

Dopamine Enhances a Glutamate-gated Ionic Current in OFF Bipolar Cells of the Tiger Salamander Retina

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The transmitter glutamate is thought to be used by all vertebrate photoreceptors to drive the second-order neurons of the retina, horizontal and bipolar neurons. Dopamine, an endogenous retinal neurotransmitter localized to amacrine and interplexiform cells, has previously been shown to enhance glutamate-gated currents in retinal horizontal cells. In the present study we demonstrate that bipolar cells, like horizontal cells, possess glutamate receptors that are modulated by dopamine. We then identify some components of the pathway through which dopamine acts.

We used whole-cell patch recording to measure how bath-applied dopamine modulated the currents elicited by puffs of transmitter solutions at bipolar cell dendrites. Excitatory amino acid-gated currents were evoked by pressure ejecting 1 mM glutamate or 10 μ M kainate for 40 msec through a micropipette positioned at the dendrites of bipolar cells. Bath-applied dopamine (20 μ M) enhanced the response to glutamate in OFF bipolar cells in the retinal slice by 40% and in isolated OFF bipolar cells by 65%.

We also explored the components of the intracellular pathway mediating this modulation. Response enhancement was blocked by the D1 receptor antagonist SCH23390, but not by the D2 receptor antagonist spiperone, suggesting that the enhancement by dopamine is mediated by a D1 receptor. GDP- β -S, a G-protein inactivator, blocked the enhancing action of dopamine, suggesting that the D1 receptor activated a G-protein to enhance the glutamate-gated current. Both 8-(4-chlorophenylthio)adenosine, a cAMP analog, and the addition of the catalytic subunit of protein kinase A (PKA) to the recording pipette enhanced glutamate-gated currents, while H-7, a PK inactivator, and PKI₂₀amide, a PKA-specific inhibitor, blocked the enhancing action of dopamine. These data suggest that dopamine acts at D1 receptors in the dendrites of bipolar cells to activate adenylyl cyclase, which through cAMP enhances a glutamate-gated current in bipolar cell dendrites. Thus, dopamine may modulate synaptic transmission from photoreceptors to OFF bipolar cells.

[Key words: vision, retina, glutamate receptors, dopamine, bipolar cell, modulation]

Recent evidence suggests that dopamine is an endogenous transmitter in retinal interplexiform cells (Dowling and Ehinger, 1975) and amacrine cells (Brecha et al., 1984; Witkovsky et al., 1984; Yang et al., 1991), and that its rate of release is controlled by light or dark stimulation and by circadian rhythms (Besharse et al., 1988; Witkovsky and Dearth, 1992; Witkovsky et al., 1993). Dopamine application to the retina has been shown to increase the contribution of cone input to cells in the outer (Yang et al., 1988; Witkovsky et al., 1989) and inner retina (Maguire and Hamasaki, 1993), and to modulate the surround inhibition mediated by horizontal cells (Mangell and Dowling, 1985). Dopamine may modify the coupling through gap junctions between horizontal cells (Piccolino et al., 1984; Lasater and Dowling, 1985) and may also modulate the sensitivity of glutamate receptors on horizontal cells (Knapp and Dowling, 1987).

Measured at the level of the retina's output, at the ganglion cells, dopamine dramatically changes their overall activity level (Jensen and Daw, 1984; Maguire and Smith, 1985), and induces an apparent shift in the ratio of rod:cone input (Maguire and Hamasaki, 1993). These observations suggest that dopamine may serve the general function of shifting sensitivity from rod to cone pathways in retinal horizontal cells, and other retinal pathways as well. Retinal dopamine could act through G-proteins (Lad et al., 1987) and/or cyclic nucleotide pathways (Caretta and Sabil, 1989) that have been localized in the outer retina. These findings raise the possibility that dopamine may exert direct effects upon bipolar cells.

The bipolar cells of vertebrate retinas can be classified into two groups based on their center-response to light: ON cells respond to an illumination increase by depolarizing and OFF cells by hyperpolarizing (Werblin and Dowling, 1969; Kaneko, 1970). Although dopamine has previously been reported to enhance the light-driven center responses of ON- and OFF-type bipolar cells (Hedden and Dowling, 1978), it is not known if the actions of dopamine were direct and through which mechanism the effect occurred.

Here, as a first step in our investigation of dopamine's actions on bipolar cells, we tested whether dopamine modulates a glutamate receptor in retinal OFF bipolar cells. We found that exogenous dopamine upmodulates the glutamate-gated current in OFF bipolar cell dendrites through a D1 receptor, G-protein, cAMP, cAMP-PK pathway, and that the endogenous effects of dopamine on the glutamate receptors of OFF bipolar cells could be blocked by SCH23390, a D1-specific antagonist. Thus, endogenous dopamine may modulate the direct glutamatergic synaptic input from photoreceptors to OFF bipolar cells by activation of the D1 pathway in the dendrites of the OFF bipolar cells.

