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Dopamine modulates the kinetics of ion channels gated by excitatory amino acids in retinal horizontal cells

(kainate/catecholamine/cell culture/patch clamp/noise analysis)

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ABSTRACT **Upon exposure to dopamine, cultured teleost retinal horizontal cells become more responsive to the putative photoreceptor neurotransmitter L-glutamate and to its analog kainate. We have recorded unitary and whole-cell currents to determine the mechanism by which dopamine enhances ion channels activated by these agents. In single-channel recordings from cell-attached patches with agonist in the patch pipette, the frequency of 5- to 10-pS unitary events, but not their amplitude, increased by as much as 150% after application of dopamine to the rest of the cell. The duration of channel openings also increased somewhat, by 20–30%. In whole-cell experiments, agonists with and without dopamine were applied to voltage-clamped horizontal cells by slow superfusion. Analysis of whole-cell current variance as a function of mean current indicated that dopamine increased the probability of channel opening for a given agonist concentration without changing the amount of current passed by an individual channel. For kainate, noise analysis additionally demonstrated that dopamine did not alter the number of functional channels. Dopamine also increased a slow spectral component of whole-cell currents elicited by kainate or glutamate, suggesting a change in the open-time kinetics of the channels. This effect was more pronounced for currents induced by glutamate than for those induced by kainate. We conclude that dopamine potentiates the activity of horizontal cell glutamate receptors by altering the kinetics of the ion channel to favor the open state.**

The amino acid L-glutamate mediates fast excitatory neurotransmission in the vertebrate central nervous system by interacting with two classes of receptors: those activated by the selective agonist *N*-methyl-D-aspartate (NMDA) and those activated by agonists such as kainate and quisqualate (1–3). It is likely that both classes of glutamate receptors are subject to multiple regulatory influences. NMDA receptors, for example, are thought to contain (in addition to the NMDA binding site) distinct modulatory sites that bind phencyclidine analogs (4), glycine (5), Mg^{2+} (6, 7), and Zn^{2+} (8, 9). These sites influence aspects of receptor function as agonist binding, ion permeation, desensitization, and voltage dependence.

Less is known about the modulation of other subtypes of excitatory amino acid receptors, although changes in the sensitivity of non-NMDA receptors have been proposed to underlie long-term modifications of synaptic efficacy (10, 11). Cultured horizontal cells isolated from teleost retinas do not express NMDA receptors but they do respond to the putative photoreceptor transmitter L-glutamate and to non-NMDA excitatory amino acid agonists (12–14). Ionic currents gated by such agonists remain constant for many minutes (15), providing a useful system in which to study regulation of glutamate receptors by second messenger systems.

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In horizontal cells isolated from white perch (*Roccus americana*), the neuromodulator dopamine has been shown to increase currents gated by L-glutamate and kainate (16). This enhancement appears to be mediated by cAMP-dependent protein phosphorylation, because in perch horizontal cells (*i*) the activation of dopamine receptors stimulates adenylate cyclase and protein phosphorylation (17, 18), (*ii*) cAMP and its analogs increase amino acid-gated currents to the same degree as does dopamine (16), and (*iii*) internal dialysis with the catalytic subunit of protein kinase A mimics the effects of dopamine and cAMP (19).

We have sought to determine which physiological properties of horizontal cell glutamate receptors are modified by dopamine. The macroscopic current produced by a given concentration of agonist is the product of the number of functional channels, the elementary conductance of an individual channel, and the proportion of time channels spend in the open state. In principle, dopamine could act to increase any or all of these parameters. In the experiments described here, we assessed these possibilities by analysis of single-channel and whole-cell currents evoked by excitatory amino acids before and after application of dopamine. The results indicate that dopamine does not substantially alter the number of glutamate-gated channels or their unitary conductance. Rather, dopamine modifies the kinetics of the channels to increase the frequency with which they open and, to a lesser extent, the amount of time they remain open.

METHODS

Cells. Horizontal cells were plated in L-15 medium (GIBCO) after mechanical dissociation of papain-digested white perch retinas (20). All recordings were made at 20°C from cone-driven cells (types H1, H2, and H3), which are responsive to dopamine (16, 21). Recordings were obtained from cells maintained in culture for 2–14 days.

