Endogenous Activation of Dopamine D2 Receptors Regulates Dopamine Release in the Fish Retina

YU WANG, KRISZTINA HARSANYI, AND STUART C. MANGEL
Departments of Neurobiology, Physiology and Biophysics, and Ophthalmology, University of Alabama School of Medicine, Birmingham, Alabama 35294

Wang, Yu, Krisztina Harsanyi, and Stuart C. Mangel. Endogenous activation of dopamine D2 receptors regulates dopamine release in the fish retina. J. Neurophysiol. 78: 439–449, 1997. In the fish retina, horizontal cell electrical coupling and light responsiveness is regulated by activation of dopamine D1 receptors that are located on the horizontal cells themselves. The effects of dopamine and dopamine D2 receptor agonists and antagonists on horizontal cell light responses were studied in vitro superfused goldfish retinas. Horizontal cell light responses and electrical coupling were assessed by monitoring responses to full-field stimuli and to small, centered (0.4 mm diam) spots of light, respectively.

INTRODUCTION

Dopamine receptors are G-protein-coupled receptors that have been classified into two general groups, D1 and D2, on the basis of their effects on adenosine 3',5'-cyclic monophosphate (cAMP) production (Civelli et al. 1993; Kebabian and Calne 1979; Seeman and Van Tol 1994). Activation of postsynaptic D1 receptors increases cAMP production, whereas activation of D2 receptors either inhibits or has no effect on adenyl cyclase activity. In addition, D1 and D2 receptors can also be distinguished by their interactions with selective pharmacological agonists and antagonists, as well as by their affinity for dopamine. That is, intact tissue D2 receptors are 2–3 orders of magnitude more sensitive to dopamine than D1 receptors (Civelli et al. 1993; Kebabian and Calne 1979; Seeman and Van Tol 1994). Finally, one D2 receptor subtype functions as a presynaptic autoreceptor whose activation inhibits dopamine release (Starke et al. 1989).

Both D1 and D2 receptors are present in the vertebrate retina (Schorderet and Nowak 1990). In many retinas, the dopaminergic cells are primarily amacrine cells. In the retinas of teleost fish and New World monkeys, however, the dopaminergic cells are interplexiform cells, a cell type that receives synaptic input from amacrine and bipolar cells in the inner plexiform layer and makes synaptic contacts onto horizontal and bipolar cells in the outer plexiform layer (Dowling and Ehinger 1975, 1978; Dowling et al. 1980; Van Haesendonck et al. 1993; Yazulla and Zucker 1988). Dopaminergic interplexiform cells thus constitute a centrifugal pathway of information transfer from the inner retina to cone horizontal cells, a type of second-order cell that also receives synaptic contact from cones.

Dopamine, by activating D1 receptors on fish cone horizontal cells, stimulates adenyl cyclase, leading to a decrease in the permeability of the electrical synapses between horizontal cells (Harsanyi and Mangel 1992; Lasater and Dowling 1985; Mangel and Dowling 1985, 1987; Teranishi et al. 1983). As a result of the uncoupling, the responses of horizontal cells to small spots of light increase in amplitude, that is, the size of their receptive fields is decreased. Although both flickering and sustained light stimulation uncouple fish horizontal cells, flicker-induced uncoupling (Umino et al. 1991), but not sustained light-induced uncoupling (Baldridge and Ball 1991), is due to an increase in dopamine release. In addition to an effect on cell coupling, dopamine application also reduces the response of cone horizontal cells to diffuse or full-field light stimulation (Hedden and Dowling 1978; Mangel and Dowling 1985, 1987).

Recent evidence indicates that the light responsiveness and receptive field size of fish horizontal cells are regulated by activation of D2 receptors, as well as by D1 receptors. Application of a specific D2 agonist increases horizontal cell receptive field size and light responsiveness, effects opposite those of D1 agonists or micromolar doses of dopamine (Harsanyi and Mangel 1992). The effects of the D2 agonist are blocked completely by prior application of a D1 agonist or after destruction of the dopaminergic interplexiform cells.
(Harsanyi and Mangel 1992). Moreover, application of D2 antagonists increases dopamine release from the fish retina (Rashid et al. 1993), and the pattern and density of D2 receptor binding over the outer plexiform layer (Wagner and Behrens 1993) is eliminated after destruction of dopaminergic neurons in the fish (Yazulla and Lin 1995). These findings together are thus consistent with the view that activation of D2 autoreceptors on dopaminergic interplexiform cells inhibits dopamine release in the fish retina.

