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Dopamine enhances both electrotonic coupling and chemical excitatory postsynaptic potentials at mixed synapses

(long-term enhancement/gap junctions/Mauthner cell)

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ABSTRACT **The transmitter dopamine reduces electrotonic coupling between retinal horizontal cells and increases their sensitivity to glutamate. Since in other systems single afferents establish mixed electrotonic and chemical excitatory synapses with their targets, dopamine might be expected there to depress one component of excitation while enhancing the other. This hypothesis was tested by applying dopamine locally in the vicinity of the lateral dendrite of the goldfish Mauthner cell (M cell) and monitoring the composite electrotonic and chemical excitatory postsynaptic potentials and currents evoked by ipsilateral eighth nerve stimulation. Dopamine produces persistent enhancements of both components of the postsynaptic response while it also increases input conductance. All these dopamine actions are prevented by superfusing the brain with saline containing the dopamine D_1 receptor antagonist SCH-23390. Postsynaptic injections of the cAMP-dependent protein kinase inhibitor (Walsh inhibitor, or PKI_{5-24}) block the dopamine-induced changes in synaptic transmission, implicating a cAMP-dependent mechanism. Furthermore, there is a dopaminergic innervation of the M cell, as demonstrated immunohistochemically with antibodies against dopamine and the rate-limiting enzyme in its synthetic pathway, tyrosine hydroxylase. Varicose immunoreactive fibers lie in the vicinity of the distal part of the lateral dendrite between the large myelinated club endings that establish the mixed synapses. As determined with electron microscopy, the dopaminergic fibers contain small vesicles, and they do not have synaptic contacts with either the afferents or the M cell, remaining instead in the synaptic bed. Taken together, these results suggest that dopamine released at a distance from these terminals increases the gain of this primary sensory input to the M cell, most likely through a phosphorylation mechanism.**

Hormones and neurotransmitters that have modulatory functions can alter the efficacy of chemical synapses by acting either on specific steps in the junctional transmission process or on intrinsic membrane properties of the postsynaptic cell (1). In contrast, little is known about the modulation of electrotonic synapses. Dopamine, a neurotransmitter that is considered to have modulatory actions, has been shown to alter both chemical synaptic transmission and electrotonic coupling via gap junctions. Its modulatory effects have been studied extensively in the retina, where it decouples horizontal cells (2–4) and enhances their responses to glutamate, the putative transmitter of the presynaptic photoreceptors (5); both actions apparently involve cAMP-dependent processes. Since some neurons establish mixed synapses with their targets (6) such that presynaptic impulses produce both coupling potentials [electrotonic-mediated excitatory postsynaptic potentials (EPSPs)] and chemically mediated EPSPs, it might be expected that there dopamine could have antagonistic effects on the two components of the composite response, preferentially reducing the functional role of coupling.

Probably the best known example of mixed synaptic transmission is that involving the eighth nerve excitatory input to the goldfish Mauthner cell (M cell) (7). Auditory eighth nerve afferents terminate as single, large, myelinated club endings that have both gap junctions and chemical synapses with this neuron's lateral dendrite (8, 9). Subthreshold extracellular stimulation of the posterior eighth nerve produces a biphasic excitatory response (7) consisting of a fast electrotonic component followed by a chemical glutamatergic EPSP (10). Recent studies of these connections demonstrated that electrotonic synapses are modifiable, in that certain patterns of afferent activity may produce persistent changes—namely, long-term potentiation and depression—of both excitatory components (11, 12). They are also enhanced by intradendritic injections of cAMP or aminophylline (13), an inhibitor of the phosphodiesterase that degrades cAMP, suggesting the presence of an endogenous system and that neurotransmitters or hormones may utilize cAMP as a second messenger there.

We report here that there is a dopaminergic innervation of the M-cell lateral dendrite and that dopamine application produces long-lasting enhancements not only of the chemical glutamatergic EPSP, but, in contrast to previous findings in neural tissue, also of the electrotonic coupling potential. Both actions apparently are mediated by a cAMP-dependent phosphorylation pathway.

