Dopamine Modulates in a Differential Fashion T- and L-Type Calcium Currents in Bass Retinal Horizontal Cells

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ABSTRACT White bass (*Roccus chrysops*) retinal horizontal cells possess two types of voltage-activated calcium currents which have recently been characterized with regard to their voltage dependence and pharmacology (Sullivan, J., and E. M. Lasater. 1992. *Journal of General Physiology.* 99:85–107). A low voltage–activated transient current was identified which resembles the T-type calcium current described in a number of other preparations, along with a sustained high threshold, long-lasting calcium current that resembles the L-type calcium current. Here we report on the modulation of horizontal cell calcium channels by dopamine. Under whole-cell voltage clamp conditions favoring the expression of both calcium currents, dopamine had opposing actions on the two types of voltage-sensitive calcium currents in the same cone-type horizontal cell. The L-type calcium current was significantly potentiated by dopamine while the T-type current was simultaneously reduced. Dopamine had no effect on calcium currents in rod-type horizontal cells. Both of dopamine's actions were mimicked with the D1 receptor agonist, SKF 38393, and blocked by application of the D1 specific antagonist, SCH 23390. Dopamine's actions on the two types of calcium currents in white bass horizontal cells are mimicked by the cell membrane–permeant cyclic AMP derivative, 8-(4-chlorophenylthio)-cyclic AMP, suggesting that dopamine's action is linked to a cAMP-mediated second messenger system. Furthermore, the inhibitor of cAMP-dependent protein kinase blocked both of dopamine's actions on the voltage-dependent calcium channels when introduced through the patch pipette. This indicates that protein phosphorylation is involved in modulating horizontal cell calcium channels by dopamine. Taken together, these results show that dopamine has differential effects on the voltage-dependent calcium currents in retinal horizontal cells. The modulation of these currents may play a role in shaping the response properties of horizontal cells.

INTRODUCTION

The vertebrate retina is a complex neuronal circuit which processes the visual signal for interpretation by the brain. Retinal horizontal cells are second-order neurons that

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receive input from photoreceptors (Trifonov, 1968; Werblin and Dowling, 1969). In the outer retina of teleost fish, horizontal cells form an electrically coupled syncytium, which provides the antagonistic surround for bipolar cell responses (Werblin and Dowling, 1969; Kameko, 1970; Sakuranaga and Naka, 1985). Widespread gap junction contacts between like horizontal cells account for the large receptive fields seen in these cells when the retina is probed with light stimuli (Yamada and Ishikawa, 1966; Kaneko, 1971). Electrical coupling in these horizontal cells is modulated by dopamine (Lasater and Dowling, 1985; DeVries and Schwartz, 1989), which is released from interplexiform cells that extend processes in a centrifugal fashion from the inner nuclear layer of the retina to the synapse on the three subtypes of cone horizontal cells (H1, H2, H3) in the outer plexiform layer (Dowling and Ehinger, 1975; Zucker and Dowling, 1987). Dopaminergic interplexiform cells do not make synaptic contact on a fourth class of horizontal cells which receive rod photoreceptor input exclusively (H4) (Dowling and Ehinger, 1978). In the cone horizontal cells, dopamine has also been shown to enhance ionic conductances gated by L-glutamate (Knapp and Dowling, 1987), the probable photoreceptor neurotransmitter (Lasater and Dowling, 1982; Ishida, Kaneko, and Tachibana, 1984; Ishida and Neyton, 1985), by altering the kinetics of channel opening (Knapp, Schmidt, and Dowling, 1990). Thus dopamine is an important retinal neuromodulator with widespread influence.

Both of these actions of dopamine on teleost horizontal cells are mediated by D1 receptors via activation of the second messenger cAMP and subsequent protein phosphorylation (O'Connor, Kropf, and Dowling, 1989). Previous studies have demonstrated that activation of second messenger systems can result in direct or indirect modulation of voltage-gated channels and is a major mechanism for the modulation of neuronal ion channels (Kaczmarek and Levitan, 1987). In this study, we have examined the effects of exogenous dopamine on voltage-activated calcium currents recorded from cultured white bass cone horizontal cells. Changes in intracellular calcium have been shown to regulate many cellular processes such as neuronal plasticity, excitotoxicity, and neurotransmitter release. Calcium ions can also act as second messengers themselves to regulate the activity of other ion channels, as during the generation of a burst of action potentials in molluscan neurons (Eckert and Chad, 1984). An elevated cytoplasmic free calcium level has also been suggested to act as a signal for the induction of long-term potentiation (Nicoll, Kauer, and Malenka, 1988). In teleost cone horizontal cells, increases in intracellular calcium may be important in retinal visual processing through regulation of the dark membrane potential and/or regulation of ion channels (Sullivan and Lasater, 1990). Little is known of the regulation of retinal calcium channels. Thus, we have studied the modulation of the voltage-sensitive calcium currents in the white bass cone horizontal cells in an effort to gain further insight into the functional role of calcium in these cells and how calcium entry into the cell is controlled.

White bass horizontal cells possess two voltage-sensitive calcium currents which have been previously characterized with regard to their voltage dependence and pharmacology (Sullivan and Lasater, 1992). One of these calcium currents resembles the T current, which has been described in other preparations (Nilius, Hess, Lansman, and Tsien, 1985; Nowycky, Fox, and Tsien, 1985; Fox, Nowycky, and Tsien, 1987). This T current activates at around −50 mV and peaks at a membrane

potential of about −20 mV, the time constant for inactivation is 15–35 ms, inactivation is voltage dependent, and the current is not carried well by barium. The other calcium current in white bass horizontal cells is similar to the sustained voltage-dependent calcium current (L-type) found in other preparations (Nowycky et al., 1985; Ozawa, Tsuzuki, Iiono, Ogura, and Kudo, 1989). Barium permeates this channel well, it is dihydropyridine sensitive, the current activates at about −30 mV, it peaks at a membrane potential of around +10 mV, and little or no inactivation occurs when the cell's membrane potential is stepped from −70 to 0 mV. This current also bears a resemblance to P-type currents (Llinas, Sugimori, Lin, and Cherskey, 1989) in that it is blocked by the funnel web spider toxin, FTX (Sullivan and Lasater, 1992).