The purpose of this study was to determine the functional role of D2 receptor activation on the light responses of horizontal cells. The results indicate that endogenous levels of dopamine activate D2 receptors linked to horizontal cell light responsiveness and receptive field size, and that D2 receptor activation is dependent on the ambient lighting conditions. Finally, the results indicate that the effectiveness of certain D2 ligands may be dependent on the concentration of divergent cations.

**METHODS**

**Preparation**

Cone horizontal cell recordings were obtained from intact, isolated goldfish (*Carassius auratus*) retinas. The fish, ∼15 cm in length, were maintained at 19°C in aerated water in a 12:12-h light:dark cycle. Experiments were performed at midday. Before the start of an experiment, the fish were dark-adapted for 20–60 min and anesthetized with methanesulfonate (100 mg/l, MS-222, Sigma). The care and use of the goldfish was in accordance with federal and institutional guidelines. After anesthesia, the fish were decapitated and pithed, and the eyes were then enucleated. Retinas were then isolated intact from the pigment epithelium and placed into a Teflon superfusion chamber photoreceptor side up.

The retinas were superfused at 1.0 ml/min with a solution that contained (in mM) 110 NaCl, 2.5 KCl, 20 NaHCO3, 20 glucose, 0.1 CaCl2, and 0.1 MgCl2 (unless otherwise noted), as described previously (Harsanyi and Mangel 1992; Harsanyi et al. 1996; Mangel and Dowling 1987). In some of the experiments (see Figs. 6A, 7A, and 8, middle), the Ringer solution contained 1.0 mM rather than 0.1 mM MgCl2. In other cases (Figs. 6B, 7B, and 8, right), the Ringer solution contained 1.0 mM rather than 0.1 mM CaCl2. In these latter cases, 130 mM rather than 110 mM NaCl was used in the Ringer solution to maintain horizontal cell dark resting membrane potential and light response amplitude and waveform (Harsanyi and Mangel 1993; cf. Rowe 1987). Oxidation with a mixture of 97% O2, 3% CO2 maintained the superfusate at pH 7.6 in the retinal chamber. Test drugs were added to the superfusate with a system of two-way stopcocks and manifold. Dopamine solutions contained 0.1 mM ascorbic acid to prevent oxidation. All dopamine agonists and antagonists were obtained from Research Biochemicals, (Natick, MA). These ligands were dissolved in either dimethyl sulfoxide (DMSO) or ethanol. Ligands dissolved in DMSO were diluted 100-fold in Ringer solution before application to the retina, whereas those dissolved in ethanol were diluted 1000-fold. Control experiments indicated that 0.1 mM ascorbic acid, 1% DMSO, or 0.1% ethanol had no effect on the light responses or membrane potential of cone horizontal cells.

Some of the fish (n = 9) received a 5-μl injection of 6-hydroxydopamine (6-OHDA) (10 g/l) and ascorbic acid (1 g/l) dissolved in an NaCl solution (9 g/l) into one eye on 2 consecutive days 7–14 days before the recording experiments (Baldridge et al. 1989; Harsanyi and Mangel 1992; Negishi et al. 1982). This procedure destroys dopaminergic cells. The same volume of the NaCl solution containing ascorbic acid was injected into the other eye as a control.
luses. Accordingly, a larger-amplitude voltage response is recorded from the illuminated cell during uncoupling.

In contrast, changes in electrical coupling should not affect the size of responses to full-field stimulation, because full-field stimulation drives every horizontal cell equivalently. When there is no membrane potential difference between coupled cells, current will not flow between the cells. Thus the finding that a drug decreases horizontal cell responses to full-field light indicates that the drug decreases the light responsiveness of the cells. In addition, the finding that a drug, such as dopamine, decreases horizontal cell responses to full-field stimuli of both low and high intensities (Dong and McReynolds 1991; Hedden and Dowling 1978; Mangel and Dowling 1987) supports the conclusion that an increase in spot response amplitude is due to an increase in coupling resistance and not to a change in synaptic and/or nonsynaptic membrane resistance.

Because dopamine affects both the coupling between horizontal cells and the light responsiveness of horizontal cells, both phenomena were studied here with the appropriate light stimuli. Control experiments indicated that the amplitude of horizontal cell responses to spot and full-field stimuli did not change when these stimuli were alternately flashed for a period of 20 min and no drug was applied. The intervals between the flashes in these control experiments were the same as those during test drug experiments.