MATERIALS AND METHODS

Adult goldfish (*Carassius auratus*) were used for electrophysiological recordings and for optical and electron microscopy; the surgical and anesthetic procedures were similar to those described (14). Intracellular recordings were obtained *in vivo* from the lateral dendrite about 250–300 μm from the M-cell soma; both current clamp and single-electrode voltage clamp (SEVC; sampling frequency from 14 to 21 kHz; Axoclamp 2; Axon Instruments, Burlingame, CA) techniques were employed. Generally, the electrodes were filled with KCl (2.5 M, 8–20 MΩ) for current clamp recordings or potassium acetate (5 M, 3–6 MΩ) for SEVC.

The composite monosynaptic EPSPs were obtained by low-strength stimulation of the posterior branch of the eighth nerve (Fig. 1*A*). The intensity was such that the range of response amplitudes was 9–30 mV for the coupling potential and 2–6.5 mV for the chemical EPSPs. Even the largest electrotonic postsynaptic potentials were subthreshold for orthodromic spike initiation at the axon initial segment, due to dendritic filtering (14). In addition, the M-cell axon was

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Abbreviations: M cell, Mauthner cell; EPSP, excitatory postsynaptic potential; PKI_{5-24}, cAMP-dependent protein kinase inhibitor; SEVC, single-electrode voltage clamp.

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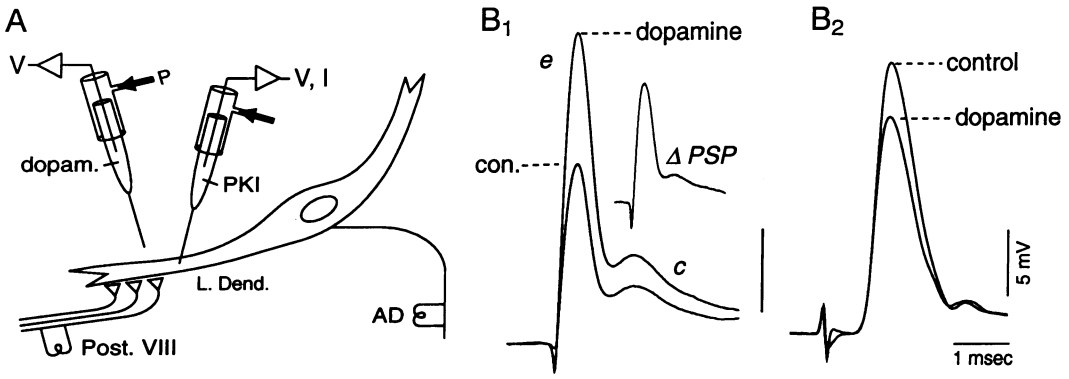

FIG. 1. Dopamine enhances synaptic excitation of the M-cell lateral dendrite. (*A*) Diagram of the experimental arrangement. L. Dend., lateral dendrite; AD, antidromic stimulation; Post. VIII, eighth nerve stimulus; V, I, intradendritic recording electrode, also used for intracellular pressure injections of the protein kinase inhibitor PKI_{5-24} (PKI); V, second electrode placed extracellularly for pressure (P) application of dopamine (dopam.). (B_1) Dopaminergic potentiation of the mixed synaptic excitatory potential. *e*, coupling potential or electrical component; *c*, EPSP or chemical component. Superimposed averages ($n = 12$) obtained in the control (con.) and 20 min after dopamine injection (dopamine) are shown. (*Inset*) ΔPSP is the computer-calculated difference between the two waveforms, which demonstrates that both components increased. (B_2) The antidromic spike height, a measure of the M-cell input resistance, is reduced by dopamine. The same experiment and recording time as in B_1 was used.

activated antidromically by an electrode on the spinal cord. The magnitude of the antidromic action potential was taken as an indicator of the M-cell input resistance, since the soma-dendritic membrane does not support spike electrogenesis (14). All measurements were made after averaging sets of 12 or more traces. Resting membrane potential (−73 to −83 mV) generally remained stable throughout each experiment, although in some cases a transient hyperpolarization of a few millivolts, possibly due to an increased potassium conductance (see below), was observed immediately after dopamine application.