When dopamine is exogenously applied to horizontal cells in conditions favoring the expression of both calcium currents (Sullivan and Lasater, 1992), we found that the transient current decreased and the sustained current simultaneously increased in the same neuron. The results presented in this study show that dopamine can regulate the entry of calcium ions into horizontal cells by acting on at least two different populations of calcium channels, and the modulation of these calcium channels occurs by activation of the cAMP second messenger system.

METHODS AND PROCEDURES

Isolation Procedures

Isolation procedures for obtaining single cells have been described in previous publications (Dowling, Pak, and Lasater, 1985; Lasater, 1986). Briefly, after dark-adapting the white bass (*Roccus chrysops*) for 2 h, the animal was enucleated and the retina was removed from the eyecup in dim red light and incubated in 10 ml of modified Leibowitz L-15 culture medium containing 1.7 mg/ml papain under sterile conditions for 40 min. After incubation, the retina was rinsed in fresh L-15. Further washing of the retina was achieved by triturating retinal pieces in a 10-ml sterile pipette and then expelling them into a fresh dish of L-15. This trituration process was repeated eight times and served to remove a majority of the photoreceptors. Retinal tissue pieces were then placed in a new culture dish containing fresh L-15 and subjected to a more vigorous agitation by triturating with a sterile Pasteur pipette. This process was repeated until the retina was completely broken up. Penicillin-streptomycin solution (GIBCO BRL, Gaithersburg, MD) was added to each culture dish (Dowling et al., 1985) and the dishes were placed in an incubator (15°C) until used. Cells were maintained in culture for 1–10 d. Old L-15 was exchanged for fresh L-15 every 3–4 d. Most experiments were performed on cells that had been maintained in culture 1–5 d.

Experimental Solutions

At the beginning of each experiment, culture medium was exchanged for bass saline consisting of 130 mM NaCl, 10 mM $CaCl_2$ or $BaCl_2$ (to enhance the calcium currents), 2.8 mM KCl, 1 mM $MgCl_2$, 10 mM glucose, 8.4 mM HEPES, 20 μM tetrodotoxin (to block the inward sodium currents), and 10 mM 4-aminopyridine (to block outward potassium currents). The saline was PH adjusted to 7.4 with NaOH.

Test agents such as dopamine, 8-(4-chlorophenylthio) adenosine 3′:5′-cyclic monophosphate (Sigma Chemical Co., St. Louis, MO), SKF 38393, SCH 23390, and quinpirole (Research

Biochemicals Inc., Natick, MA) were added to bass saline solution and pressure ejected onto selected horizontal cells from a separate blunt-tipped micropipette positioned very near the cell (Lasater, 1987). Dopamine solutions were made up fresh every 2–4 h or ascorbic acid was added to the dopamine solution to slow oxidation. Results obtained from the two procedures were identical.

The patch pipette solution consisted of either 130 mM K^+ gluconate (with 4 mM NaCl, 11 mM EGTA, 1 mM $MgCl_2$, 8.4 mM HEPES, 1 mM $CaCl_2$, 20 mM TEA, and 1 mM ATP) or 120 mM CsCl (with 11 mM EGTA, 2 mM $MgCl_2$, 10 mM HEPES, 1 mM $CaCl_2$, 20 mM TEA, and 1 mM ATP). Neither of these pipette solutions completely suppressed potassium currents evoked by large depolarizations. However, the CsCl pipette solution was much more effective than the K^+ gluconate solution at blocking outward potassium currents in the horizontal cells after 5 min. Even so, results obtained in the experiments using the CsCl pipette solution were identical to the results obtained when K^+ gluconate was used, suggesting that potassium currents are not interfering with our observation of dopamine's effect on calcium currents. The free calcium concentration of these solutions was calculated to be 4.2 nM. In one set of experiments (see below), 20 μM protein kinase inhibitor (Sigma Chemical Co.) was added to the patch pipette solution and allowed to diffuse into the cell for 5 min after seal formation and membrane rupture. For some experiments, the perforated-patch technique was used. Nystatin (Sigma Chemical Co.), a pore-forming antibiotic, was added to the patch pipette solution and allowed to perforate the horizontal cell's membrane (3–5 min) after obtaining a seal (Horn and Marty, 1988). This approach prevents the loss of cytoplasmic components that are needed for preservation of calcium channel activity. In some cell types, a shift in the current–voltage characteristics of calcium currents is seen to occur as the cytoplasmic contents are exchanged with the electrode solution in whole-cell experiments (Fenwick, Marty, and Neher, 1982). The use of the nystatin technique avoids this. We found the current–voltage characteristics obtained from whole-cell recordings to be identical to those obtained with perforated patches. Thus, no shift in operating range occurred as a result of internal perfusion of our cells.

Recording Procedures

Patch pipettes were pulled from borosilicate glass using a vertical microelectrode puller (Narishige Instruments, Tokyo, Japan) and were used uncoated and unpolished. The electrode tip resistance used in these experiments was between 4 and 8 MΩ when measured in Ringer's bath solution. Cells in culture were easily identified based on their characteristic morphology (Dowling et al., 1985). Individual cells were voltage clamped using the whole-cell patch clamp technique (Hamill, Marty, Neher, Sakmann, and Sigworth, 1981) except in those instances when the nystatin patch technique was used (Horn and Marty, 1988). Recording was carried out using an Axopatch 1B amplifier (Axon Instruments, Inc., Foster City, CA). Series resistance and capacitive artifacts were compensated for using the amplifier controls. Series resistance could be adjusted to 90% or better and thus little voltage error occurred even for large command voltages. Leakage currents were not corrected for in these studies since data were only obtained from cells with relatively small leakage currents and care was taken to reject data from cells in which the leak changed by more than a few millivolts over the course of an experiment.