In addition to an assessment of horizontal cell coupling and light responsiveness, an additional response measure, the ‘‘response ratio,’’ was used to quantify the response of horizontal cells to dopamine ligands, such as eticlopride, sulpiride, and quinpirole. This response measure is defined as the ratio of spot response size to full-field response size (Harsanyi et al. 1996; Perlman and Ammermuller 1994). Because this measure utilizes responses to both spot and full-field stimuli, it does not quantify the extent of changes in either horizontal cell coupling or horizontal cell responsiveness. Rather, the response ratio quantifies the action of dopamine ligands on horizontal cells. Because the response to full-field stimuli does not depend on the extent of horizontal cell coupling (Dong and McReynolds 1991; Mangel and Dowling 1985, 1987), and because each dopamine ligand has opposite effects on spot and full-field responses, measurements of the horizontal cell response to dopamine ligands in which the response ratio was used were as sensitive as or more sensitive than measurements in which either spot or full-field responses alone would have been utilized. Thus relatively small effects of dopamine ligands on horizontal cells are detectable with the use of the response ratio. The size of each response was calculated from the peak of the response. Before application of dopamine ligands, the intensities of spot and full-field stimuli were adjusted to generate responses of approximately equal amplitude. Thus the response ratio under control conditions was approximately equal to 1. Subtracting the response ratio obtained before drug superfusion (control) from the ratio obtained during superfusion of a test drug yields the change in response ratio.

The Wilcoxon two-sample test was used for all statistical comparisons between control and experimental conditions.

**RESULTS**

Data were obtained from 152 L-type (H1) cone horizontal cells and from 41 C-type (H2 and H3) cone horizontal cells. No obvious differences in the effects of D2 ligands on these cell types were observed, and for this reason all data were pooled.

**Effects of D2 agonists and antagonists**

Figure 1 illustrates the differential effects of dopamine (A), a dopamine D2 agonist (B), and a dopamine D2 antagonist (C) on the light responses of cone horizontal cells. As reported previously, application of dopamine (0.2–10 μM) increased horizontal cell responses to spots of light and decreased responses to full-field light (Fig. 1A). The finding that dopamine application decreases horizontal cell responses to full-field light indicates that dopamine decreases the light responsiveness of the cells (see METHODS). In addition, the increase in the cells’ responses to small spot stimuli centered within the receptive field, at the same time as the full-field response decreases, indicates that dopamine application decreases electrical coupling between the horizontal cells (see METHODS). In contrast, application of quinpirole (0.1–10 μM), a specific D2 agonist, produced effects opposite those of dopamine (0.2–10 μM), as reported previously (Dong and McReynolds 1991; Harsanyi and Mangel 1992). That is, quinpirole (n = 35) decreased spot responses and increased full-field responses (Fig. 1B), indicating that horizontal cell coupling and light responsiveness have increased. Application of eticlopride (10–50 μM), a specific D2 antagonist, affected horizontal cell light responses (Fig. 1C) in a manner opposite that of quinpirole, but similar to that of dopamine, indicating that it decreased horizontal cell coupling and light responsiveness (n = 15). These latter findings indicate that endogenous dopamine in the goldfish retina activates D2 receptors.

Because activation of postsynaptic D2 receptors has been shown to decrease cAMP levels in the retina (Deary and Burnside 1986; Iuvone 1986), it is possible that the above effects of quinpirole and eticlopride result from activation of D2 receptors on the horizontal cell postsynaptic membrane. To test whether goldfish horizontal cells themselves possess D2 receptors, the effects of eticlopride were examined following prior application of the specific D1 antagonist SCH23390 (10 μM). SCH23390 application increased horizontal cell coupling (data not shown), after which full-field light intensity was decreased so that full-field and spot stimuli generated approximately equal amplitude responses. Eticlopride was then applied, during which the concentration of SCH23390 was maintained throughout. Figure 2 illustrates that during application of SCH23390, eticlopride had no effect (6 of 6 cases). These results suggest that the D2 receptors linked to horizontal cell coupling are not on the postsynaptic membrane of horizontal cells.

