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Dopamine alters glutamate receptor desensitization in retinal horizontal cells of the perch (*Perca fluviatilis*)

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ABSTRACT **The patch-clamp technique in combination with a fast liquid filament application system was used to study the effect of dopamine on the glutamate receptor desensitization in horizontal cells of the perch (*Perca fluviatilis*). Kinetics of ligand-gated ion channels in fish horizontal cells are modulated by dopamine. This modulation is presumably mediated by a cAMP-dependent protein phosphorylation. Before incubation with dopamine, the glutamate receptors of horizontal cells activate and desensitize with fast time constants. In the whole-cell recording mode, fast application of the agonists L-glutamate, quisqualate, or α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid prior to the dopamine incubation gives rise to fast transient currents with peak values of about 200 pA that desensitize within 100 ms. Kainate as agonist produced higher steady-state currents but no transient currents. After incubation of the cells with dopamine for 3 min, the desensitization was significantly reduced and the agonists L-glutamate, quisqualate, or α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid induced steady-state currents with amplitudes that were similar to the previously observed transient currents. Kainate-induced currents were only slightly affected. Fast desensitizing currents upon fast application of L-glutamate were also recorded from outside-out patches that were excised from horizontal cells before incubation with dopamine. The currents from excised patches desensitized to a steady-state level of about 0.2 of the peak amplitude with time constants of less than 2 ms. When the outside-out patches were excised from cells after dopamine incubation, steady-state currents were enhanced and no transient currents were observed. The results may indicate that the dopamine-dependent modulation of glutamate-induced currents, which is presumably mediated by a protein phosphorylation, is due to an alteration of the desensitization of the glutamate receptors.**

In cone-driven horizontal cells of fish, dopamine acts as a neuromodulator via a second-messenger system (1). This system includes an adenylate cyclase and a cAMP-dependent protein kinase (2). Dopamine has been shown to play a major role in light and/or dark adaptation of the retina (3). At the level of the horizontal cells, dopamine effects the electrical coupling and the permeability of gap junctions (4, 5) and the formation of spinules (6), and alters excitatory amino acid-induced currents (7). In fish horizontal cells, mainly glutamate receptors of the non-*N*-methyl-D-aspartate type were found (8, 9). The gating kinetics of these channels is altered by dopamine treatment (10).

In a previous study, we quantified (11) the effect of dopamine on amino acid-gated steady-state currents by measurement of concentration–response curves and found that the dopamine-dependent enhancement of amino acid-gated currents was abolished after preincubation with the plant lectin concanavalin A (Con A) or with the nootropic drug aniracetam. Con A and aniracetam block the fast desensitization of ionotropic glutamate receptors. It is therefore likely that the modulation of glutamate-gated currents in cone-driven horizontal cells is related to a switch in the fast receptor desensitization.

In the project presented here, a fast liquid filament application system was used for a direct test of this hypothesis. Currents gated by excitatory amino acids were recorded in the whole-cell recording mode and from outside-out patches. Pretreatment of horizontal cells with dopamine abolished or reduced the fast desensitization of glutamate receptors. Therefore, the modulation of amino acid-gated steady-state currents in cone-driven horizontal cells in the teleost retina is at least partly based on an altered receptor desensitization.

METHODS

Experiments were performed on isolated horizontal cells of the perch (*Perca fluviatilis*). Animals were dark-adapted, decapitated, and enucleated under dim red light. After enzymatic treatment with papain, the isolated retina was mechanically dissociated as described (12), and horizontal cells were plated in L-15 medium (GIBCO) and kept at 12°C in culture for 2–5 days. Cells were superfused with extracellular solution containing 145 mM NaCl, 2.5 mM KCl, 20 mM $NaHCO_3$, and 2.5 mM $CaCl_2$ (pH 7.6). Patch pipettes were filled with intracellular solution containing 64 mM potassium gluconate, 40 mM KCl, 11 mM EGTA, 1 mM $CaCl_2$, 10 mM Hepes, and 3 mM MgATP (pH 7.5). Experiments were carried out at room temperature. For application of agonist, a liquid-filament switch operated by a piezo translator was used that can exchange solution within a few milliseconds (13). For whole-cell measurements, dopamine was applied with the background solution of the application system so that it was possible to measure membrane currents from a single cell before and after dopamine application. When measuring from excised patches, dopamine was applied to the bath and a patch was pulled from the same cell before and after dopamine treatment. Concentration of dopamine was 500 μM, and incubation time was at least 5 min. Concentration and time were sufficient to activate the dopamine-dependent second messenger system maximally (2, 11).