Data collection was controlled by an NEC personal computer in conjunction with a LabMaster data acquisition board. The data were low-pass filtered with a Bessel filter at 2 kHz (−3 dB) before being sampled. All data analysis was done using the pClamp suite of programs (Axon Instruments, Inc.).

RESULTS

Effect of Dopamine on Calcium Currents

As already mentioned, bass retinal horizontal cells possess two types of voltage-activated calcium currents. Fig. 1 reiterates this point and illustrates the effect of dopamine on each of these currents. In conditions favoring calcium current expression, an H2 horizontal cell's membrane potential was changed in a rampwise fashion from −90 to +90 mV. In this example, a large transient calcium current that peaked at −20 mV was present, while the sustained calcium current peaked at a more depolarized potential of +10 mV. Fig. 1 *A* illustrates the calcium currents in the

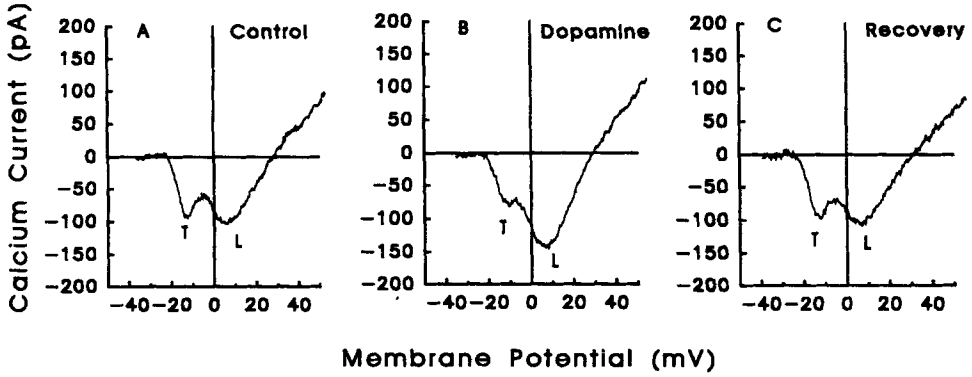

FIGURE 1. Effect of dopamine on horizontal cell transient and sustained voltage-sensitive calcium currents. In this figure the membrane potential of an H2 cone horizontal cell was shifted in a rampwise fashion from −90 to +90 mV over 500 ms (Sullivan and Lasater, 1992) and clearly showed both voltage-sensitive calcium currents. Sodium and potassium currents have been pharmacologically suppressed (see Methods and Procedures). Only the portion of the current trace from −50 to +50 mV is illustrated. In *A*, the control transient (*T*) and sustained (*L*) currents are illustrated. The effect of 7 μM dopamine on the magnitude of these two calcium currents 3 min after application is shown in *B*. The transient current was dramatically reduced while the sustained current was simultaneously augmented. Recovery of dopamine's effect is illustrated in *C* 15 min after dopamine application.

absence of dopamine. Fig. 1 *B* demonstrates the effect of dopamine application on the two calcium currents. Surprisingly, dopamine had an opposing action on the two types of voltage-dependent calcium currents in the same neuron. When 7 μM dopamine was pressure ejected onto this H2 horizontal cell, the transient current decreased by 38%, whereas the sustained L-type current simultaneously increased by 35%. Similar results were obtained from all cone horizontal cells examined (n = 17; Table I). Peak effects were reached over a period of ~3 min. To ensure that the reduction of the transient current was not due to depletion of cytoplasmic components necessary for calcium current activity, separate experiments were performed on cone horizontal cells using the nystatin patch technique (see Methods and Proce-

TABLE I

Effect of Dopamine and Dopamine-related Agents on Voltage-dependent Calcium Currents in White Bass Cultured Horizontal Cells under Various Conditions

	Mean percent decrease in T current	Mean percent increase in L current
DA	56 (± 16; n = 17)	95 (± 13; n = 32)
SKF	35 (± 15; n = 3)	105 (± 37; n = 8)
SCH and DA	6 (± 2; n = 4)	12 (± 13; n = 9)
8-CPT	38 (± 12; n = 6)	135 (± 53; n = 11)
PKI and DA	No significant change (n = 5)	

Results presented here were obtained when cone horizontal cells were held at a membrane potential of −90 mV and stepped to −20 mV to activate the T current or to +10 mV to activate the L current. Similar results were obtained when the cell's membrane potential was changed in a rampwise manner from −90 to +90 mV over 0.5 s. Values are expressed as the mean percentage change from control transient and sustained currents. The standard deviations and number of cells the various agents were tested on are indicated to the right. Note: not all cone horizontal cells demonstrated a measurable transient current. On the other hand, all cone horizontal cells demonstrated a sustained calcium current when depolarized to within the sustained current's activation range. The absence of the T current in some cells is puzzling, but may be due to such things as the isolation procedure. DA, dopamine; SKF, SKF 38393; SCH, SCH 23390; 8-CPT, 8-(4-chlorophenylthio)adenosine 3′:5′ cyclic monophosphate; PKI, protein kinase inhibitor.

dures). The results of these experiments did not differ from experiments performed using the whole-cell patch technique. In addition, in those instances where we were able to hold the cell for a sufficient period of time (> 10 min), recovery was observed (Fig. 1 *C*). Thus, the effects we saw were due to the application of dopamine to the cells.