To test this possibility further, retinas were pretreated with intraocular injections of 6-OHDA (see METHODS), a procedure that destroys dopaminergic neurons (Baldridge et al. 1989; Harsanyi and Mangel 1992; Negishi et al. 1982). Figure 3, top, shows that eticlopride had no effect following 6-OHDA treatment in all cases tested for both L-type and C-type cone horizontal cells (n = 12). In contrast, as reported previously (Harsanyi and Mangel 1992; Harsanyi et al. 1996; Teranishi et al. 1983), application of dopamine (10 μM) uncoupled horizontal cells and reduced their light responsiveness (n = 6) following 6-OHDA pretreatment (data not shown). Eticlopride application to control retinas that were pretreated with saline/ascorbate injections (Fig. 3, bottom) uncoupled horizontal cells and reduced their light responsiveness (n = 9) in a manner indistinguishable from that observed in normal retinas (see Fig. 1C). These results thus indicate that the effects of eticlopride depend on the presence of viable dopaminergic interplexiform cells.
FIG. 1. Differential effects of dopamine and dopamine D2 agonists and antagonists on light responses of fish retinal cone horizontal cells. Effects of dopamine (A), quinpirole (B), a D2 agonist, and eticlopride (C), a D2 antagonist, on responses of 3 different L-type cone horizontal cells to spot and full-field stimuli. Centered spot (0.4 mm diam) and full-field stimuli were alternately flashed and initially adjusted in intensity to generate responses of similar amplitude. In each trace for this and all subsequent figures, 1st spot stimulus is indicated by dot placed just below response; spot was always 1st of stimulus pair. Portions of trace were expanded ~5-fold by increasing penwriter speed so that response waveform could be viewed. Dopamine and eticlopride increased response of cone horizontal cells to small spots of light, an effect indicative of uncoupling action (see text). At same time, they also decreased response of cells to full-field stimuli, an effect that suggests that dopamine and eticlopride decrease responsiveness of horizontal cells to light. In contrast, quinpirole decreased response of cells to small spots of light and increased full-field responses. These results indicate that endogenous dopamine activates D2 receptors that are linked to horizontal cell coupling and light responsiveness. Time delay between initiation of drug applications and cells’ responses: delay for exchange of superfused fluids. Intensity of spot: −1.0 log (A), −1.3 log (B), −1.2 log (C). Intensity of full-field stimulus: −5.0 log (A), −5.2 log (B), −5.1 log (C).

results further suggest that endogenous dopamine activates D2 receptors, thus decreasing dopamine release from dopaminergic interplexiform cells onto cone horizontal cells.

Functional role of D2 receptor activation

Although both flickering and sustained light stimulation uncouple fish cone horizontal cells, flicker-induced uncoupling (Harsanyi et al. 1996; Umino et al. 1991), but not sustained light-induced uncoupling (Baldrige and Ball 1991), is due to an increase in dopamine release. In fact, it has been reported that flickering light is more effective than sustained light stimulation in increasing dopamine release in the fish retina (Kirsch and Wagner 1989; Weiler et al. 1997). We thus tested whether the differential effects of flickering and sustained light stimulation on dopamine release would alter the effectiveness of quinpirole, a D2 agonist. As shown in Fig. 4A, quinpirole had no effect on the light responses of horizontal cells when applied after a period of sustained light stimulation (see METHODS), but was effective in increasing coupling and light responsiveness (Figs. 1B and 4B) when applied after a period of flickering light stimulation (see METHODS). Figure 4B illustrates further that the coupling action of quinpirole could be partially reversed
Fig. 2. Prior application of SCH23390, a D1 antagonist, blocks effects of eticlopride, a D2 antagonist, on cone horizontal cells. Conventions as in Fig. 1. Application of SCH23390 (10 μM, 4 min) decreased spot response and increased full-field response of L-type cone horizontal cell (not shown), after which full-field intensity was decreased so that full-field and spot stimuli generated approximately equal amplitude responses. Eticlopride (20 μM) was then applied; concentration of SCH23390 in Ringer solution was maintained throughout. Eticlopride had no effect in presence of SCH23390. These results suggest that D2 receptors that are linked to horizontal cell coupling and light responsiveness are not on postsynaptic membrane of horizontal cells. Intensity of spot: −1.0 log. Intensity of full-field stimulus: −5.3 log.

by a period of flickering light stimulation (−4 log I₀ at 0.5 Hz for 40 s, 50% duty cycle).

Similar results were obtained with eticlopride application. Eticlopride (10–50 μM) decreased horizontal cell coupling and light responsiveness following flickering light stimulation (see Fig. 1C), but not following sustained light stimulation.

6-OHDA

Fig. 3. Eticlopride, a D2 antagonist, uncouples cone horizontal cells and reduces their light responsiveness by increasing release of dopamine from interplexiform cells. Conventions as in Fig. 1. Top: After 6-hydroxydopamine (6-OHDA) pretreatment, eticlopride (20 μM) application had no effect on spot and full-field light responses of cone horizontal cells. Bottom: in control experiments following pretreatment with intraocular injections of saline, eticlopride (20 μM) application uncoupled cone horizontal cells and reduced their light responsiveness. Intensity of spot: −1.0 log (top), −1.2 log (bottom). Intensity of full-field stimulus: −5.2 log (top), −5.0 log (bottom).