Single-Channel Recording. Single-channel currents were measured from cell-attached patches (22) using Sylgard-coated glass pipettes containing 150 mM CsCl (to suppress potassium channels), 10 mM Hepes (pH 7.5), and 2–10 μM kainate or 20–50 μM L-glutamate. In these experiments, the extracellular solution was chosen to maintain the cellular membrane potential near 0 mV and contained 140 mM potassium gluconate, 20 mM $MgCl_2$, 10 mM EGTA, and 10 mM Hepes (pH 7.5). The patch potential was held at −100 mV to allow resolution of small-conductance channels. Dopamine (200 μM) plus ascorbic acid (1 mg/ml) was applied by pressure ejection from a narrow-bore patch pipette placed 100 μm from the cell. Currents were filtered at 2 kHz and

Abbreviation: NMDA, *N*-methyl-D-aspartate.

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digitized at 5 kHz; quantitative analysis was done with FETCHAN and pSTAT programs (Axon Instruments, Burlingame, CA).

Whole-Cell Recordings. Isolated cells were voltage-clamped in the whole-cell configuration by using fire-polished patch pipettes (5–10 MΩ) containing 72 mM potassium gluconate, 48 mM KF, 4 mM KCl, 1 mM $CaCl_2$, 11 mM EGTA, 1 mM Mg-ATP, and 10 mM Hepes, adjusted to pH 7.5 with KOH. The holding potential was set at −50 mV to minimize voltage-dependent currents. Voltage-clamped cells were locally superfused (23) with a solution containing 145 mM NaCl, 2.5 mM KCl, 2.5 mM $CaCl_2$, 1 mM $MgSO_4$, 10 mM glucose, and 20 mM $NaHCO_3$ (pH 7.6), equilibrated with 97% O_2/3% CO_2. During recording, the superfusate was switched between the control extracellular solution, a similar solution containing a non-NMDA agonist (kainate or L-glutamate), and one containing the same concentration of agonist plus freshly prepared 200 μM dopamine with ascorbic acid (1 mg/ml) added to retard oxidation. This concentration of dopamine maximally stimulates accumulation of cAMP in teleost horizontal cells (24). In some experiments haloperidol (50 μM) was added to the superfusate. The rate of exchange of solutions was sufficiently slow (≈30 s) to allow recording of whole-cell currents throughout the accumulation and washout of agonists. Currents were low-pass filtered at 1 kHz with an 8-pole Bessel filter and digitized online at 2 kHz by PCLAMP software (Axon Instruments).

Noise Analysis. Whole-cell currents were analyzed in two ways. For mean vs. variance analysis, records were divided into 128-ms segments whose mean current (I) and variance (σ^2) were calculated. To reduce scatter in the variance measurements, estimates from segments with similar mean values were binned together (binwidth, 10 or 50 pA) and averaged. Least-squares parabolic fits to the resulting values were used to derive estimates of single-channel properties according to the relations (25)

$$I = Npi \quad [1]$$

and

$$\sigma^2 = Ii - (I^2/N), \quad [2]$$

where N is the number of agonist-gated channels, i is the current through a single channel, and p is the probability that a channel is open at any given instant. According to these equations, current variance is a parabolic function of mean current if the number of functional channels and the single-channel conductance remain constant while the open probability varies.

For spectral analysis, records were divided into 512-ms segments and power-density spectra were calculated by the maximum entropy method (26) using 20 poles. For frequencies between 2 and 500 Hz, background current fluctuations contributed <10% of the spectral power observed in the presence of agonist and were not corrected for. Multiple Lorentzian functions were fit to the average of 30 spectra by a nonlinear least-squares algorithm (Levenberg–Marquardt).

RESULTS

Enhancement of Single-Channel Currents. When cell-attached patches were clamped at −100 mV, unitary inward currents were observed in most experiments (≈70% of stable patches) when the patch electrode contained L-glutamate (20–50 μM) or kainate (2–10 μM). Channel activity was never seen in experiments in which the pipette did not contain agonist (n = 10). Fig. 1 *A* and *B* (upper traces) shows examples of channel openings recorded with 2 μM kainate or 20 μM L-glutamate, respectively, in the pipette. As reported for other preparations (27, 28), channels gated by non-NMDA agonists in horizontal cells had multiple conductance levels. With low agonist concentrations, the vast majority (>90%) of unitary events had amplitudes of 5–10 pS, although larger (12–20 pS) events occurred with lower frequency. Because transitions between conductance levels resemble superpositions of independent channel openings, it was not generally possible to determine the number of channels present in a patch. When the patch was depolarized from −100 mV, the channel openings became smaller, reversing near 0 mV (data not shown).