A second pipette situated 40–100 μm above the M cell's lateral dendrite and about the same distance from the soma as the intracellular electrode (Fig. 1*A*) was used for local pressure ejections (5–15 psi; 1 psi = 6.89 kPa; 1–45 sec) of dopamine (10 mM; dissolved in 130 mM NaCl/10 mM Hepes, pH 7.2; given the distance between the electrode and the M cell, volume dilution, and difficulties inherent to extracellular pressure ejection, we estimate that the effective dopamine concentration was at least 2 orders of magnitude less). In one set of experiments, the inhibitor of the catalytic subunit of the cAMP-dependent protein kinase (Walsh inhibitor, or PKI_{5-24} amide; kindly provided by A. Nairn from Rockefeller University, New York) was pressure injected intracellularly through the recording electrode (Fig. 1*A*). Its concentration was 300–900 μM (dissolved in 0.5 M KCl/10 mM Hepes, pH 7.2). In another, the dopamine D_1 receptor blocker SCH-23390 [(*R*)-(+)-SCH-23390 hydrochloride, 50 μM in saline] was applied by superfusion.

For light microscopy, fish were perfused intracardiacally with 4% paraformaldehyde in phosphate buffer (0.12 M at pH 7.4) for 15 min. In the case of EM, 0.1% glutaraldehyde was added, and this step was followed by 5 min of paraformaldehyde alone. Brains were kept for 6 hr in the corresponding fixative and overnight in phosphate-buffered saline, and they were then sectioned with either a cryomicrotome (50 μm) for light microscopy or a vibratome (80 μm) for EM.

The presence of dopamine was revealed with a polyclonal anti-dopamine antibody (Geffard, Bordeaux, France), and that of tyrosine hydroxylase was determined with an anti-tyrosine hydroxylase antibody (Incstar Corp., Stillwater, MN). Immunoglobulin binding sites were labeled with an immunoperoxidase reaction using a secondary biotinylated anti-rabbit and avidin-biotin-horseradish complex (ABC Vectastain kit; Vector Laboratories). For light microscopy, the sections were mounted on gelatin-coated slides and viewed with Nomarski optics, whereas for EM they were embedded in araldite and resectioned (gray/silver) for viewing with a Phillips Electronic Instruments (Mahwah, NJ) 400 microscope. Controls in the absence of the primary antibody lacked the corresponding staining.

RESULTS

Enhancement of Both Excitatory Synaptic Components by Dopamine. Application of the amine outside the lateral den-

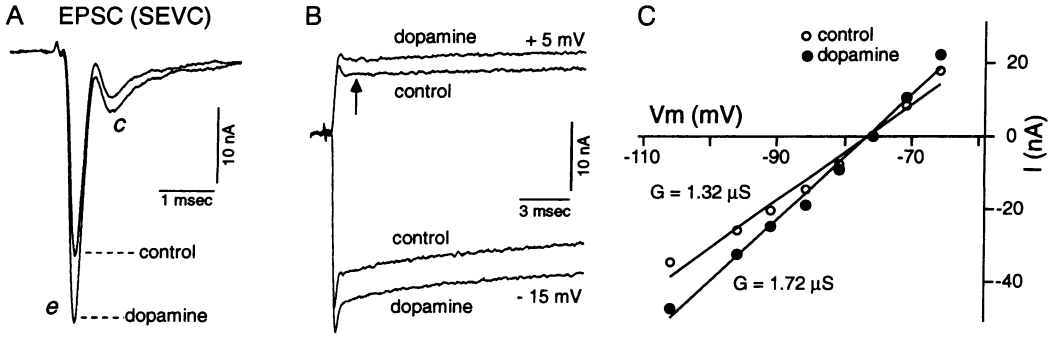

FIG. 2. Evidence that dopamine enhances junctional conductance and excitatory synaptic currents. Records are from one SEVC (chopping frequency = 15.5 kHz) experiment. (*A*) Excitatory postsynaptic currents (EPSC) obtained while clamping at resting potential (−76 mV) before and ≈10 min after dopamine application. (*B*) Currents evoked by command pulses of +5 and −15 mV from resting potential in the same two conditions; note that dopamine increases the current responses to the voltage steps. The arrow indicates the time at which currents were measured for the voltage–current relationship plotted in *C* for a larger range of command pulses. Note the increased conductance (*G*; calculated from the slopes of the fitted lines) produced by dopamine.