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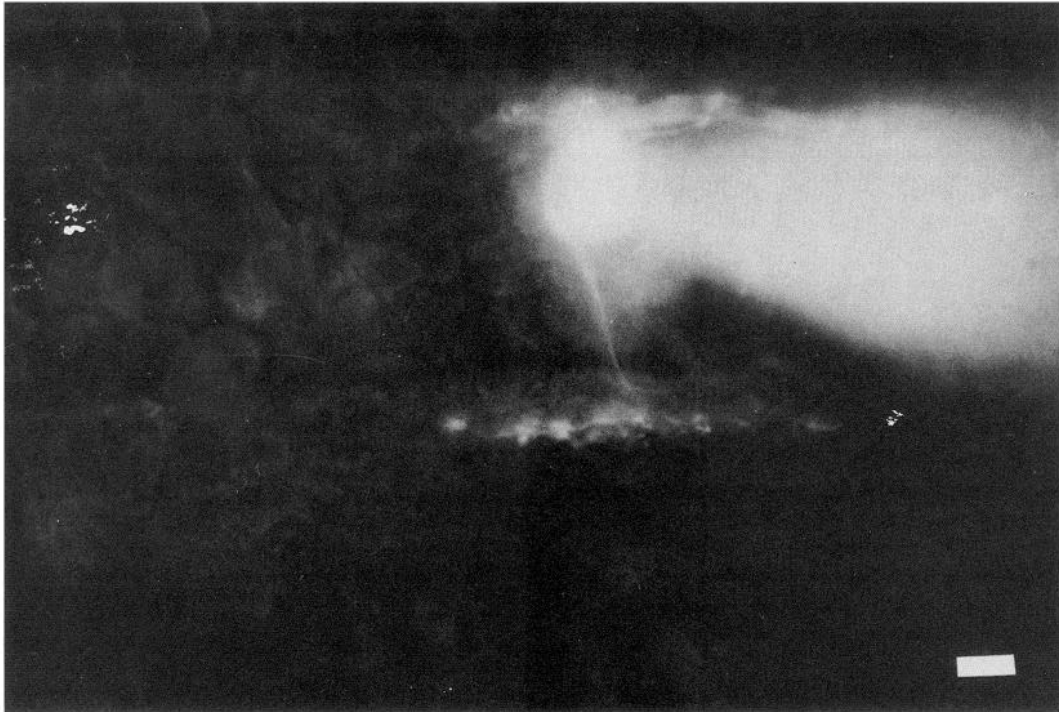


Figure 1. Photomicrograph of a Lucifer yellow-filled OFF-type bipolar cell in the living retinal slice. All bipolar cells in the retinal slice were recorded with conventional whole-cell patch-clamp techniques and were filled with Lucifer yellow from the micropipette containing the electrode during the recording session. All cells included in this study displayed a bipolar cell morphology with axon terminals ramifying in the outer half of the inner plexiform layer, that is, sublamina *a* of the IPL. Scale bar, 10 μ m.

Materials and Methods

Slices of larval tiger salamander (*Ambystoma tigrinum*; Kon's Scientific) retina were prepared according to the method described by Werblin (1978), without the use of enzymes. Animals were maintained in aquaria with continuous filtration at 12°C on a 12 hr/12 hr light/dark cycle. Slices were viewed under 400 \times Hoffman Modulation Contrast, using a 40 \times water immersion objective. Retinal neurons were dissociated by placing the isolated retina in a papain (Worthington) solution (1 mg/ml) for 30 min and then into a 5% serum solution for 10 min, followed by several rinses in normal ringer and gentle mechanical trituration. The experiments on isolated cells were otherwise similar to those for the slices. Conventional whole-cell patch recordings (Hamill et al., 1981) were done as described by Maguire et al. (1990) using either an EPC-7 or Axopatch 200 amplifier and pCLAMP software. Patch electrodes with resistances of 3–4 M Ω were filled with the following solution (in mM): 12 KCl, 104 potassium gluconate, 1 EGTA, 4 HEPES, 0.1 CaCl₂, brought to pH 7.4 with KOH. The bathing solution consisted of (in mM) 120 NaCl, 2 KCl, 3 CaCl₂, 1 MgCl₂, 4 HEPES, 3 glucose, brought to pH 7.5 with NaOH. Cells in the slice were filled with Lucifer yellow (1% solution) from the patch pipette, and viewed using a Nikon mercury fluorescent epilluminate with a B filter package (Stewart, 1978). Glutamate (1 mM) or kainate (10 μ M) was pressure ejected (15 psi) for 40 msec through a micropipette (tip bore < 0.5 μ m) positioned at the bipolar cell dendrites. A background luminance of 15 mW/cm² (white light) was used throughout, and the light step was a full field white light with a luminance of 18 mW/cm². SCH23390 was purchased from Research Biochemicals Inc. (Natick, MA); PKI₂₀amide, from Peninsula Labs (CA); and the rest of the chemicals, from Sigma (St. Louis, MO).

Results

The general strategy of these experiments involved comparing the ionic currents elicited by focal puffs of glutamate or kainate at the dendrites of retinal bipolar cells in the presence and absence of bath-applied dopamine or its antagonists, along with blockers and activators of other components in the pathway mediating the modulatory effects of dopamine on the glutamate-gated channel.