To facilitate the analysis of single-channel kinetics, channel openings were identified as excursions beyond a fixed threshold of 4 pS (approximately 4 times the standard deviation of the baseline noise). Channel openings were short, with many events faster than the frequency response of the apparatus. Prior to dopamine treatment, the mean channel open time (calculated from single exponential fits of measureable open durations) was always ≤1 ms regardless of the dose or species of agonist used. However, unitary events became more frequent (the mean interval between events decreased) with higher doses of agonist (data not shown).

When dopamine was applied to the cell membrane outside the patch electrode, the frequency of channel openings induced by kainate or by L-glutamate increased by 50–150% (n = 11). The time course of this effect was similar to that seen for the enhancement of whole-cell currents (ref. 16 and see below), typically taking 2–3 min. Lower traces in Fig. 1 *A* and *B* show examples of the elevated levels of channel activity recorded 2.5 min after application of dopamine to the cells. In these experiments, dopamine did not have access to the patch of membrane exposed to agonist; therefore, the enhancement of channel activity must be mediated by an intracellular second messenger, presumably cAMP (see *Discussion*).

Channels activated by kainate opened significantly more frequently after exposure of the cell to dopamine ($P < 0.05$, paired t test). With 2 μM kainate in the pipette, the interval between channel openings (in ms) decreased from 10.49 ± 4.99 to 5.38 ± 0.82 (n = 3); with 5 μM kainate the interval decreased from 8.20 ± 3.64 to 5.52 ± 3.87 (n = 4). In addition, dopamine increased the duration of channel openings induced by kainate by ≈20% (from 0.92 ± 0.22 to 1.13 ± 0.14 for 2 μM kainate, n = 3; from 0.99 ± 0.19 to 1.20 ± 0.23 for 5 μM kainate, n = 4), but these differences were not statistically significant. Fig. 1*C* shows dwell-time distributions for events recorded from the patch of Fig. 1*A*. In contrast to its effects on channel kinetics, dopamine did not significantly affect the conductance of channels activated by kainate. Fig. 1*D* shows distributions of unitary current amplitudes obtained before and after application of dopamine to the same patch as in Fig. 1 *A* and *B*. Although the total number of events recorded after dopamine was larger, the shape of the distribution did not change.

The interval between channel openings induced by L-glutamate (20 μM) also decreased after application of dopamine (from 11.37 ± 6.52 to 6.21 ± 3.04, n = 4, $P < 0.05$), again with little change in the single-channel amplitude. Dopamine increased the duration of channel openings activated by 20 μM glutamate by ≈30% (from 0.70 ± 0.25 to 1.03 ± 0.3, n = 4, $P < 0.05$). Open and closed times did not change when recording from patches on untreated cells or when dopamine-free extracellular solution was applied to the cells by pressure ejection (n = 8).

Noise Analysis of Whole-Cell Currents. To evaluate whether the changes in channel activity observed in membrane patches are sufficient to explain the enhancement of whole-cell currents by dopamine (16), fluctuations of agonist-gated whole-cell currents were analyzed before and after application of dopamine. Slow superfusion of kainate or glutamate onto cells voltage clamped at −50 mV caused concentration-