RESULTS

Fig. 1 shows recordings of whole-cell currents induced by various excitatory amino acids (at 0.1 mM–1 mM) before and after treatment of the horizontal cells with dopamine (0.5 mM). Before dopamine treatment, L-glutamate and the glutamate agonists quisqualate and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) elicited peak membrane currents in the range of 60–120 pA. These currents desensitized within a few milliseconds to a steady-state level

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Abbreviation: AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid.

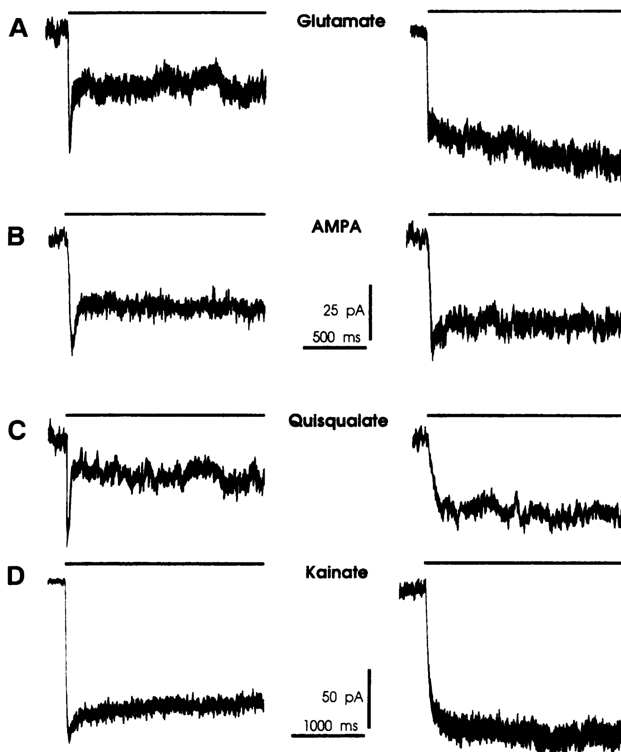

FIG. 1. Excitatory amino acid-induced whole-cell currents recorded from a single cone-driven horizontal cell. (*Left*) Membrane currents recorded before treatment of the cell with dopamine. (*Right*) Currents recorded after dopamine treatment. Currents induced by 0.5 mM L-glutamate (*A*), 0.5 mM quisqualate (*B*), or 1 mM AMPA (*C*) display a fast desensitization to a pulse of agonist before dopamine incubation, whereas 0.1 mM kainate (*D*) produces currents with no or only very little desensitization. Incubation with dopamine changes the desensitization behavior. For glutamate- and quisqualate-induced currents, no desensitization was observed after dopamine treatment; the desensitization of AMPA currents was reduced. Kainate-induced currents were only slightly affected. In this experiment, the cell was manually driven into the liquid filament.

of 20–40 pA. The desensitization was most pronounced when quisqualate was used as agonist. As it was observed before in other preparations, kainate produced larger peak and steady-state currents than any other agonist. Kainate-gated currents had peak amplitudes in the range of 100–200 pA and displayed no or only a very slight desensitization. The peak currents that were obtained in the measurements with the fast application system were relatively small compared to previous experiments (7, 10, 11). Two reasons may account for this result. (*i*) Relatively small cells were chosen for the experiments presented here, and (*ii*) it is possible that only a part of the membrane instead of the whole cell was exposed to the agonist.

In the 10 horizontal cells that were examined before and after preincubation with dopamine, the desensitization behavior was significantly altered. After treatment with 500 μM dopamine in the extracellular solution for 5 min, the fast desensitization of glutamate- and quisqualate-induced whole-cell currents was completely abolished, and the steady-state currents were significantly enhanced. The amplitudes of the steady-state currents were in the range that was observed for the peak currents prior to a dopamine incubation. When AMPA was applied after dopamine, the desensitization was reduced, although not completely abolished, and the steady-state current was significantly enhanced. Peak currents induced by AMPA were not changed upon dopamine treatment. Kainate-induced currents were only slightly affected by dopamine treatment, but in the few experiments where a small desensitization of kainate-gated currents was observed, it was abolished after dopamine treatment. Means and standard deviations of peak and steady-state currents induced by the various agonists before and after treatment with dopamine are given in Table 1. As it was shown (7, 10, 11) in other preparations, dopamine was ineffective in the perch horizontal cells when it was applied with the dopamine antagonist haloperidol.

Currents from excised patches in the outside-out configuration were also recorded in addition to the whole-cell measurements. Membrane patches were excised from the same horizontal cell before and after dopamine incubation. Prior to dopamine treatment, a pulse of glutamate generated a fast transient current (Fig. 2*A*). The glutamate-dependent ion channels displayed a fast desensitization and activated and inactivated completely within less than 5 ms. We never observed reopenings of the channels. The resensitization time was also very short. When a train of glutamate pulses with intervals of 10 ms was applied, each pulse activated a fast transient current.