In an effort to determine the concentration range over which dopamine was active, a dose–response curve was generated by measuring the amount of enhancement of the sustained current at different concentrations of dopamine. Fig. 2 shows the

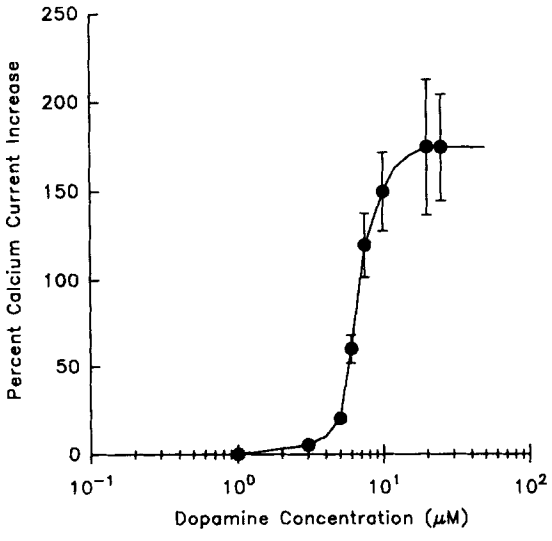

FIGURE 2. Dose–response curve obtained from isolated cone horizontal cells. Various concentrations of dopamine were pressure ejected onto cells. After a waiting period, the cells were stepped to +10 mV from a membrane potential of −90 mV to activate the L-type sustained calcium current. Data points represent the average percentage of current increase 3 min after dopamine application. Points were curve-fit using the Hill equation. Data points represent the means from 5–10 cells and vertical bars represent standard deviations from the mean.

dose–response curve generated when various concentrations of dopamine were pressure ejected onto H2 cone horizontal cells voltage clamped at a holding potential of −70 mV and stepped to a membrane potential of +10 mV, which favored sustained calcium current expression. Dopamine, at concentrations as low as 2 μM, potentiated the sustained calcium current. The data points were curve-fit using the Hill equation:

$$[Ca^{+2}\text{ current increase}] = ([Ca^{+2}\text{ current increase}]_{max} \times ([A]^n/EC_{50}^n + A^n))$$

where A is the dopamine concentration applied, EC_{50} is the concentration of dopamine producing a half-maximal response, and n is the Hill coefficient. From this curve, the EC_{50} was calculated to be 7 μM, the Hill coefficient and slope of the curve

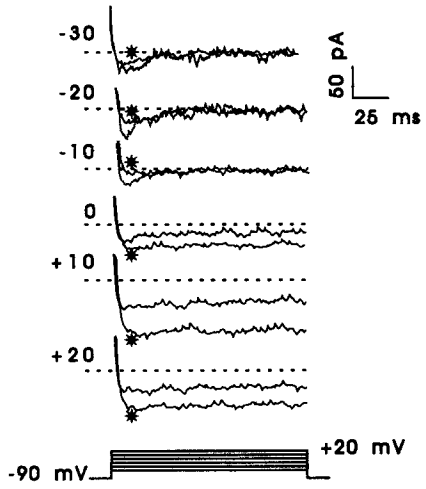

FIGURE 3. Effect of dopamine on voltage-sensitive calcium currents on an H3 horizontal cell voltage clamped at a membrane potential of −90 mV and stepped to various depolarized membrane potentials. The transient calcium current was apparent when the H3 cell was stepped to −30, −20, and −10 mV, whereas the sustained current was elicited at step commands to 0, +10, and +20 mV. Two traces have been superimposed at each command potential. The unmarked current trace illustrates the control current evoked when the cell was stepped to the membrane potential value, listed at the left of the figure, from the holding potential. An asterisk marks the current traces obtained after exposing the cell to 7 μM dopamine. Note that dopamine reduces the transient calcium current and increases the sustained calcium current. The voltage command regime is shown below the current traces in this and all similar figures that follow. Capacitative artifacts have been removed from the current traces for clarity.

were 6 and the maximum percentage change in sustained calcium current was an increase of 175%. We should point out that the concentration plotted on the abscissa is the concentration of dopamine in the application pipette. The concentration reaching the cell will be less than this due to dilution effects (Lasater and Dowling, 1982). Thus, the curve of Fig. 2 may actually be shifted somewhat to the left.

The steep slope of this curve suggests subsequent amplification of the signal, which leads to an increase in the calcium current after dopamine binding to membrane receptors. Signal amplification after agonist binding may result from activation of a second messenger system, which can directly or indirectly influence current permeation through voltage-sensitive calcium channels (see below).

A more detailed view of the action of dopamine is shown in Fig. 3, where the membrane potential of an H3 horizontal cell was voltage clamped at a holding

potential of −90 mV and stepped to various depolarized potentials. Each step illustrated contains two traces: a control trace and a trace obtained 2 min after dopamine had been applied. The transient current was apparent when the membrane potential was stepped to −30, −20, and −10 mV from the holding potential. Dopamine decreased this transient calcium current by ~50% when the cell was stepped to −30 and −20 mV, and almost eliminated it at −10 mV. The sustained current was significantly enhanced by dopamine, as seen at the membrane potential values of 0, +10, and +20 mV. Similar results were obtained from all cone horizontal cells examined (n = 17; Table I).

The differential modulation by dopamine of the two voltage-sensitive calcium channels in cone horizontal cells did not occur for rod-dominated horizontal cells (H4), which lack dopaminergic innervation from interplexiform cells (Fig. 4). The lack of effect of dopamine on a rod horizontal cell is shown in Fig. 4. In Fig. 4 *A*, two current traces are present which represent an H4 neuron voltage clamped at a holding potential of −90 mV and stepped to −20 mV before and after dopamine was applied. At a membrane potential of −20 mV, only the transient current is activated.