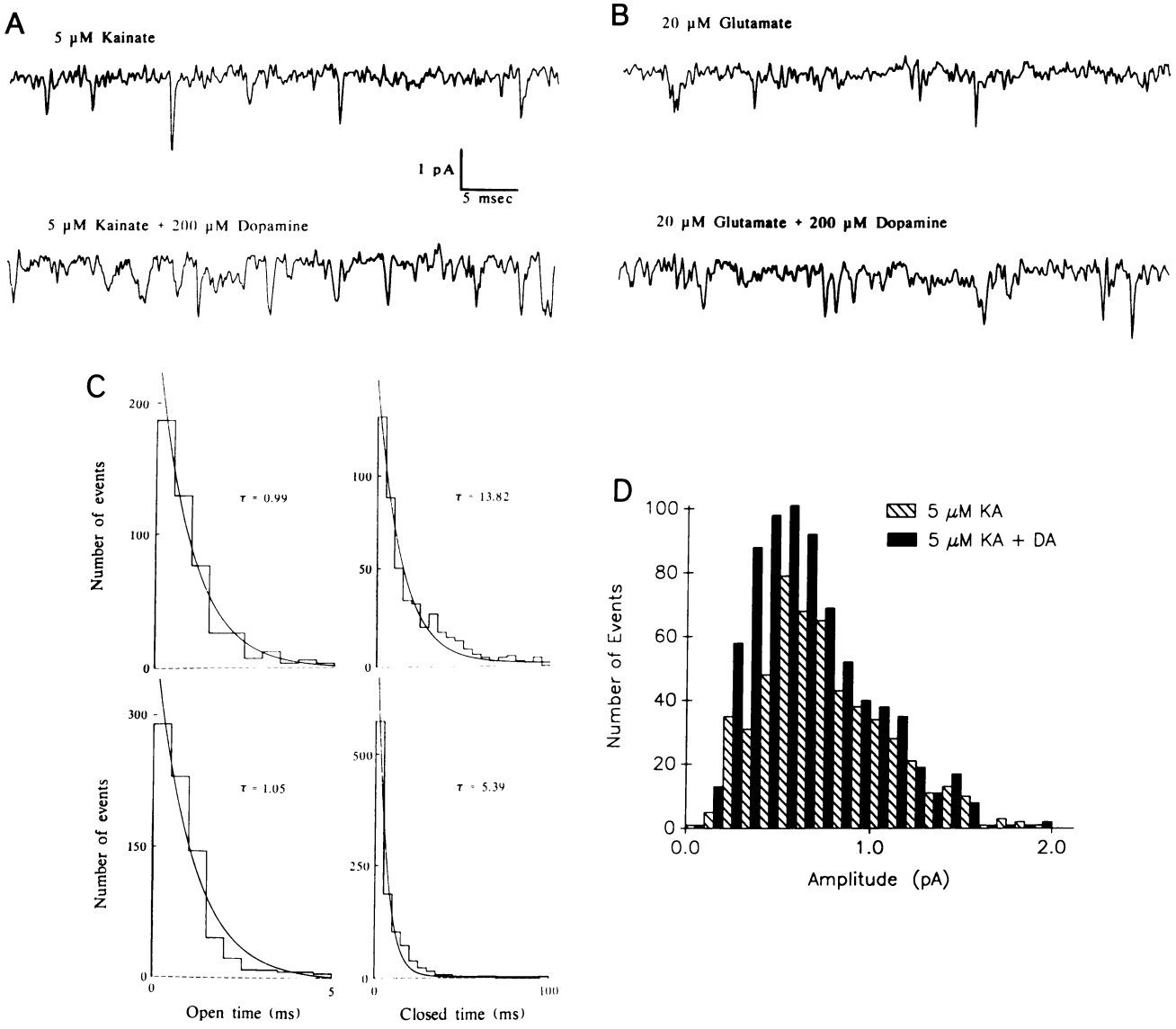

FIG. 1. Effect of dopamine on single-channel currents recorded from two cell-attached patches exposed to 5 μM kainate (*A*) or 20 μM L-glutamate (*B*). Upper traces of *A* and *B* were recorded before and lower traces were recorded 2.5 min after application of 200 μM dopamine to the cell outside the membrane patch. The holding potential was −100 mV in both cases. (*C*) Dwell-time distributions for the channel activity recorded from the same patch as in *A* (openings induced by kainate). Upper histograms were derived from events recorded prior to application of dopamine; lower histograms were from events starting 2.5 min after the start of dopamine application. For this patch, the distribution of open times changed only slightly after application of dopamine, but the closed times became much shorter. Note that different time scales are used for open and closed times. Distributions are fit by single exponentials. (*D*) Amplitude distributions for the same patch. KA, kainate; DA, dopamine.

dependent agonist-specific inward currents accompanied by characteristic changes in membrane current noise, and exposure of horizontal cells to dopamine reliably enhanced currents evoked by nonsaturating concentrations of both kainate and L-glutamate. Overall, the maximal current observed in the presence of agonist increased by 186 ± 45% after exposure to dopamine (n = 32). No change in mean or variance occurred when the superfusate contained the dopamine antagonist haloperidol (Table 1).