The current responses to pulses of glutamate changed significantly when the patches were pulled from identical cells after dopamine treatment. When a patch was excised from a cell that was incubated with 500 μM dopamine for 5 min, the glutamate receptors displayed no fast inactivation. A steady-state current that lasted during the whole pulse duration was recorded instead of the transient current that was recorded from a patch excised from the same cell before treatment with dopamine (Fig. 2*B*). Glutamate-induced steady-state currents without transients were never observed when membrane patches were pulled from cells that were not incubated with dopamine. The peak currents that were recorded from patches excised before (10.2 ± 3.1 pA) and after (9.4 ± 4.2 pA) dopamine treatment did not differ significantly, whereas the steady-state currents before (2.0 ± 1.1 pA) and after (8.5 ± 3.1 pA) dopamine incubation were significantly

Table 1. Whole-cell peak and steady-state currents induced by various agonists before and after treatment of the cells with dopamine

	Current, pA			
	Before dopamine treatment		After (1 min) dopamine treatment	
Agonist	Peak	Steady state	Peak	Steady state
Glutamate (n = 10)	81 ± 26	38 ± 14	79 ± 27	73 ± 23
AMPA (n = 8)	78 ± 28	36 ± 12	76 ± 24	74 ± 25
Quisqualate (n = 9)	76 ± 20	19 ± 7	75 ± 26	70 ± 21
Kainate (n = 10)	184 ± 23	178 ± 19	182 ± 17	175 ± 15

Steady-state currents recorded upon application of glutamate, AMPA, and quisqualate were significantly ($P < 0.01$, two-tailed t test) different before and after incubation with dopamine, whereas the peak currents were nearly the same in both situations. The steady-state currents recorded upon application of kainate did not differ significantly with and without dopamine. Data are the mean ± SD.

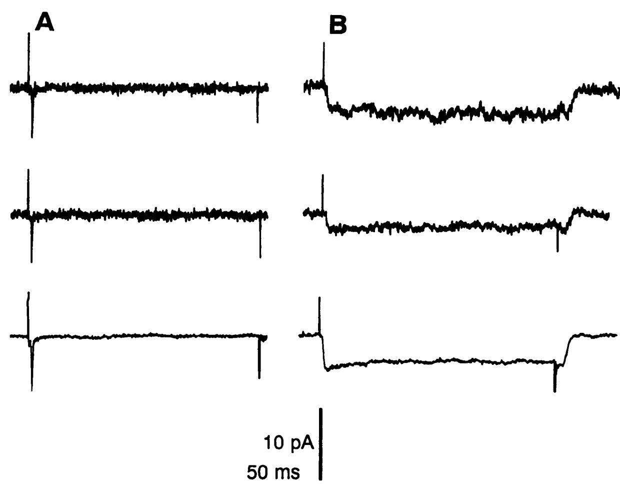

FIG. 2. Glutamate-induced currents recorded from outside-out membrane patches excised from the same cell before (*A*) and after (*B*) treatment of the cell with dopamine-containing extracellular solution. The upper two traces are single recordings; the lowest trace of each column represents 50 averaged responses. Before dopamine treatment, a fast transient current response was recorded upon application of glutamate (1 mM) to the outside-out patch. When a patch was excised from the same cell after incubation with dopamine, a steady-state current was recorded upon application of glutamate (1 mM) and no desensitization was observed. The channels opened during the whole time of L-glutamate application. Such a response was never seen in outside-out patches pulled from cells before dopamine treatment. The artifact at the beginning and end of the agonist application is due to the sudden changes in the piezo control voltage.

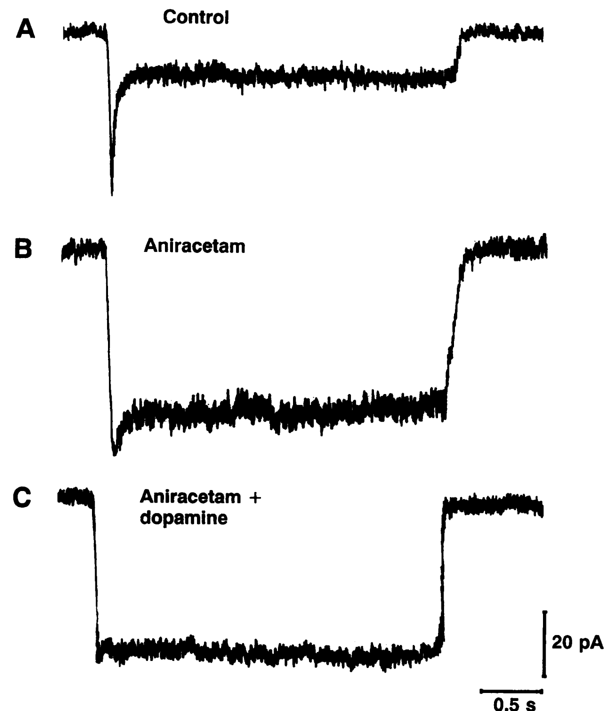

FIG. 3. Whole-cell currents induced by L-glutamate (0.5 mM) recorded from a cone-driven horizontal cell. (*A*) Membrane current recorded under control conditions. (*B*) Glutamate-induced current recorded after application of aniracetam (1 mM). (*C*) Aniracetam plus dopamine (0.5 mM) was applied for 1 min. In this experiment, the cell was manually driven into the liquid filament.