To verify that our responses were being measured from OFF-type bipolar cells (Fig. 1) we first measured the light response of morphologically identified bipolar cells (Fig. 2*A*), and then puffed glutamate at their dendrites to measure the evoked current ($N = 6$). Bipolar cells in the slice (Fig. 1) could be identified by their morphology that included a soma in the outer half of the inner nuclear layer (INL) and dendrites that project into the outer plexiform layer (OPL), and an axon that ramifies in the inner plexiform layer (IPL). Bipolar cells also lack a voltage-gated sodium current as previously described (Maguire et al., 1989*a*), and the OFF cells in the slice responded to the onset of light with an outward current that showed little or no relaxation during the light step (Maguire et al., 1990), which is in contrast to IPCs (Maguire et al., 1990) and sustained amacrine cells (Maguire et al., 1989*b*). Further, OFF bipolar cells in the slice send their axon terminals to sublamina *a* of the IPL, whereas the axons of ON bipolar cells ramify deeper in the IPL, in sublamina *b* (Hare et al., 1986). The ON-type bipolar cells responded to puffs of glutamate with either (1) a conductance decrease with a reversal potential near 0 mV, or (2) with a conductance increase and a reversal potential near -70 mV (Maguire and Werblin, 1991). When stimulated by a puff of glutamate or kainate, the OFF cells responded with an excitatory current that reversed at +10 mV (Fig. 2*B*). Cells in isolation could be identified as OFF bipolar cells by their bipolar morphology, voltage-gated currents that lacked an inward sodium current, and a normally inward glutamate-gated current that reversed at +10 mV.

Dopamine acts at a D1 receptor

Dopamine had no measurable effects on the resting membrane current of isolated cells over a wide range of holding potentials

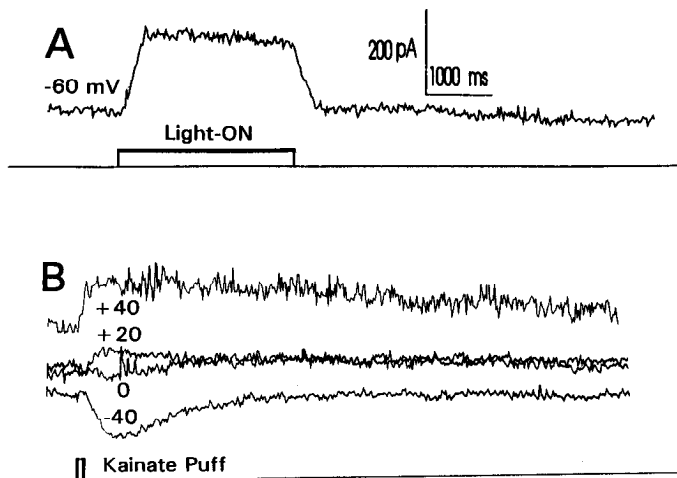


Figure 2. Light-driven synaptic currents and voltage-gated currents from an OFF-type bipolar neuron. *A*, An OFF-type bipolar cell was recorded using conventional whole-cell patch-clamp methods and the membrane was held at -60 mV. A step of increased full-field illumination (white light) was used to stimulate the photoreceptors, and the resulting current recorded in the bipolar cell. As previously reported for OFF-type bipolar cells, this cell responded with an outward current during the illumination increase. This sustained light response is a signature for the OFF bipolar cell, being unlike those of other “sustained cells” in the retina whose synaptic currents show relaxation during an equivalent light step. *B*, Kainate ($10 \mu\text{M}$)-gated current recorded from the same OFF bipolar cell in the slice as for *A*. This response had a reversal potential of $+10$ mV and is similar to that observed in the isolated OFF bipolar cells.

indicating that the dopamine receptors in these cells are not directly linked to an ion channel, in agreement with similar observations on fish horizontal cells (Knapp and Dowling, 1987). Dopamine also had no effect on the resistance of the cell membrane of isolated bipolar cells as measured by applying small (10 mV) hyperpolarizing or depolarizing steps to the isolated cells (not shown). These data serve as controls indicating that dopamine did not act to open a channel or change the membrane resistance of the bipolar cell to mediate the modulatory effects observed in bipolar cell glutamate-gated currents.

Glutamate (1 mM) or kainate ($10 \mu\text{M}$) puffed for 40 msec elicited currents of about 150 pA that were repeatable over a period of about 30 min if the puffs were delivered at interstimulus intervals of about 1 min or longer. Puffs of shorter interstimulus intervals caused the response to decrease in amplitude. Figure 3*A* shows responses for a bipolar cell in the slice, and Figure 3*B* shows responses for an isolated bipolar cell. We found that glutamate at 1 mM and kainate at $10 \mu\text{M}$ in the pipette elicited consistent responses of submaximal amplitude; 10 mM glutamate and $100 \mu\text{M}$ kainate elicited responses of larger amplitudes in the same neurons. Recent estimates suggest that glutamate reaches a maximal concentration of 1 mM in the synaptic cleft of neurons (Clements et al., 1992; Colquhoun et al., 1992). It is probable that the 1 mM glutamate in the pipette used in the present study is diluted before it reaches its target sites, and thus the concentration of glutamate that we presented exogenously was below the maximum physiological concentration.