Fig. 2 shows mean vs. variance plots for currents recorded during slow superfusion of kainate or L-glutamate before and after application of dopamine. When the agonist was 10 μM kainate (Fig. 2*A*), steady-state variance was already close to its peak value prior to the application of dopamine (i.e., open probability was near 0.5). After application of dopamine, the enhancement of mean current was accompanied by a decrease in variance. Moreover, values of mean and variance obtained after exposure to dopamine fell along the same parabola as those measured during the initial superfusion of agonist alone. That is, dopamine acted to extend the parabola along the mean axis without changing its initial slope or its amplitude. During

Table 1. Enhancement by dopamine of whole-cell currents induced by kainate and glutamate

Agonist	μM	n	Steady-state current, pA	
			Control	Dopamine (200 μM)
Kainate	10	7	432 ± 116	841 ± 237*
	20	4	787 ± 276	1191 ± 390*
	100	8	1266 ± 835	1224 ± 860
Glutamate	100	5	145 ± 123	356 ± 200*
	1000	5	198 ± 83	678 ± 355*
Kainate + haloperidol	10 50	4	599 ± 215	587 ± 219

Data are mean ± SD.
*$P < 0.05$, two-tailed t test.

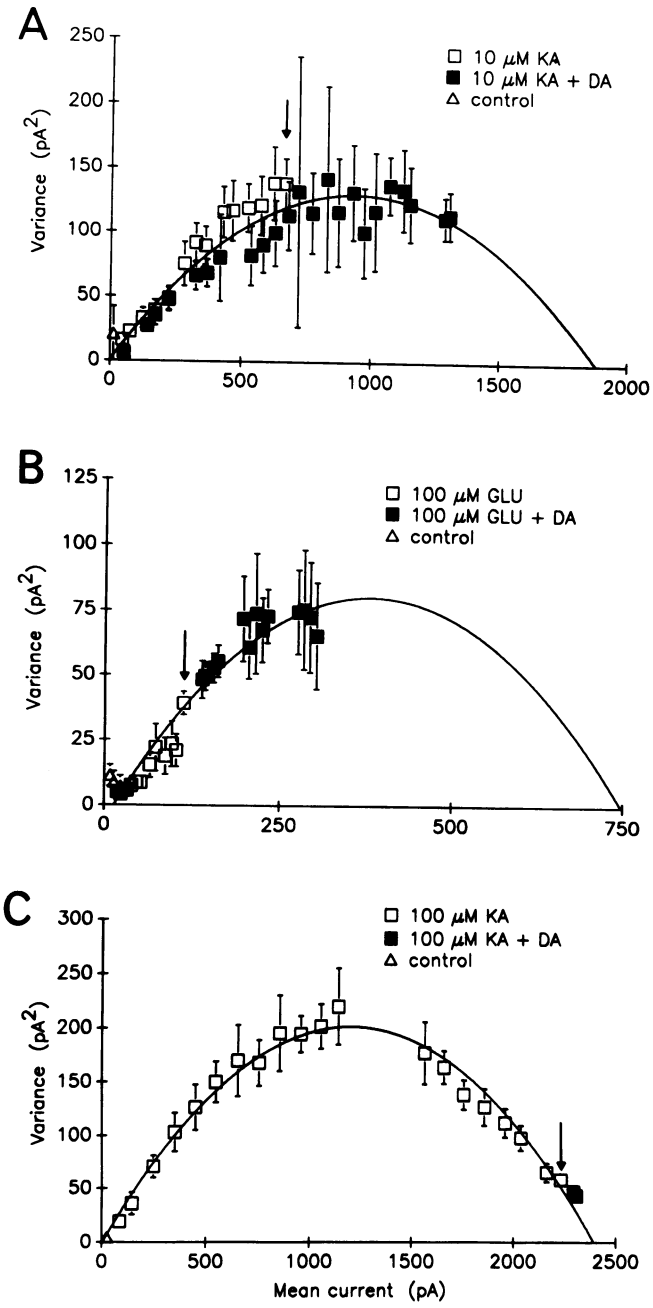

FIG. 2. Mean-variance plots for whole-cell currents induced by slow superfusion. △, Mean and variance in the absence of agonist; □, estimates obtained during application of agonist alone; ■, estimates obtained during application of the same concentration of agonist plus 200 μM dopamine. Curves are parabolic fits to the combined data and were used to estimate single-channel conductance (g) and number of channels (N). (*A*) Kainate (10 μM); mean of four cells with similar steady-state currents; g = 5.3 pS; N = 7128 channels. Arrow, steady-state mean and variance. (*B*) L-Glutamate (100 μM); mean of four cells; g = 8.7 pS; N = 1704. (*C*) Kainate (100 μM); results from a single cell (similar results were obtained for seven other cells—see Table 1); g = 6.1 pS; N = 7834.

application of 100 μM L-glutamate alone (Fig. 2*B*), variance varied approximately linearly with mean, indicating that this concentration of drug opens channels with low probability. After exposure to dopamine, the mean vs. variance plot became more nearly parabolic, with the initial portion of the parabola once again having the same slope as the original (predopamine) relation. These results for nonsaturating concentrations of both kainate and L-glutamate argue that dopamine does not act by increasing the single-channel conductance, because such a mechanism would be expected to increase the initial slope of the mean-variance plots.

The possibility that dopamine may enhance whole-cell currents by increasing the number of agonist-gated channels was excluded by examining the effects of dopamine on the currents produced by a large concentration of kainate, which opens glutamate channels with high probability. If dopamine increases the number of channels, a large enhancement should still occur regardless of the initial open probability. On the other hand, if dopamine acts by increasing the probability of