The responses of L-type and C-type cone horizontal cells to quinpirole and eticlopride were quantified with the use of a response measure, the response ratio (see METHODS), which incorporated both spot and full-field response values. The differential effects of quinpirole and eticlopride following flickering compared with sustained light stimulation are shown in Fig. 5. The change in response ratio is obtained.
by subtracting the response ratio obtained during control conditions from the response ratio obtained during drug application. As illustrated in Fig. 5, the effect of eticlopride was significantly reduced ($P < 0.01$) when it was applied after sustained ($n = 9$) compared with flickering ($n = 15$) light stimulation. Similarly, the effect of quinpirole was also significantly reduced ($P < 0.01$) when it was applied after sustained ($n = 8$) compared with flickering ($n = 35$) light stimulation.

These results are thus consistent with the view that the actions of quinpirole and eticlopride depend on the presence of a flicker-induced basal level of dopamine (Harsanyi and Mangel 1992). Because D2 receptors respond to levels of dopamine $\approx$ 2–3 orders of magnitude lower than D1 receptors (Civelli et al. 1993; Kebabian and Calne 1979; Seeman and Van Tol 1994), the level of endogenous dopamine must have been sufficient to activate D1 receptors on horizontal cells for the effects of D2 ligands to be detectable in these experiments (see DISCUSSION). In addition, the level of endogenous dopamine must not have saturated the D2 receptors for quinpirole, a D2 agonist, to be effective (Starke et al. 1989).

### Effects of divalent cations

Because previous work has shown that the effectiveness of dopamine ligands at D2 receptors is modulated by the divalent cations $\text{Mg}^{2+}$ and $\text{Ca}^{2+}$ (Hamblin and Creese 1982; Sibley and Creese 1983; Watanabe et al. 1985), we tested whether changes in the concentration of divalent cations in the Ringer solution would alter the effects of D2 ligands on horizontal cells. As described in METHODS, control retinas were superfused with a Ringer solution that contained 0.1 mM $\text{MgCl}_2$ and 0.1 mM $\text{CaCl}_2$. In experiments that tested whether the $\text{Mg}^{2+}$ concentration alters the effectiveness of D2 ligands, a concentration of 1.0 mM rather 0.1 mM $\text{MgCl}_2$ was used; all other Ringer solution components remained the same. However, in experiments that tested whether the $\text{Ca}^{2+}$ concentration alters the effectiveness of D2 ligands, the test Ringer solution contained 1.0 mM rather 0.1 mM $\text{CaCl}_2$, and 130 mM rather than 110 mM NaCl. This latter procedure was used to maintain horizontal cell dark resting membrane potential and light response amplitude within normal levels (Harsanyi and Mangel 1992; cf. Rowe 1987). Control experiments indicated that an increase of NaCl from 110 to 130 mM, while CaCl$_2$ remained constant (0.1 mM), had no effect on the dark resting membrane potential (cf. Harsanyi and Mangel 1993) or on the amplitude of spot and full-field light responses (data not shown).

Eticlopride uncoupled horizontal cells and reduced their light responsiveness (Figs. 1C and 3B) regardless of the concentration of divalent cations in the superfusate. That is, the effects of eticlopride were the same whether the total concentration of divalent cations was at control (0.2 mM) levels ($0.1 \text{ mM } \text{Mg}^{2+}$ and 0.1 mM $\text{Ca}^{2+}$) or whether it was 1.1 mM ($1.0 \text{ mM } \text{Mg}^{2+}$ and 0.1 mM $\text{Ca}^{2+}$ or vice versa).
In contrast, sulpiride (0.5–20 µM) uncoupled horizontal cells and decreased their light responsiveness when the total concentration of divalent cations (Mg²⁺ and Ca²⁺) in the Ringer solution was 1.1 mM (Fig. 6A: 1.0 mM Mg²⁺ and 0.1 mM Ca²⁺; Fig. 6B: 0.1 mM Mg²⁺ and 1.0 mM Ca²⁺), but coupled the cells and increased their light responsiveness when the concentration of divalent cations was 0.2 mM (Fig. 6C: 0.1 mM Mg²⁺ and 0.1 mM Ca²⁺). The effects of spiperone (0.25–10 µM) also depended on the concentration of divalent cations. Spiperone uncoupled horizontal cells and decreased their light responsiveness when the total concentration of divalent cations (Mg²⁺, Ca²⁺) in the Ringer solution was 1.1 mM (8 of 9 cases), but had no effect (5 of 5 cases) when the concentration of divalent cations was 0.2 mM.