To mimic the increased release of dopamine associated with increases in retinal illumination (Kramer, 1971; Witkovsky et al., 1993) we bath applied dopamine at a concentration of $20 \mu\text{M}$, the level at which cAMP in retinal horizontal cells has been

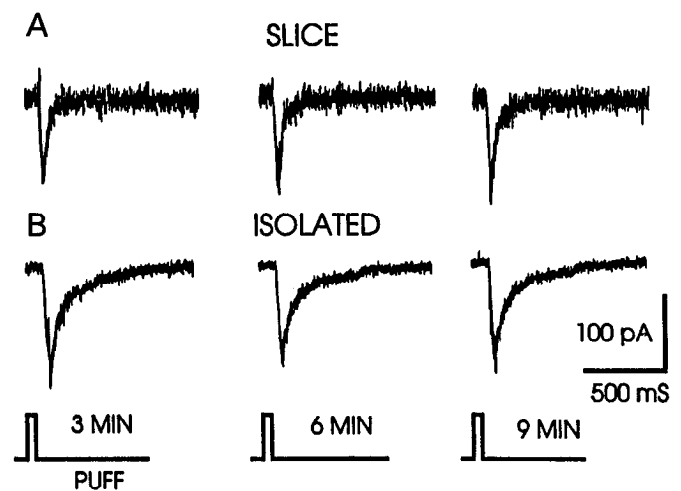


Figure 3. Glutamate-gated currents in OFF bipolar cells are stable over time. The pressure ejection (40 msec puff duration) of glutamate (1 mM) from a micropipette (pipette tip lumen was approximately $0.5 \mu\text{m}$) onto the dendrites of OFF bipolar cells either in the slice (*A*) 3 min, 6 min later, and then 9 min after obtaining the whole-cell configuration. Glutamate-gated currents from a bipolar cell in isolation (*B*) 3 min, 6 min, and 9 min following obtaining the whole-cell configuration. The currents were repeatable over many minutes (up to 30 min) if the interstimulus interval is about 1 min or longer. Shorter intervals between the puffs of glutamate resulted in nonrepeatable currents. The holding potential in each case was -60 mV, the equilibrium potential for any chloride-mediated conductances.

shown to be half-maximally activated (Van Buskirk and Dowling, 1981), although DeVries and Schwartz (1989) have shown that even lower concentrations are physiologically active. Dopamine caused an increase in inward currents elicited by glutamate puffs. Figure 4 shows that the bath application of $20 \mu\text{M}$ dopamine induced a twofold increase in the size of the glutamate-gated current over its control value (Fig. 4*A*) in an isolated OFF bipolar cell within 3 min of dopamine's application (Fig. 4*B*). The effect could be reversed by washing in normal bathing solution (Fig. 4*C*).

The enhancement was always larger in bipolar cells in isolation than in the slice possibly because of endogenous stores of dopamine in the slice preparation that had already enhanced the response. Consistent with this, the bath application of the D1 dopamine receptor blocker, SCH23390, in the slice led to a decrease in the amplitude of the glutamate-gated currents (42% decrease, $N = 5$). Figure 4*D* shows a control response to glutamate application, followed by the reduced response in the presence of bath-applied SCH23390 (Fig. 4*E*). The reduction in response could then be reversed by washing with normal bathing solution (Fig. 4*F*).

To determine whether the enhancing effects were specific to dopamine, similar measurements were made with $20 \mu\text{M}$ melatonin, another retinal amine. Melatonin caused no enhancement of the glutamate-gated current in isolated bipolar cells ($N = 3$), supporting the conclusion that the enhancement was due to an action of dopamine and not simply due to a perfusion artifact, and suggesting that melatonin, which often opposes the effects of dopamine (Dubokovich, 1988), was ineffective at this site.

Figure 5 shows the current–voltage relations, measured at the peak of the glutamate-gated currents, for both the control and dopamine enhanced conditions. The responses have a common

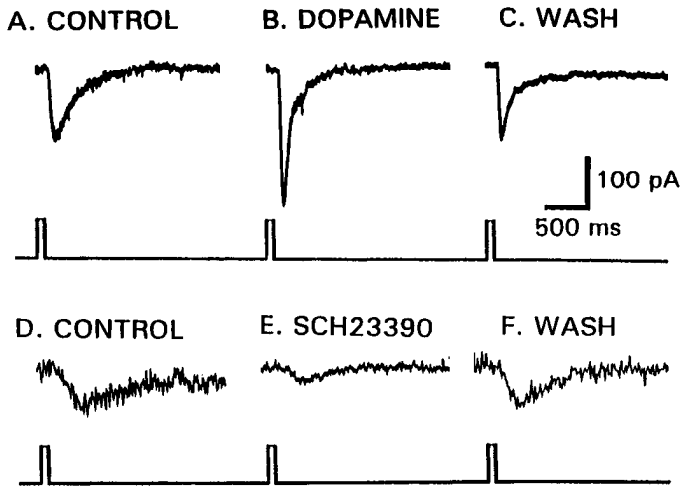


Figure 4. Dopamine upmodulates a ligand-gated current in the dendrites of OFF bipolar cells. *A*, Control response of isolated bipolar cell to a puff of 1 mM glutamate at its dendrites. *B*, Response to a similar glutamate puff, but in the presence of 20 μ M dopamine applied to the bathing medium. The peak response increased from 225 pA to more than 400 pA in the presence of dopamine. *C*, Response amplitude to a puff of glutamate is reduced to near control levels within about 3 min following the removal of dopamine from the bathing solution. *D*, Control response of bipolar cell in the retinal slice to a puff of 10 μ M kainate. *E*, SCH23390 was applied to the bath and the response elicited again, showing that SCH23390 was able to block endogenous dopamine and to depress the glutamate elicited current. *F*, Reversal of the effects of SCH23390 by changing the bathing solution back to normal. Holding potential in *A–F* was -60 mV.

reversal potential near $+10$ mV, suggesting that under both conditions glutamate application opens similar nonspecific cation channels. This suggests that dopamine acts to modulate the activity of the same glutamate receptors previously gated by glutamate in the absence of dopamine, and does not gate or modulate another channel in addition to the glutamate-gated channels. This, along with our findings of no changes in the input resistance of the isolated cells when exposed to bathed dopamine, suggests that the effects of dopamine are specific and modulate a single type of glutamate receptor/channel complex. This rules out the possibility that dopamine alters the membrane resistance at a site somewhere between the recording electrode and the glutamate receptors.