drite produces a long-lasting enhancement of both components of the mixed EPSP (Fig. $1B_1$). This dual facilitation could be clearly seen by subtracting the control waveform from that recorded after dopamine injection (ΔPSP in Fig. $1B_1$). In 20 experiments, the increases ranged from 7.6% to 72.6% of the control for the coupling potential (mean = 28.78%, SEM = 3.48%) and from 6.8% to 85.7% for the chemical EPSP (mean = 41.91%, SEM = 4.31%). It took about 3–8 min for these modifications to begin, and they lasted throughout the recording period in all experiments; the longest duration was 90 min after injection. Generally, dopamine did not increase the two components by the same percentage, and there was no quantitative correlation between the effects on the two. As indicated by the average enhancements, it often had a greater effect on the chemical EPSP, although in some cases the increase in the coupling potential was more prominent. The magnitude of this effect seems to be related to the amplitude of the control response. In 7 of these experiments, a second weaker test strength was used in addition to the standard one; the control amplitude ranged from 3.27 to 11.46 mV and from 0.52 to 2.01 mV for the coupling potential and EPSP, respectively. The percent enhancements of the two components were approximately twice as large for the weak test as for the stronger one (enhancement ratios = 2.0 and 1.85, for coupling and the chemical EPSP, respectively).

We examined the possibility that dopamine may have facilitated the chemical EPSP presynaptically via spike broadening (15), since in these afferents this process increases both the incidence and amount of transmitter release (16). Presynaptic spike duration, as inferred from the half-width of the electrotonic EPSP, was virtually unchanged by dopamine, averaging 99.58% (SEM = 3.64%, n = 6) of the control value, thereby ruling out this possible mechanism.

Increases in both components of the mixed EPSP could be produced by a separate action on both electrotonic and chemical synapses or, instead, by simply reducing the M cell's input conductance. To explore this last possibility, we asked if dopamine also increases the antidromic spike amplitude, which would signal a decreased input conductance. However, the spike height (Fig. $1B_2$) actually was generally decreased, on average by 7.8% (SEM = 1.38%, n = 20), suggesting that the enhanced synaptic potentials cannot be explained by a change in nonsynaptic membrane properties. This conclusion was confirmed with SEVC recordings. As shown in Fig. 2*A*, both components of the synaptic current were increased by dopamine. The average enhancements were about the same as those found in current clamp, 38.2% (SEM = 10.3%) for the coupling potential and 27.8% (SEM = 10.4%) for the chemically mediated EPSP (n = 4). In the same experiments, current responses to voltage pulses were used to quantify the action of dopamine on M-cell membrane properties. After the application, the currents elicited by the voltage steps were increased (Fig. 2*B*), as was the slope of the voltage–current relationship (Fig. 2*C*). Overall, the resting input conductance was augmented by 20.1%, from 1.05 to 1.27 μS (n = 5). Thus, the enhanced synaptic responses are due to specific actions of dopamine on gap junctions and on chemical transmission.