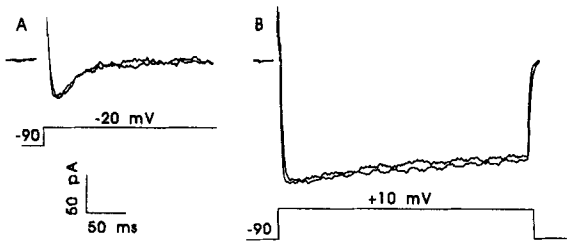

FIGURE 4. Dopamine has no effect on rod-dominated horizontal cells. In this example, an H4 horizontal cell was voltage clamped at −90 mV and stepped to −20 mV in *A* to demonstrate the transient calcium current and to +10 mV in *B* to illustrate the sustained current. In each example two current traces have been superimposed on each other. One current trace represents the control calcium current elicited by the command potential. The second trace represents the current response after 7 μM dopamine was pressure ejected onto the cell. Dopamine had no significant effect on either the transient or the sustained calcium current.

In Fig. 4 *B*, the same H4 horizontal cell was held at a membrane potential of −90 mV and stepped to +10 mV, which fully activated the sustained L-type calcium current. The two current traces in Fig. 4, *A* and *B*, are very similar, demonstrating that dopamine had no effect on either current when compared with the control trace. In four other H4 horizontal cells, 7 μM dopamine elicited no significant change in either the transient current or the sustained current.

Time Course of Dopamine's Action

Dopamine's effect on the sustained L-type current peaked at 2–3 min in all cells tested. The action of dopamine on the transient calcium current was more variable. In one-third of cone horizontal cells tested, dopamine's reduction of the transient current peaked sooner at 1 min, while the effect on sustained currents didn't peak until 2 or 3 min. Recovery of the sustained calcium current occurred in all cells that were held for 15–20 min. However, transient current reduction due to dopamine only partially recovered in 25% of cells that were held for an extended period of time.

Dopamine's Action Is via the D1 Receptor

Since dopamine's effects on horizontal cell electrical coupling and kainate-induced currents (Knapp and Dowling, 1987) are mediated via a D1 receptor mechanism, it seemed plausible that a D1 mechanism mediated the alterations we observed in the calcium currents. To determine if dopamine's action was carried through the D1 type of dopamine receptor, the specific D1 agonist, SKF 38393 (1 μM), was pressure ejected onto H2 horizontal cells. In Fig. 5 *A*, the effect of SKF 38393 on the T-type transient calcium current of an H2 cell is shown. Similar to dopamine, SKF 38393 reduced the transient current by 40% within 2 min. Fig. 5 *B* demonstrates the effect of SKF 38393 on the sustained current in the same cell. 3 min after the agonist was pressure ejected onto the H2 neuron, the sustained current increased by 100%. Thus, SKF 38393 mimicked the opposing action of dopamine on the two types of calcium currents. SKF 38393 had similar effects on calcium currents in seven other cone horizontal cells (See Table I).

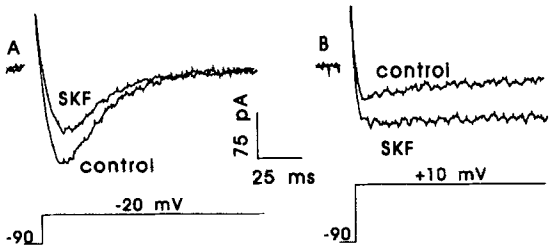

FIGURE 5. Effect of a D1 receptor agonist on the transient and sustained calcium currents. In *A*, an H2 horizontal cell was voltage clamped at −90 mV and the membrane potential stepped to a value of −20 mV to evoke the transient calcium current. The bottom current trace represents the control response to the step command potential. The D1 agonist, SKF 38393, reduced this current response after it was pressure ejected onto the cell. In *B*, the same H2 horizontal cell was stepped to +10 mV from a holding potential of −90 mV to elicit the sustained calcium current. In this example, the top current trace illustrates the control current response to the command potential, whereas the bottom trace illustrates the large potentiating effect of SKF 38393 on the sustained calcium current.

If dopamine is working through D1 receptors, as the above data suggest, then a D1 antagonist should block or attenuate the action of dopamine. We tested this idea using SCH 23390, and Fig. 6 illustrates the effect of the D1 specific antagonist on the two inward calcium currents present in an H3 horizontal cell. The left trace shows the resultant current recorded when the membrane potential was moved in a rampwise fashion from −90 to +90 mV. Both transient and sustained calcium currents are present in this control trace. The right current trace is from the same cell 3 min after a mixture of 7 μM dopamine and 10 μM SCH 23390 was applied to the H3 neuron. The SCH 23390 completely blocked dopamine's typical effect on the two types of calcium current. Neither the transient current nor the sustained calcium current was affected by dopamine when SCH 23390 was allowed to bind to D1 receptors alone. SCH 23390 virtually eliminated dopamine's effect on voltage-activated calcium currents in eight other cone horizontal cells (Table I). These results, in combination with the previous data, indicate that dopamine's modulation of both the transient

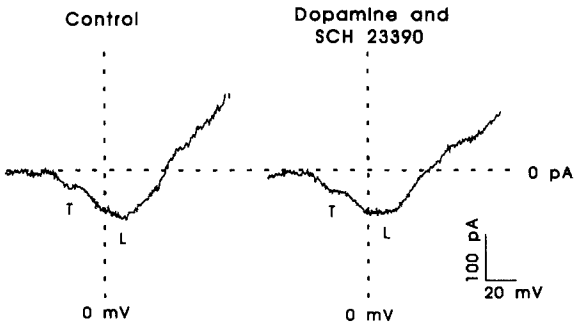

FIGURE 6. Effect of a D1 receptor antagonist on transient and sustained calcium currents. The left control current–voltage trace was generated from an H3 horizontal cell whose membrane potential was changed in a rampwise fashion from −90 to +90 mV over 500 ms. Only the membrane potential range from −50 to +50 mV is illustrated. Both transient (*T*) and sustained (*L*) currents are labeled. The right current–voltage trace was generated from the same cell 3 min after a mixture of dopamine (10 μM) and SCH 23390 (10 μM) was pressure ejected onto the cell. The SCH 23390 completely blocked both actions of dopamine.

and the sustained calcium currents is specific and operates through a D1 receptor–mediated mechanism.