We used dopamine receptor antagonists to determine which dopamine receptor types were involved in modulating the glutamate-gated currents. Figure 6 shows that the increase in amplitude of the glutamate-gated current due to dopamine (20 μ M) was suppressed in the presence of SCH23390 (20 μ M; $N = 5$), a dopamine D1 receptor antagonist (Kebabian and Calne, 1979; Billard et al., 1984). However, there was no suppression of the dopamine-mediated enhancement of the glutamate-gated current in the presence of spiperone (20 μ M; $N = 4$), a D2 receptor antagonist (Arnett et al., 1985; Seeman and Grigoriadis, 1987). These results suggest that the dopamine enhancement of the glutamate-gated response is mediated by a D1, but not a D2 receptor.

Components of the intracellular pathway activated by dopamine

The identity of some components of the intracellular pathway mediating the dopamine modulated increase in glutamate-gated

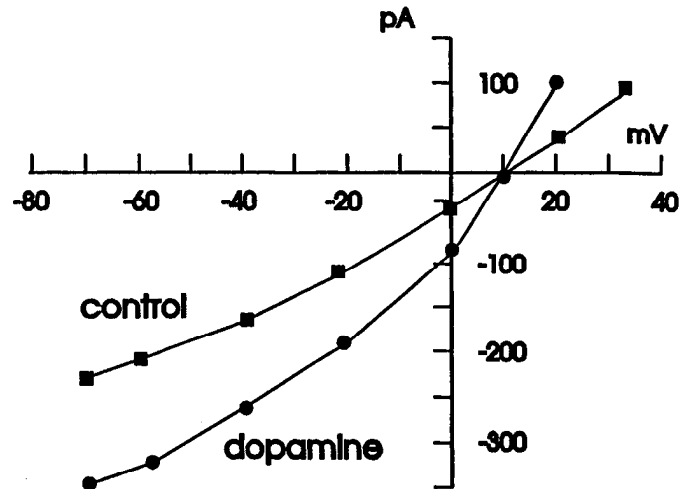


Figure 5. Current-voltage curves for the peak responses in the presence and absence of dopamine appear to have a common reversal potential near $+10$ mV, suggesting that dopamine does not activate a separate population of glutamate activated channels.

currents were inferred from the following measurements. Previous studies have shown that the D1 receptor fits into a superfamily of receptors that have seven transmembrane domains and are coupled to membrane-bound GTP-binding proteins (Deary et al., 1990). Therefore, we tested whether the effects of dopamine could be blocked when G-proteins in the recorded cell were inactivated. Figure 7*A* shows a control glutamate-gated current, and that dopamine (Fig. 7*B*) was ineffective in increasing the response to glutamate when the patch pipette contained GDP- β -S (500 μ M; $N = 5$), a GTP-binding protein inhibitor (Gilman, 1987; Kaziro et al., 1991). This suggests that a second messenger pathway initiated by a membrane-bound G-protein is normally involved in this modulation.

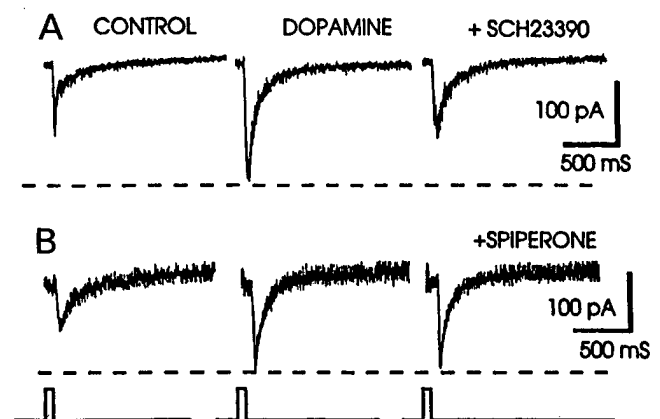


Figure 6. Dopamine acts at a D1 receptor to enhance a glutamate-gated current in OFF bipolar cells. *A*, Control response to a puff of glutamate to which the bath application of dopamine (20 μ M) increases the amplitude of the glutamate-gated current. The addition of the D1 receptor antagonist, SCH23390 (20 μ M), in combination with dopamine (20 μ M) blocks the enhancing action of dopamine, suggesting that a D1 receptor mediates the enhancing action on the glutamate channel. *B*, Control response to a puff of glutamate is enhanced by dopamine (20 μ M) added to the bath. The addition of spiperone (20 μ M), a D2 receptor antagonist, and dopamine (20 μ M) to the bath enhances the glutamate elicited response, suggesting that a D2 receptor is not involved in modulating the glutamate receptor.

