perch retina (26) but not in perch horizontal cells, has no

effects on horizontal cell coupling. Second, L-glutamate, which powerfully depolarizes isolated horizontal cells (15), has minimal effects on coupling. Indeed, the small effect of L-glutamate on coupling (i.e., see Fig. 5) can probably be accounted for by the decrease in nonjunctional membrane resistance that accompanies the large inward flow of current induced in these cells by this agent. It is interesting to note the difference in time course of the action of L-glutamate and dopamine on horizontal cells. That is, a short pulse of L-glutamate induces a depolarization of these cells that lasts seconds, while a short pulse of dopamine induces changes in electrical coupling between the cells that may last 15 min or more.

A particularly interesting feature of the present system under study is that it represents the modulation of an electrical synapse via a chemical synapse. In the teleost retina, dopamine is found in the interplexiform cells, which are known to make synapses on horizontal cells (16). Furthermore, dopamine, presumably reflecting interplexiform cell action, has been shown to decrease lateral inhibitory effects in the outer plexiform layer mediated by the horizontal cells (20). Decreasing coupling between horizontal cells, and thus restricting receptive field size of these inhibitory interneurons, will obviously decrease the effectiveness of these cells in mediating lateral inhibition.

In summary, the present experiments provide direct evidence that dopamine acts on horizontal cells to decrease the conductance of the electrical junctions between horizontal cells. Although cyclic AMP appears to be involved in mediating the effects of dopamine, the mechanism by which this occurs is not known. It is possible that, as in other cyclic AMP-mediated systems, protein phosphorylation is involved (28), and some recent evidence indicates that a cyclic AMP-dependent protein kinase phosphorylates a protein (MP26) believed to be a component of lens gap (electrical) junctions (29). Factors that are presently known to modify electrical coupling between cells include intracellular Ca^{2+} concentration (25, 30), intracellular pH (31, 32), and transmembrane voltage (24, 33). Preliminary experiments indicate that horizontal cell coupling does not appear to be affected by membrane voltage (unpublished observations), but we have no information on other possible factors. Further studies on coupled horizontal cells in culture appears to be a promising way to understand the role of dopamine and cyclic AMP in modulating electrical coupling between horizontal cells.

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