Quinpirole consistently coupled the cells and increased their light responsiveness when the concentration of divalent cations in the Ringer solution was 0.2 mM (Fig. 1B: 0.1 mM Mg²⁺ and 0.1 mM Ca²⁺), but generally had no effect when the divalent cation concentration was 1.1 mM (Fig. 7A: 1.0 mM Mg²⁺ and 0.1 mM Ca²⁺; Fig. 7B: 0.1 mM Mg²⁺ and 1.0 mM Ca²⁺). Averaged data from both L-type and C-type cone horizontal cells, which illustrate that the actions of sulpiride and quinpirole, but not that of eticlopride, depend on the concentration of divalent cations, are shown in Fig. 8. Although the magnitude of the change in the response ratio due to eticlopride application did not depend on the Mg²⁺ or Ca²⁺ concentration, the action of sulpiride did. Specifically, the action of sulpiride was similar to that of eticlopride when the total divalent cation concentration was 1.1 mM, but the average change in response ratio when sulpiride was added to Ringer solution containing 0.1 mM Mg²⁺ and 0.1 mM Ca²⁺ (n = 23) was reversed in polarity. The effect of sulpiride when it was added to Ringer solution containing 0.1 mM Mg²⁺ and 0.1 mM Ca²⁺ was significantly different from what occurred when sulpiride was added to Ringer solution containing a concentration of divalent cations of 1.1 mM (1.0 mM Mg²⁺ and 0.1 mM Ca²⁺, n = 15; 0.1 mM Mg²⁺ and 1.0 mM Ca²⁺, n = 8; P < 0.01 in each case).

The effect of quinpirole also depended on Mg²⁺ and Ca²⁺ concentrations, as revealed by use of the response ratio. The average change in response ratio following quinpirole application was significantly reduced when the divalent cation concentration was raised from control levels (0.2 mM) to 1.1 mM (1.0 mM Mg²⁺ and 0.1 mM Ca²⁺, n = 16; 0.1 mM Mg²⁺ and 1.0 mM Ca²⁺, n = 10; P < 0.05 in each case).

DISCUSSION

The principal findings of this study are fourfold. First, application of the specific dopamine D2 receptor antagonist eticlopride uncouples the electrical synapses between fish retinal cone horizontal cells and decreases their light responsiveness, effects that are similar to those of dopamine (0.2–10 µM) but opposite those of quinpirole, a D2 agonist. Second, the effects of eticlopride are completely blocked by application of SCH23390, a D1 antagonist, or eliminated by prior treatment of the retinas with 6-OHDA, a procedure that destroys dopaminergic neurons. Third, the effects of D2 agonists and antagonists occurred following flickering, but not sustained, light stimulation. Fourth, although the effects of eticlopride did not depend on the concentration of the divalent cations Mg²⁺ and Ca²⁺, the effects of quinpirole and two D2 antagonists, sulpiride and spiperone, did. These findings thus indicate that endogenous levels of dopamine activate D2 receptors linked to horizontal cell light responsiveness and receptive field size. In addition, the findings suggest that D2 receptor activation is greater following flickering compared with sustained light stimulation.

The present findings, in conjunction with previous studies on fish retina (Harsanyi and Mangel 1992; Lasater and Dowling 1985; Rashid et al. 1993; Van Buskirk and Dowling 1981), further suggest that the postsynaptic membrane of fish cone horizontal cells contains D1 and not D2 receptors, and that the D2 receptors function as D2 autoreceptors, whose activation inhibits the release of dopamine from dopaminergic interplexiform cells. Activation of these D2 receptors by application of quinpirole thus causes a decrease in dopamine release so that the horizontal cells couple more completely. This view is supported by the following evidence from the fish retina: 1) the uncoupling effects of dopamine (0.2–10 µM) and SKF38393, a D1 agonist, still occur after 6-OHDA-induced destruction of dopaminergic cells (Harsanyi and Mangel 1992; Teranishi et al. 1983); 2) the effects of quinpirole are eliminated by 6-OHDA-induced destruction of dopaminergic cells and are blocked by nonsaturating doses of SKF38393 (Harsanyi and Mangel 1992); 3) the effects of eticlopride are eliminated by 6-OHDA-
induced destruction of dopaminergic cells and are blocked completely by prior application of SCH23390, a D1 antagonist (Figs. 2 and 3); and 4) application of sulpiride, a D2 antagonist, increases both baseline [3H]-dopamine release and [3H]-dopamine release induced by an increase in extracellular potassium concentration (Rashid et al. 1993). In addition, studies on rabbit retina have shown that D2 agonists inhibit and D2 antagonists increase calcium-dependent electrically evoked dopamine release (Dubocovich and Weiner 1985), thus confirming the suggestion that endogenous dopamine, by activating D2 receptors, regulates dopamine release from dopaminergic cells. The present findings are also supported by a study on amphibian retina that reported that quinpirole increases horizontal cell coupling and light responsiveness (Dong and McReynolds 1991).