channel openings, then little or no enhancement should occur when the channels are already open most of the time. Fig. 2*C* shows a mean-variance plot for an experiment in which 100 μM kainate was superfused over a horizontal cell and then the superfusate was switched to one containing 100 μM kainate and 200 μM dopamine. In this case, there was only a slight increase in the mean current, and the mean and variance of the enhanced current clearly fell along an extension of the predopamine relationship. This result suggests that, at least for kainate, dopamine acts primarily by increasing the open probability produced by a given concentration of agonist without changing the number of channels or the single-channel conductance. Analogous experiments using saturating doses of L-glutamate could not easily be interpreted because the mean-variance relationship for L-glutamate deviates from that expected for a homogeneous population of channels when high concentrations are used (ref. 29 and unpublished observations).

Power Spectra of Whole-Cell Currents. To examine further the increase in open probability observed after exposure to dopamine, power spectra for steady-state whole-cell currents were calculated before and after application of dopamine. Fig. 3 shows pairs of spectra for currents elicited by 10 μM kainate and by 100 μM L-glutamate. The power spectra for all cells studied were well fit by the sum of two Lorentzians with time constants near 1 ms and 9 ms. Dopamine treatment did not change the cutoff frequency of either component: in eight experiments with kainate the time constants were (in ms) 1.25 ± 0.34 and 8.5 ± 2.5 before exposure to dopamine and 1.18 ± 0.15 and 9.2 ± 2.6 after. Similar results were obtained in two experiments with glutamate. However, the relative amplitude of the slow component increased after dopamine treatment. For kainate, the amplitude ratio of the two components (slow/fast) was 0.66 ± 0.34 before dopamine and 2.20 ± 1.85 afterwards (n = 8). For glutamate the ratio was 0.76 ± 0.28 before and 11.95 ± 3.3 after (n = 2). These results provide additional evidence that dopamine alters the open time kinetics of these channels whether they are activated by kainate or by glutamate.

DISCUSSION

This study has demonstrated that non-NMDA channels in isolated white perch horizontal cells have elementary conductances in the range of 5–10 pS and that dopamine enhances responses to non-NMDA agonists primarily by altering the kinetics of channel openings promoted by a given concentration of agonist. Although the single-channel events were attenuated somewhat by the degree of filtering necessary to resolve them, the amplitudes of the events observed were close to estimates derived from noise analysis and were similar to conductance values reported for kainate- and glutamate-gated channels in other preparations (27, 28, 30, 31), including retinal horizontal cells of other teleosts (14, 29). Neither the single-channel nor the whole-cell experiments provided any evidence for a change in elementary conductance after dopamine treatment.