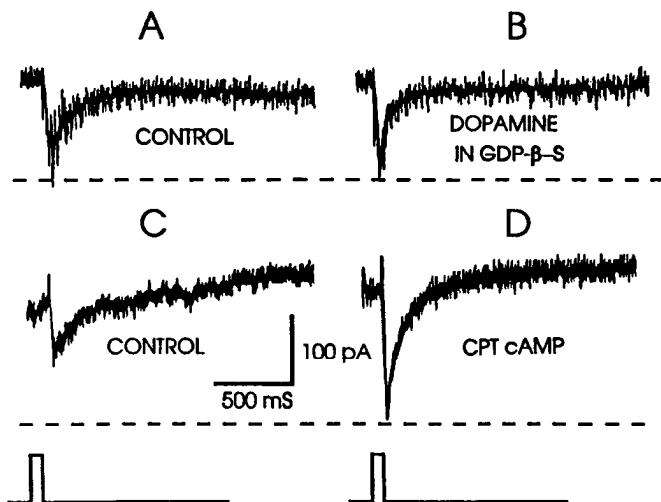


Figure 7. The D1 receptor appears to act via a G-protein, cAMP, and PKA. *A*, Control response, with GDP- β -S in the patch pipette, to a puff of glutamate. *B*, The enhancing effect of dopamine (20 μ M) applied to the bathing medium, is blocked by the inclusion of GDP- β -S in the recording pipette. *C*, Control response to a puff of glutamate. *D*, In the presence of the membrane-permeable cAMP analog CPT cAMP (300 μ M), the response to glutamate is more than doubled.

A primary function of these D1 receptors in many systems is to increase the activation of cAMP production when bound by dopamine (Brown and Makman, 1972; Kebejian et al., 1972). Therefore, we artificially raised the levels of intracellular cAMP to see if this would mimic the enhancing effects of dopamine on the glutamate activated currents. Figure 7*C* shows that the control response to glutamate increased from 50 pA to 90 pA by the inclusion of 300 μ M CPT cAMP (Fig. 7*D*; $N = 4$), a membrane-permeable analog of cAMP (Heuschneider and Schwartz, 1989), to the bathing medium. This result suggests a role for cAMP in the modulation of the glutamate receptor.

We tested whether a cAMP-dependent protein kinase A (PKA) was involved in the pathway between D1 receptor and glutamate receptor. Increased PKA activity was mimicked by increasing the intracellular levels of the PKA catalytic subunit. Figure 8*A* shows that the inclusion of the catalytic subunit of PKA (500 nM to 1 mM) in the patch pipette, allowing it to dialyze the recorded cell, also led to an increase in the glutamate elicited response ($N = 4$). In this measurement the amplitude of the current increased from 50 pA to about 200 pA over a period of 3 min. The time course of this effect probably reflects the time required for the catalytic subunit to diffuse from the patch pipette to the bipolar cell dendrites.

To better test whether an endogenous PKA was involved in this pathway, we used two agents known to block PKA activity, H-7 and PKI₂₀amide, to determine whether they would block dopamine's enhancement of the glutamate-gated current. Figure 8*B* shows that the inclusion of H-7 (300 μ M), a membrane-permeable PKA and PKC inhibitor (Malenka et al., 1989; Wang et al., 1991), in the bathing solution along with dopamine (20 μ M) appeared to suppress any upmodulation of the glutamate-gated current for as long as 9 min following the application of dopamine ($N = 4$). And finally, Figure 8*C* shows that PKI₂₀amide, a PKA-specific inhibitor (Greengard et al., 1991), that was included in the recording pipette to dialyze the recorded cell, suppressed the enhancing effects of 20 μ M bath-applied dopamine ($N = 5$). These data suggest that an endogenous PKA is a