D1 Receptor Activation Is Linked to a cAMP Second Messenger System

Previous studies have demonstrated that dopamine reduces gap junction permeability between teleost horizontal cells by activation of the cAMP second messenger system after D1 receptor binding (Lasater and Dowling, 1985; DeVries and Schwartz, 1989). To determine if dopamine's effect on voltage-dependent calcium channels in

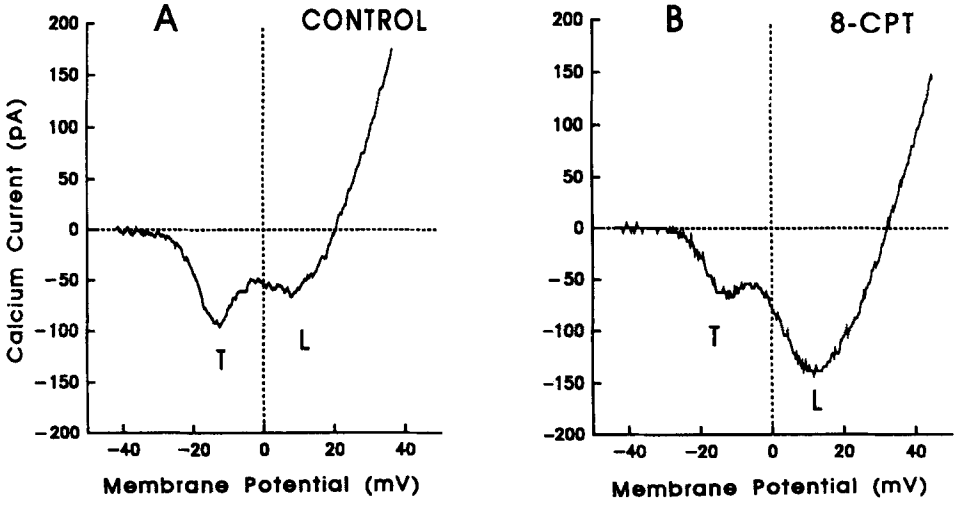

FIGURE 7. Effect of a cAMP derivative on the sustained calcium current. *A* represents a control current–voltage trace generated when an H2's membrane potential was changed from −90 to +90 mV over 500 ms in a rampwise fashion. Transient (*T*) and sustained (*L*) calcium currents are labeled. *B* illustrates the effect of 50 μM 8-CPT on both types of calcium currents 3 min after application. 8-CPT decreased the transient current while simultaneously increasing the sustained current. Only the portion of the current trace from −50 to +50 mV is illustrated.

white bass horizontal cells is mediated by cAMP, the membrane-permeable cAMP derivative 8-(4-chlorophenylthio)-cyclic AMP (8-CPT) was pressure ejected onto cone horizontal cells (n = 11; Table I). In Fig. 7, the effect of 8-CPT (50 μM) on the two types of voltage-activated calcium currents recorded in an H2 horizontal cell is shown. In this example, the H2 neuron's membrane potential was changed in a rampwise fashion from −90 to +90 mV. 8-CPT decreased the magnitude of the

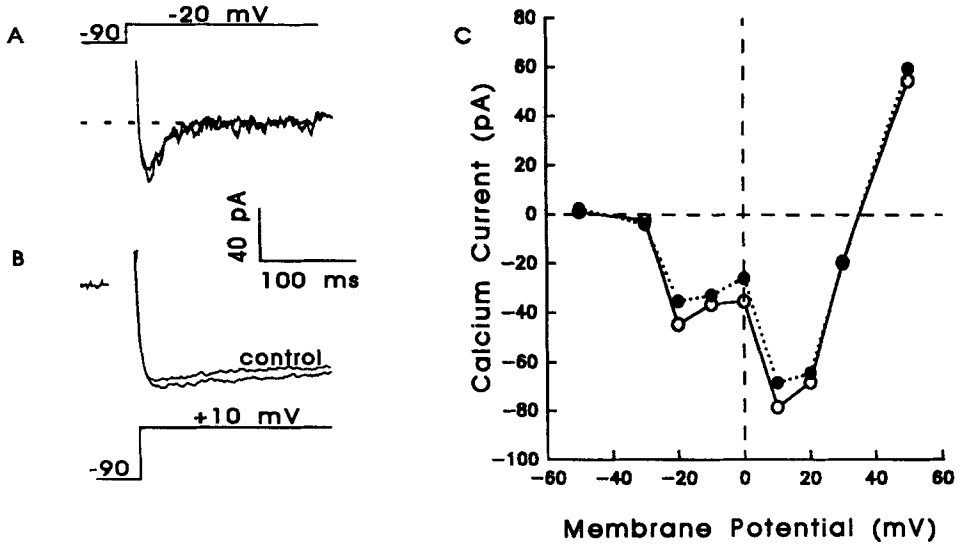

FIGURE 8. Effect of PKI on the voltage-sensitive calcium currents. In this example, 20 μM of the peptide PKI was allowed to diffuse into an H3 cell for 5 min before initiating experiments. In *A*, the horizontal cell was stepped from a holding potential of −90 mV to −20 mV to obtain the transient current. The two current traces represent a control response to the command potential and the current response 3 min after 7 μM dopamine application. With PKI in the cell, dopamine failed to elicit a significant change in the magnitude of the transient current. In *B*, the same cell was stepped to a membrane potential of +10 mV from the holding potential to activate the sustained calcium current. The two current traces represent a control current response to the step command and the current response after 7 μM dopamine was applied. Dopamine increased the sustained calcium current by only 5%. *C* illustrates the current–voltage relationship obtained for the same cell when it was stepped to various membrane potentials from the holding potential. The filled data points represent the control current–voltage curve. The open circles represent the effect of dopamine at each membrane potential with PKI in the cell. PKI blocked dopamine's effect on calcium currents at each command potential. Why the trace obtained after dopamine application is slightly larger overall from the control trace is not known. A reduction in leakage current or some similar phenomenon could account for this.