Although the above evidence clearly indicates that the D2 receptors in the fish retina, which are linked indirectly to horizontal cell coupling and light responsiveness, function as D2 autoreceptors, the exact site of these receptors cannot be known with certainty. However, recent findings suggest that these D2 receptors may indeed reside on the presynaptic terminals of interplexiform cells. Yazulla and Lin (1995) reported that the pattern and density of specific [3H]-spiperone binding (D2 receptors) over the outer plexiform layer of the fish retina was eliminated by 6-OHDA-induced destruction of dopaminergic interplexiform cells, whereas the pattern and density of [3H]-SCH23390 binding (D1 receptors) was not affected.

Our results confirm previous findings that flickering light is more effective than sustained light stimulation in increasing dopamine release in the fish retina (Baldridge and Ball 1991; Harsanyi et al. 1996; Kirsch and Wagner 1989; Umino et al. 1991; Weiler et al. 1997). The level of endogenous dopamine must be sufficient to activate D1 receptors to be detectable in our measurements of horizontal cell coupling and light responsiveness. Thus the finding that D2 ligands are effective following flickering, but not sustained, light stimulation may reflect the fact that flickering light increases dopamine levels so that D1 receptors on horizontal cells are activated. In addition, the level of endogenous dopamine must not saturate D2 receptors for D2 agonists to be effective at D2 autoreceptors (Starke et al. 1989). This seems plausi-
FIG. 7. Action of quinpirole, a D2 agonist, on cone horizontal cell light responses depends on concentration of divalent cations Mg$^{2+}$ and Ca$^{2+}$. Conventions as in Fig. 1. A and B: application of quinpirole (1 μM) had no effect on light responses of horizontal cells when total divalent cation concentration in Ringer solution was 1.1 mM (A: 1.0 mM Mg$^{2+}$ and 0.1 mM Ca$^{2+}$; B: 0.1 mM Mg$^{2+}$ and 1.0 mM Ca$^{2+}$). See text for details. Intensity of spot: $-1.1 \log (A)$, $-1.0 \log (B)$. Intensity of full-field stimulus: $-5.0 \log (A)$, $-4.9 \log (B)$.

ble in the intact fish retina, because previous work has shown that D2 receptors in the intact fish retina respond to low nanomolar levels of dopamine (Deary and Burnside 1986; Mangel and Wang 1996), whereas the threshold of D1 receptor activation in the intact fish retina is 2 orders of magnitude higher (Harsanyi and Mangel 1992). Thus the finding that quinpirole is effective following flickering, but not sustained, light stimulation (Figs. 4 and 5) is consistent with the view that endogenous dopamine increases to a level after flickering light stimulation sufficient to activate D1 receptors but not saturate D2 receptors. Endogenous dopamine levels may in fact be sufficient to activate D2 autoreceptors during sustained light stimulation, but may not be detectable with the use of measurements of horizontal cell coupling and light responsiveness. However, because flickering light increases dopamine levels but does not saturate D2 receptors, the find-

FIG. 8. Actions of sulpiride and quinpirole, but not eticlopride, depend on Mg$^{2+}$ and Ca$^{2+}$ concentrations. Average change in response ratio was calculated as described in Fig. 5. Action of eticlopride on cone horizontal cells was not affected by Mg$^{2+}$ and Ca$^{2+}$ concentrations. In contrast, action of sulpiride was similar to that of eticlopride at total Mg$^{2+}$/Ca$^{2+}$ concentration of 1.1 mM, but was reversed in polarity at Mg$^{2+}$/Ca$^{2+}$ concentration of 0.2 mM. Magnitude of action of quinpirole was significantly greater in control Ringer solution (0.1 mM Mg$^{2+}$ and 0.1 mM Ca$^{2+}$) than when it was added to Ringer solution containing Mg$^{2+}$/Ca$^{2+}$ concentration of 1.1 mM. Error bars: means ± SE.
ings of this study also suggest that flickering light stimulation activates D2 receptors to a greater extent than sustained light. The finding that the effectiveness of D2 agonists and antagonists depends on the light stimulus conditions, and presumably on the level of endogenous dopamine present, further substantiates the view that these D2 receptors function as presynaptic autoreceptors (Starke et al. 1989).