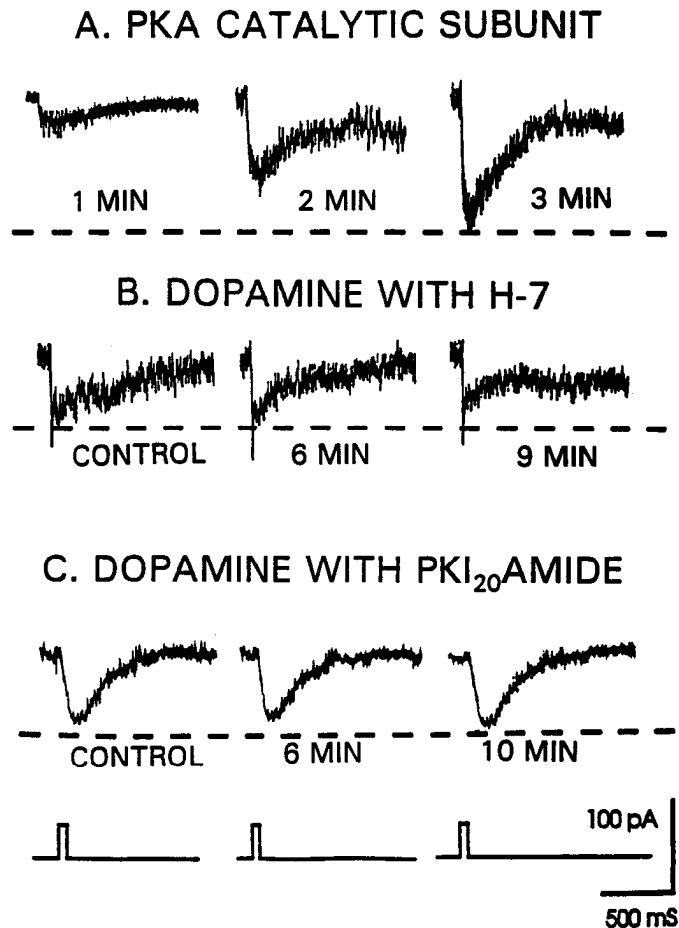


Figure 8. The PKA catalytic subunit enhances the glutamate elicited responses. *A*, Approximately 1 min after obtaining the whole-cell patch configuration a response to a puff of glutamate is recorded. This current gradually grows, and at 2 min after obtaining the whole-cell configuration the amplitude of the current has more than doubled in size, and at 3 min the current has more than tripled in size. The PKA inhibitor H-7 blocks the enhancing action of dopamine. *B*, Control response to a puff of glutamate is compared to a response obtained approximately 6 min after bathing the bipolar cell in a combination of dopamine (20 μ M) and H-7 (300 μ M) the amplitude of the response was unchanged, as was the case at 9 min later. This suggests that the inhibition of PKA was able to shunt the dopamine activated pathway affecting the glutamate channel. *C*, PKI₂₀amide (1 μ M), a PKA-specific inhibitor, was added to the recording pipette and blocked the enhancing effects of dopamine (20 μ M bath applied) on the glutamate-gated currents recorded in OFF bipolar cells.

necessary component in the dopamine regulation of the glutamate receptor. A summary of these data are presented in Table 1.

Discussion

In addition to dopamine's previously described actions in the vertebrate retina, here we have identified an additional mechanism through which dopamine can act, by upmodulating some of the glutamate receptors in OFF-type bipolar cells. Although D1 and D2 receptors are localized throughout the retina, including the outer plexiform layer where the bipolar cells receive their photoreceptor inputs (Wagner and Behrens, 1993; Wagner et al., 1993), our results suggest that dopamine acts at a D1 receptor in a classical manner to activate a stimulatory G-protein (Andersen et al., 1990) to mediate the enhancement of the

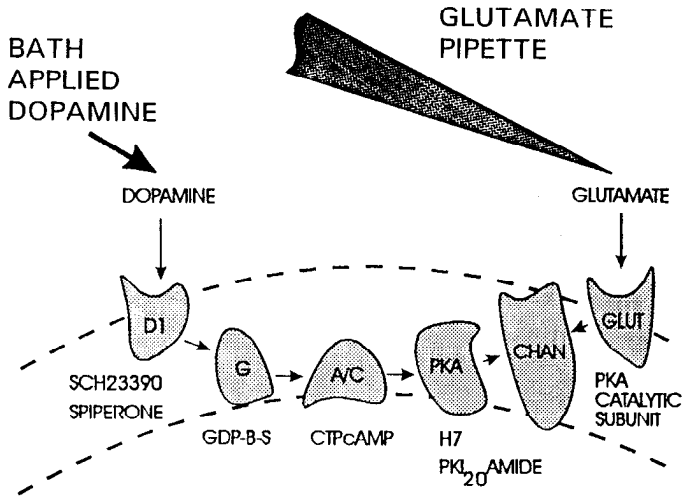


Figure 9. Model summarizing the components of an intracellular pathway in the dendrites of bipolar cells through which dopamine acts to modulate a glutamate receptor. (1) D1 dopamine receptor. Because SCH23390, a D1 antagonist, was able to block the enhancing actions of dopamine, while spiperone, a D2 antagonist, was ineffective in blocking dopamine's actions, it is probable that a D1 receptor, not a D2 receptor, mediates the effects of dopamine. (2) G-Protein. The inclusion of GDP- β -S, a G-protein inactivator, to the intracellular milieu blocked the actions of dopamine on the glutamate receptor, suggesting that dopamine acts through a G-protein. (3) Adenylate cyclase. Enhancement of the glutamate-gated current was observed following bath application of a cAMP analog, CPT cAMP. (4) Protein kinase A. Inclusion of the catalytic subunit of PKA in the intracellular milieu increased the amplitude of the glutamate-gated current, while bath application of H-7, a PKA inhibitor, blocked the enhancing actions of dopamine on the glutamate-gated current. These two experiments suggest that dopamine acts through PKA to upmodulate the glutamate-gated current. We do not yet know whether PKA acts directly on the glutamate receptor, or whether it acts via another intracellular protein such as DARPP or ARPP, or another kinase.

glutamate-gated current in OFF bipolar cells. Our results also suggest that the G-protein then activates adenyl cyclase to form cAMP, leading to the increased dissociation of the catalytic subunit of protein kinase A and the upmodulation of the cation current flowing through the glutamate-gated channels in the dendrites of OFF bipolar cells. This is similar to a mechanism observed in cultured fish horizontal cells (Knapp and Dowling, 1987). We have not attempted to determine whether the recently cloned D5 dopamine receptor, which exhibits a pharmacological and physiological profile similar to that of the D1 receptor, is involved in this pathway (Sunahara et al., 1991). Future molecular and pharmacological studies should be able to address this issue.

PKA could act directly on the glutamate-gated channel to phosphorylate it, or it could act to phosphorylate an intermediate protein, such as one of the DARPP (Girault et al., 1990) or ARPP proteins (Hemmings et al., 1986), which would then regulate the channel by inhibiting protein phosphatase-1 (Halpain et al., 1990). The actual phosphorylation of the glutamate receptor could then be accomplished by another kinase, such as protein kinase C or CaM-kinase. Although we have not studied how dopamine effects the glutamate-gated channel, others have shown in retinal horizontal cells that the frequency and time of channel openings are increased by dopamine while the number and unitary conductance of the channels are unaffected (Knapp et al., 1990).