transient current by 30% from control and increased the magnitude of the sustained calcium current by 133% from control. This result suggests that dopamine's action on sustained calcium currents in cone horizontal cells is linked to an increase in intracellular cAMP.

The above results raise the question of whether or not cAMP activates a cAMP-dependent protein kinase to alter calcium currents (Kaczmarek and Levitan, 1987).

The effects of dopamine on horizontal cell electrical coupling (Lasater and Dowling, 1985; DeVries and Schwartz, 1989) and on excitatory amino acid–activated currents (Knapp and Dowling, 1987; Knapp et al., 1990) appear to be due to a cAMP-dependent mechanism involving a protein kinase (Lasater, 1987; O'Connor et al., 1989; DeVries and Schwartz, 1992); cAMP analogues and internal perfusion of cells with the catalytic subunit of protein kinase A all mimic the effect of dopamine. To determine if a cAMP-dependent protein kinase is involved in dopamine's effect on voltage-dependent calcium currents, the peptide protein kinase inhibitor (PKI) was introduced into cone horizontal cells to block the enzymatic activity of protein kinase A.

Fig. 8 demonstrates that PKI can eliminate dopamine's dual effects on voltage-sensitive calcium currents in white bass horizontal cells. In this example, an H3 horizontal cell was voltage clamped at a holding potential of −90 mV for 5 min while 20 μM PKI was allowed to diffuse into the cell from the patch pipette solution. After PKI treatment, dopamine's effect on transient (Fig. 8 *A*) and sustained (Fig. 8 *B*) calcium currents was measured. With PKI in the cell, 7 μM dopamine reduced the transient current by only 3%, which is significantly less than the average reduction of 56% in the absence of PKI (Table I). Likewise, the sustained calcium current increase due to dopamine was nearly eliminated when PKI was in the cytoplasm of the cell. In Fig. 8 *C*, the effect of dopamine on a PKI-treated cell can be seen as the cell is stepped to various membrane potentials from the holding potential. The effect of dopamine on transient and sustained calcium currents was blocked at every membrane potential. PKI also blocked dopamine's effect on calcium currents in four other cone horizontal cells examined (Table I). These results suggest that dopamine's dual effect on voltage-sensitive calcium channels in white bass cone horizontal cells involves a cAMP-dependent protein kinase.

DISCUSSION

Calcium Currents

Calcium currents are a general feature of retinal horizontal cells and, in fact, of all neurons. In teleosts such as goldfish (Tachibana, 1983), catfish (Shingai and Christensen, 1983), white perch (Lasater, 1986), and white bass (Sullivan and Lasater, 1992) an inward sustained calcium current has been described. These currents have similar properties and resemble the L-current characterized in chick dorsal root ganglion cells (Fox et al., 1987). In the white bass, we have demonstrated that dopamine modulates this L-type calcium current and potentiates calcium influx. The transient calcium current (or T-current) of white bass horizontal cells has not been seen in other teleost horizontal cell preparations, although it has characteristics in common with transient currents found in tiger salamander bipolar cells (Maguire, Maple, Lukasiewicz, and Werblin, 1989), mouse bipolar cells (Kaneko, Pinto, and Tachibana, 1989), and rat (Karschin and Lipton, 1989) and turtle (Liu and Lasater, 1993) ganglion cells. Dopamine also modulates this current in the white bass horizontal cell, but, unlike the L-type current, it acts to diminish the transient calcium current.

Neurotransmitters and Bass I_{ca}

Many neurotransmitters and neuromodulators have been found to affect the activity of voltage-activated ion channels in both peripheral and central neurons through a wide variety of mechanisms (for review see Scott, Pearson, and Dolphin, 1991). Voltage-activated calcium channels, in particular, are modulatory targets for a large number of neurotransmitters and neuromodulators, most of which act to inhibit the voltage-sensitive currents (Scott et al., 1991). A few neuroactive agents have been found to increase voltage-sensitive calcium currents (Artalejo, Ariano, Perlman, and Fox, 1990; Scott et al., 1991). Our results, however, demonstrate that dopamine can have a dual effect on two types of voltage-activated calcium currents in the same cell. Along the same lines, dopamine was shown to affect both the high and low threshold calcium currents in sensory neurons (Marchetti, Carbone, and Lux, 1986). However, in these cells dopamine has similar inhibitory actions on both types of calcium currents. Thus, with regard to calcium currents, dopamine appears to have a unique action in the retina.

It is very interesting that in teleost horizontal cells dopamine has now been shown to initiate at least four D1 receptor–mediated events. Previous studies have shown that dopamine reduces gap-junctional permeability between like horizontal cells and increases an excitatory amino acid–activated conductance (Lasater and Dowling, 1985; Knapp and Dowling, 1987). Both of these actions were shown to be mediated through a cAMP-dependent pathway. The studies presented here show that dopamine has opposing effects on two types of voltage-sensitive calcium currents that are also mediated via a cAMP-dependent mechanism. Thus, activation of the cAMP cascade in these horizontal cells leads to a wide variety of intracellular actions after D1 receptor activation. An interesting question is whether the cell can regulate the different actions independent of one another, or whether they are all a part of a more general event that leads to a specific functional state of the cell.