The presence of functional D2 autoreceptors in the fish retina may serve to accentuate the effect of endogenous dopamine on horizontal cell coupling and light responsiveness. Because D2 receptors in the intact fish retina respond to low nanomolar levels of dopamine (Dearry and Burnside 1986; Mangel and Wang 1996), whereas the threshold of D1 receptor activation in the intact fish retina is 2 orders of magnitude higher (Harsanyi and Mangel 1992), D2-autoreceptor-induced inhibition of dopamine release from interplexiform cells may dominate during periods of relatively low dopamine release such as occur during the day under sustained light background conditions (Kirsch and Wagner 1989; Mangel and Wang 1995; Weiler et al. 1997). However, during periods of greater dopamine release, such as occur when background illumination is rapidly changing (e.g., flickering light), both D1 receptors and D2 autoreceptors may be activated. The presence of both receptor types at the interplexiform/horizontal cell synapse thus might reduce the effect of dopamine on horizontal cell coupling and light responsiveness under sustained light background conditions and might accentuate the effect of dopamine on horizontal cell coupling and light responsiveness as the dopamine concentration increases during rapid changes in the visual environment.

Previous reports have indicated that divergent cations, such as Mg$^{2+}$ and Ca$^{2+}$, regulate binding at D2 receptors in the striatum (Hamblin and Creese 1982) and pituitary (Sibley and Creese 1983; Watanabe et al. 1985). Our findings are consistent with such a role for divergent cations in the fish retina, because the effectiveness of the D2 agonist quinpirole increases, and the effectiveness of sulpiride and spiperone as D2 antagonists decreases, when total divalent cation (Mg$^{2+}$ and Ca$^{2+}$) concentration is reduced from 1.1 to 0.2 mM. Interestingly, the effectiveness of eticlopride is not altered by changes in Mg$^{2+}$ or Ca$^{2+}$ concentration. The effectiveness of sulpiride is affected more significantly than spiperone by divergent cation concentration. That is, although both antagonists uncouple horizontal cells at higher divergent cation concentrations, sulpiride increases coupling and spiperone has no effect when a lower divergent cation concentration is used. Thus divergent cations differentially alter the effectiveness of some D2 ligands in the fish retina.

Although the exact basis for the dependence of D2 ligand effectiveness on divergent cation concentration in the fish retina remains to be determined, these results may account for some dopamine release findings recently reported in the fish retina (Rashid et al. 1993). Using a total divergent cation concentration of 5.7 mM, these workers reported that quinpirole had no effect on baseline $[^{3}H]$-dopamine release, but that sulpiride increased it. On the basis of the evidence presented here, it is thus possible that the reported differential effectiveness of quinpirole and sulpiride on baseline dopamine release is due to the divergent cation concentration employed.

Finally, the dependence of D2 ligand effectiveness in the fish retina on Mg$^{2+}$ and Ca$^{2+}$ concentrations probably does not involve N-methyl-d-aspartate (NMDA) receptors. As reported previously, NMDA application decreases horizontal cell coupling and light responsiveness by increasing dopamine release in the fish retina (Harsanyi et al. 1996). Moreover, application of Mg$^{2+}$, a noncompetitive blocker of NMDA receptors, can result in effects opposite those of NMDA or dopamine (Harsanyi et al. 1996). However, NMDA receptor activation is blocked by Mg$^{2+}$, but not by Ca$^{2+}$ (Mayer and Miller 1990). Therefore, because the effectiveness of D2 ligands in modulating horizontal cell coupling and light responsiveness depends on Ca$^{2+}$, as well as on Mg$^{2+}$, the dependence of these ligands on divergent cations probably does not involve NMDA receptors.

In summary, endogenous levels of dopamine in the fish retina activate D2 receptors that are linked indirectly to horizontal cell electrical coupling and light responsiveness. These receptors function as presynaptic autoreceptors in that their activation inhibits the release of dopamine from dopaminergic interplexiform cells. Activation of these receptors is also dependent on the ambient lighting conditions. In addition, the effectiveness of certain D2 ligands is dependent on the concentration of the divergent cations Mg$^{2+}$ and Ca$^{2+}$.

This investigation was supported in part by grants to S. C. Mangel from the National Eye Institute (EY-05102), the National Science Foundation (IBN-9312878), and the Plum Foundation, and by CORE Grant P30 EY-03039 to the University of Alabama at Birmingham. Y. Wang was supported in part by a Postdoctoral Fellowship from the Helen Keller Eye Research Foundation. S. C. Mangel was a Robert E. McCormick Scholar of Research to Prevent Blindness, New York.

Present address of Y. Wang: Dept. of Physiological Optics, University of Alabama, Birmingham, AL 35294.

Address for reprint requests: S. C. Mangel, Dept. of Neurobiology, University of Alabama School of Medicine, 1719 Sixth Ave. South, CIRC 425, Birmingham, AL 35294-0021.

Received 14 June 1996; accepted in final form 20 March 1997.

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