different ($P < 0.01$, $n = 12$, two tailed t test). The time course of the response onset was not significantly different in patches excised before and after dopamine incubation, although recordings from different patches cannot be compared directly with one another.

In excised patches, the effect of dopamine appears as an all-or-none phenomenon, and intermediate changes in the rate of desensitization were not observed. The transition from one state to the other was relatively fast. When patches where excised 20 s after the beginning of dopamine application, no effect was observed and patches excised after 40 s of dopamine treatment always displayed a complete removal of desensitization. The time course of the dopamine effect on the basis of sustained whole-cell currents has been analyzed (11).

In principle the results described above can be explained by two mechanisms. The disappearance of the desensitization could be due either to a dopamine-dependent modulation of the kinetic behavior of a single channel receptor complex or to an activation of a second receptor population with more sustained kinetics. The experiments described in the following section were performed to discriminate between these two explanations.

The activation of a second receptor type could cause an alteration of the reversal potential. Current–voltage relations were, therefore, recorded before and after dopamine incubation in three horizontal cells. The shape of the current–voltage curve was not altered after dopamine application and the reversal potential (+5 mV) was the same before and after dopamine incubation. The effect of an AMPA/quisqualate antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (100 μM) on whole-cell currents elicited by glutamate before and after dopamine treatment was also tested. 6-Cyano-7-nitroquinoxaline-2,3-dione blocked the response to the agonists completely and reversibly. The modulated response to the agonists after dopamine incubation (see Fig. 1) was also completely and reversibly blocked. This result reveals no evidence for a pharmacological discrimination of two receptor types. The nootropic drug aniracetam is known to reduce glutamate-receptor desensitization in hippocampus cells (14). The activation of a covert receptor population by dopamine would be effective also after removing the desensitization of the first receptor population. In Fig. 3*B*, it is shown that the desensitization of the glutamate receptors in horizontal cells is partly removed by aniracetam. The application of dopamine was almost ineffective when it was applied after treatment of the cells with aniracetam. As shown in Fig. 3 *B* and *C*, the steady-state currents were not enhanced by dopamine when it is applied with aniracetam. Thus the results indicate that dopamine modulates the desensitizing behavior of a single channel receptor complex rather than that a second channel population is unmasked.

DISCUSSION

We have demonstrated that the enhancement of steady-state currents as it has been observed before in fish horizontal cells (7, 10) is at least partly related to an altered desensitization of excitatory amino acid-induced currents. This may answer the question of why dopamine shows no effect after treatment of the cells with substances that are known to reduce glutamate receptor desensitization such as Con A or aniracetam (11).

The results show that dopamine significantly reduces the desensitization of the ionotropic glutamate receptor in retinal horizontal cells. Accumulating evidence demonstrates that protein phosphorylation is a mechanism for modulating the function of ligand-gated channels (15). Some recent results demonstrate direct phosphorylation of the glutamate receptor subunit GluR6 by the cAMP-dependent protein kinase (16, 17). It is not known which subunit composition exists in horizontal cells, although there is evidence that these cells

express at least the subunits GluR1 and GluR2 (18). Since GluR6 is the only glutamate receptor subunit with a strong consensus site for the cAMP-dependent protein kinase (19), it seems very likely that the non-*N*-methyl-D-aspartate receptors in horizontal cells possess this subunit.

An enhancement of steady-state currents via activation of a cAMP-dependent protein kinase and direct phosphorylation of the channel protein was measured in hippocampus neurons (20, 21). Here it is demonstrated that such a modulation can be related to an alteration of the desensitization behavior.

The glutamate receptors in perch horizontal cells respond to AMPA and to kainate, and it is, therefore, likely that these receptors belong to a family of AMPA/kainate receptors (22). The desensitization behavior of these receptors is determined by two alternatively spliced sequences flip and flop. The desensitization behavior of the glutamate receptors in horizontal cells matches the properties of an A-flip/B-flop type that displays fast inactivation upon AMPA application and no inactivation upon application of kainate. Therefore, it is not necessary to assume that more than one type of non-*N*-methyl-D-aspartate receptor exists in horizontal cells, although on the basis of our results, we cannot exclude the existence of different receptor subtypes with certainty.

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