Modulation of Calcium Currents

Modulation of the voltage-dependent calcium currents in white bass horizontal cells may occur by direct action on the ion channels or by an indirect effect, say on a regulatory protein. Current evidence favors a direct effect on the ion channel protein. In cardiac muscle, beta-adrenergic stimulation has been shown to enhance an L-type calcium conductance. This enhancement was mediated by cAMP and by a cAMP-dependent protein phosphorylation (Osterrieder, Brun, Hescheler, Trautwein, Flockerzi, and Hofman, 1982). Biochemical analysis of the phosphorylation event demonstrated that the catalytic subunit of cAMP-dependent protein kinase directly phosphorylated ion channel subunits from transverse tubule membranes (Curtis and Catterall, 1985). Thus, in white bass horizontal cells, modulation of the voltage-dependent calcium currents by dopamine may also be due to direct phosphorylation of a channel subunit resulting from activation of protein kinase A. The opposing actions of dopamine on the two calcium currents may be due to the phosphorylation of different subunits on the two channel types. It is interesting that the action of dopamine is specific to cone horizontal cells. We saw no evidence of an effect of dopamine or dopamine agonists on rod-driven horizontal cells. This makes sense in

that rod-driven H4 horizontal cells are not in synaptic contact with dopamine-releasing interplexiform cells (Dowling and Ehinger, 1975, 1978).

However, our results do raise the question of whether or not the H4 calcium channels are modulatable, particularly through a cAMP-dependent pathway. That is, a different neuromodulator may be able to activate adenylate cyclase to modulate the H4 calcium currents. We tested the notion that cAMP could modulate rod-driven horizontal cell calcium currents by applying 8-CPT to H4 horizontal cells ($n = 4$). Surprisingly, the 8-CPT had no effect; both calcium currents remained unchanged. This indicates that there may be fundamental differences in the way in which calcium currents in different cells are modulated. However, it may simply be that the H4 currents are not modulated through any mechanism in the retina's normal course of processing visual information. This remains to be determined.

Physiological Significance

At present, it is unclear what role the simultaneous modulation of the two calcium currents might play in the processing of the visual signal. In the dark, photoreceptors are depolarized and continuously release neurotransmitter. The probable photoreceptor neurotransmitter is L-glutamate (Marc and Lam, 1981; Lasater and Dowling, 1982; Ishida et al., 1984; Kageyama and Meyer, 1989), which induces a current in isolated horizontal cells that has a reversal potential of ~0 mV (Tachibana, 1985; Hals, Christensen, O'Dell, Christensen, and Shingai, 1986). Nevertheless, in the dark, horizontal cells rest at between −15 and −30 mV. In response to a light stimulus, photoreceptor transmitter release is attenuated or terminated and the horizontal cell hyperpolarizes. With a bright stimulus the membrane potential can reach as low as −60 to −70 mV. In the case of the transient calcium current, this current might be activated upon the offset of a bright light stimulus (Sullivan and Lasater, 1992) after the cell has been hyperpolarized to below −40 mV or so. The current would activate as the membrane begins to depolarize and might augment the depolarization. In this respect the current would be similar to the transient low threshold calcium current described in other preparations, which serves to enhance low threshold depolarizations (Llinas and Yarom, 1981; Jahnsen and Llinas, 1984). Under the influence of dopamine, light-activated calcium influx would be reduced or eliminated and the return to a depolarized potential slowed. Thus, dopamine could alter the kinetics of the cell's response after a flash of light. It would also affect any other calcium-dependent processes that rely on calcium entry through the T-type channels.

An enhancement of the L-type current may also have an impact on the photic response of cone horizontal cells. The activation range of the current falls, for the most part, outside the normal operating range of the cell, although the foot of the current's current–voltage curve does intrude on the upper portion of the cell's operating range. Thus enhancement of the current would increase the amount of calcium entering the cell at these voltage levels; in some cells the amount of calcium entering would more than double. The purpose for doing this is unknown. We have speculated (Sullivan and Lasater, 1992) that the L-current plays a role in maintaining the membrane potential of the horizontal cell at depolarized levels in the dark. The current has been shown to be essential in these cells for maintaining a long

depolarized plateau in response to a brief exogenous glutamate pulse (Lasater and Dowling, 1982) or to an injected current pulse (Shingai and Christensen, 1986; Sullivan and Lasater, 1990). If this is the case, dopamine's role may be to increase the intracellular calcium level in the cell to depolarize it and reduce the amount of neurotransmitter required to maintain the dark resting potential.

The increase of intracellular calcium ions due to the action of dopamine on the sustained current is probably also linked to other cellular events. Increases in intracellular calcium ions can activate enzyme systems that control protein phosphorylation, resulting in ion channel regulation (Ewald and Levitan, 1987), cell plasticity, and the activation of long-term potentiation (Nicoll et al., 1988). In all probability intracellular increases (or decreases) in calcium ions help regulate cellular events in horizontal cells. Possibly calcium plays a role in the events associated with light or dark adaptation. In fact, increases or decreases in intracellular calcium levels may be in part responsible for the slowing and diminution in the light-evoked response observed in white perch cone horizontal cells when dopamine is applied to the intact retina (Yang, Tornqvist, and Dowling, 1988). In any case, the results of this study have raised a number of interesting questions. To better understand the role of dopamine and its modulation of horizontal cell I_{Ca}'s, more work remains to be carried out. Nevertheless, the picture of dopamine that we now have is that of an important neuromodulator with a broad range of actions in the retina.

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