In the absence of dopamine, the durations of channel openings promoted by L-glutamate and kainate were brief (≤1 ms) and varied little over the range of concentrations for

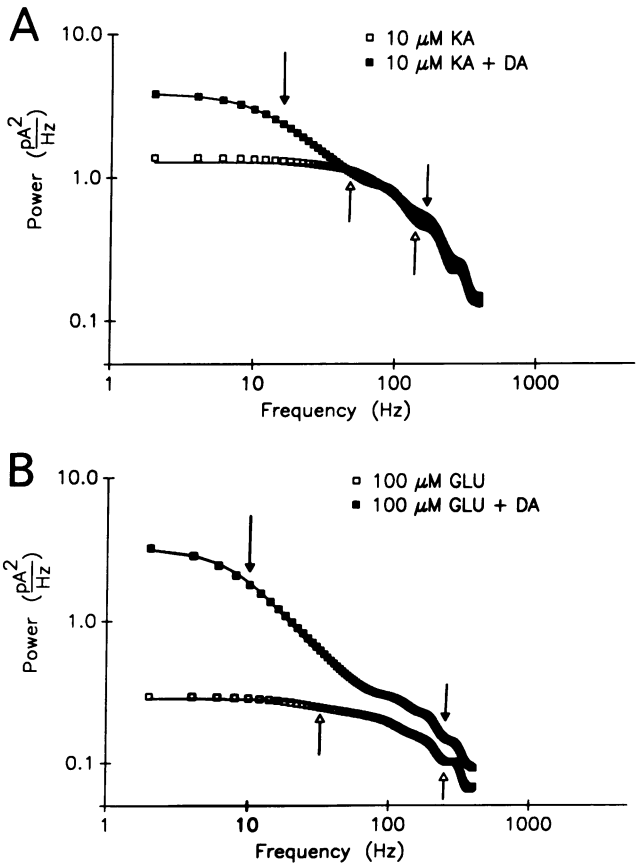

FIG. 3. Power spectra of whole-cell currents induced by 10 μM kainate (*A*) or 100 μM L-glutamate (*B*) before (□) and after (■) enhancement by dopamine. Curves are best-fit sums of two Lorentzians; arrows indicate the cutoff frequencies of the two components.

which discrete events could be resolved. The cutoff frequencies of whole-cell current power spectra were similarly concentration-independent. Thus, the open probability of glutamate channels in horizontal cells, as in other neurons (32), appears to depend primarily upon the frequency of channel openings rather than the time spent in the open state.

Interestingly, the enhancement of glutamate responses by dopamine also involved mainly an increase in the frequency of channel openings. In cell-attached patches, this effect could not be distinguished from the recruitment of additional functional channels. However, such a possibility was ruled out with whole-cell experiments by using high concentrations of kainate, where the number of channels could be determined by mean-variance analysis.

After dopamine treatment, the duration of channel openings activated by L-glutamate and by kainate increased somewhat, although the difference was statistically significant only for 20 μM glutamate. Dopamine also clearly enhanced a slow spectral component of whole-cell currents produced by both agonists, providing qualitative evidence for a change in channel open duration. Further experiments, using additional agonists over a wider range of concentrations, are necessary to clarify the extent to which dopamine affects channel open time.

The biochemical mechanism by which dopamine alters the gating properties of glutamate receptors remains to be determined. Dopamine's actions on horizontal cells, including the enhancement of glutamate responses, appear to be mediated exclusively through cAMP-dependent protein phosphorylation but the target proteins have not yet been identified. In other neurons, phosphorylation has been shown to alter the kinetics of a variety of voltage-dependent ion channels. The situation is less clear for ligand-gated channels, although phosphorylation of the nicotinic acetylcholine receptor has been shown to increase its rate of desensitization (33). The simplest explanation for the changes in channel-gating behavior reported here is that they result from phosphorylation of the channels themselves.

Our laboratory has shown (34) that dopamine decreases electrical coupling between white perch horizontal cells by reducing the open time of gap junctional channels. The results reported here demonstrate that dopamine can exert opposite effects (increase vs. decrease) on two different kinds of synaptic transmission (chemical vs. electrical). In both cases, however, dopamine acts by altering gating kinetics and not by changing conductance states or the number of channels.

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