Table 1. Effects of neuroactive drugs on glutamate-gated currents

Drug	Control response (pA)	Drug response (pA)	Number trials	Statistic
Dopamine	150 (20)	260 (35)	18	$p < 0.05$ <i>t</i> test
Dopamine + SCH23390	131 (22)	139 (27)	5	$p = 0.406$ Wilcoxon
SCH 23390	55 (10)	25 (10)	5	$p = 0.062$ Wilcoxon
Dopamine + spiperone	125 (20)	210 (25)	4	$p = 0.062$ Wilcoxon
Dopamine + GDP- β -S	140 (19)	145 (18)	5	$p = 0.312$ Wilcoxon
CPT cAMP	110 (10)	200 (20)	4	$p = 0.062$ Wilcoxon
PKA catalytic subunit	70 (15)	160 (10)	4	$p = 0.062$ Wilcoxon
Dopamine + H-7	120 (6)	130 (11)	4	$p = 0.219$ Wilcoxon
Dopamine + PKI ₂₀ amide	75 (20)	70 (15)	5	$p = 0.312$ Wilcoxon

SEMs are shown in parentheses. These data reflect experiments performed in the slice and in isolation: the dopamine experiments were performed on isolated cells and cells in the slice; the experiments with SCH23390 alone were done only in the slice; all other experiments were performed on isolated cells.

Glutamate receptor types

Molecular studies of glutamate receptors have thus far identified seven ionotropic glutamate receptor subunits in the mammalian retina based on *in situ* hybridization of mRNA made from cDNA of cloned receptor subunits from the rat brain (Hamasaki-Britto et al., 1993). Five of the subunits, excluding subunits GluR2 and GluR3, can possibly be assigned to the bipolar cells, with all five being possibilities for the OFF bipolar cells because none of the five is the clone for the APB receptor. The APB receptor is associated with the ON bipolar cell response (Slaughter and Miller, 1985) or a conductance thought to underlie the ON bipolar cell response (Hirano and MacCleish, 1991), and a recent molecular study suggests that it belongs to the superfamily of seven transmembrane spanning metabotropic receptors (designated mGluR6; Nakajima et al., 1993).

Although the known number of glutamate receptor subunits is growing, based on what we know at this time the OFF bipolar cells may contain natively expressed glutamate receptors consisting of some combination of the five subunits either through homologous or heterologous expression to form the multimeric glutamate receptor. The complexity of the ionotropic receptors is increased by mechanisms that alter the sequence of GluR2, -5, and -6 between the genomic and the cDNA forms (Sommer et al., 1991) and also by alternative splicing in the sequence between transmembrane domains III and IV, the two forms being designated flip and flop and conferring different functional properties on the two subunit forms (Sommer et al., 1990).

Dopamine-glutamate receptor interactions

However, with more studies describing the molecular and physiological properties of the glutamate receptors in bipolar cells, we shall have a clearer understanding of the pathway between dopamine receptor and glutamate receptor. For example, al-

ready we know that the GluR1–GluR4 subunits lack consensus phosphorylation sites for PKA, while GluR6 does contain such a site (Raymond et al., 1993). PKA acts at that site to increase the amplitude of the glutamate-gated currents from these GluR6 recombinants when transiently expressed in mammalian cells. Thus, if OFF bipolar cells are definitely found to express GluR6, then they are candidates for a pathway involving the direct phosphorylation of their glutamate receptors by PKA, leading to enhanced glutamate-gated currents, while those cells not expressing GluR6 would necessarily utilize a different intracellular pathway if they are to accomplish a similar enhancement of glutamate-gated currents by dopamine. Further, this sets the stage for the possibility of a single OFF bipolar cell to express combinations of glutamate receptors, with some glutamate receptors containing GluR6 and others not. Thus, even in the same cell, dopamine could phosphorylate, through PKA, some glutamate receptors and not others.

Dopamine's interaction with glutamate receptors in rod and cone pathways

Recent studies indicate a diversity of glutamate receptors in the outer retina (Nawy and Jahr, 1990; Shiells and Falk, 1990; Gilbertsen et al., 1991; Hirano and MacLeish, 1991; Hughes et al., 1992; Hamasaki-Britto et al., 1993), and suggest that separate populations of glutamate receptors on bipolar (Nawy and Copenhagen, 1987) and horizontal cells (Kim and Miller, 1992) may be differentially sensitive to rod and cone input. The studies of Dowling and coworkers (Yang et al., 1988) indicate that glutamate receptors on cone horizontal cells are upmodulated by dopamine, while those on rod horizontal cells are unaffected. The bipolar cells in this study probably receive both rod and cone inputs (Hare et al., 1986), but dopamine may regulate only those receptors associated with cones.

Preliminary studies in ON bipolar cells indicate that dopamine differentially modulates two different types of glutamate receptors, upmodulating a glutamate-gated current that reverses at -70 mV while having no effect on a glutamate-gated current that reverses at 0 mV (Maguire and Werblin, 1991). Whether different glutamate receptor subtypes in bipolar cells can be associated with rod and cone inputs, and whether dopamine upmodulates the cone associated glutamate receptor and not the rod associated glutamate receptor as it does in horizontal cells remains to be demonstrated. It will also be of interest to determine whether the different glutamate receptor types associated with rod and cone synaptic inputs to bipolar and horizontal cells are composed of subunits that contain or lack the consensus phosphorylation sites for PKA or other kinases, and whether this is a mechanism for dopamine's differential regulation of rod and cone inputs to the second-order neurons of the vertebrate retina.

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