Implication of a Postsynaptic cAMP-Dependent Pathway. Given the similarities between the actions of dopamine and the consequences of raising cAMP levels in the lateral dendrite (13), we explored the possibility that activation of a dopamine D_1 receptor, which increases the concentration of this second messenger, is involved (17). Fig. 3 *Top* illustrates the typical time course of the dopamine effects on the synaptic potentials and the antidromic spike height, in control saline. In contrast, when dopamine was applied 30 min or more after beginning superfusion with a specific dopamine D_1 receptor antagonist, SCH-23390 (50 μM), all three responses remained constant (Fig. 3 *Middle*). In eight experiments with the blocker, the electrotonic coupling potential, chemical

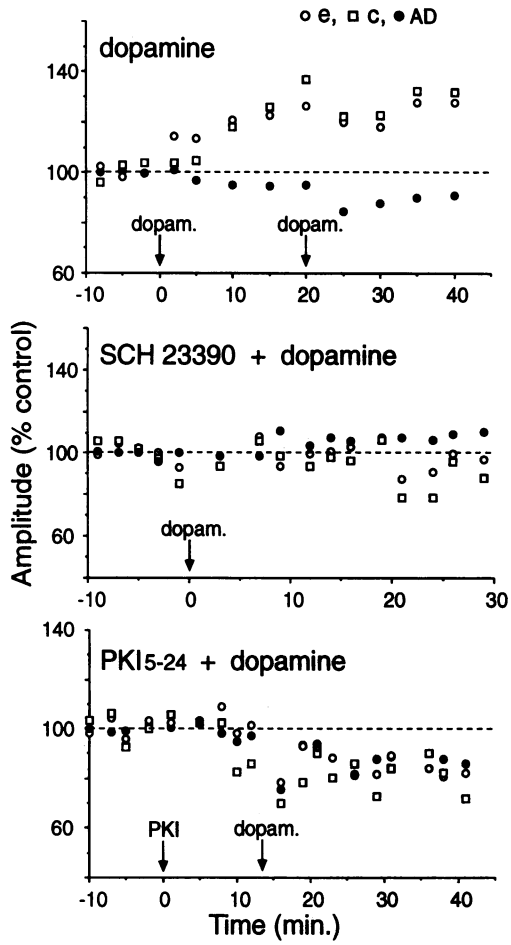

FIG. 3. Pharmacological evidence that the dopamine effects on synaptic transmission are cAMP dependent. Plots of time course of changes in the coupling potential (e; ○), chemical EPSP (c; □), and antidromic spike (AD; ●) amplitudes produced by dopamine application are shown; all magnitudes were expressed as the percentages of their control values. (*Top*) Typical effect of dopamine—namely, sustained increases in both components of the mixed EPSP and a decreased antidromic spike height. (*Middle*) Block of all actions of dopamine by superfusing the fish brain with the D_1 receptor blocker SCH-23390 (50 μM). (*Bottom*) Injection of PKI_{5-24} (first downward arrow) had no effect on the measured parameters, but subsequent application of dopamine (second downward arrow) now reduced all responses, consistent with an increased membrane conductance. dopam., Dopamine.

EPSP, and spike height averaged 96.1% (SEM = 3.03%), 97.3% (SEM = 3.36%), and 97.8% (SEM = 2.25%) of their control values, respectively. Thus, the dopamine receptor mediating these actions is most likely of the D_1 type.

More direct evidence implicating the cAMP pathway was obtained when PKI_{5-24} (300–900 μM) was injected intracellularly prior to applying dopamine. As shown in Fig. 3 *Bottom*, PKI_{5-24} itself had no effect on the measured responses, and the subsequent application of dopamine decreased all of them. Specifically, in the presence of PKI_{5-24}, the average reductions (n = 7) were 10.4% for the coupling potential, 9.8% for the chemical EPSP, and 9.3% for the antidromic spike. These reductions, which are of comparable magnitudes, are therefore due to an increased input conductance that, in contrast to the dopamine-induced enhancements of synaptic transmission, most likely is not mediated via increased cAMP levels in the M cell.

Dopaminergic Innervation of the M-Cell Lateral Dendrite. In view of these findings, it was important to determine if such effects could be of physiological significance, a possibility

requiring the presence of a dopaminergic input at the level of the M cell's lateral dendrite. This possibility was confirmed immunohistochemically by staining with antibodies against tyrosine hydroxylase and dopamine (Fig. 4 *A* and *B*, respectively). At the light microscopic level, the patterns produced by the two were the same, with large diameter fibers (5–10 μm) forming a bundle that runs from the vestibular complex towards the midline, passing dorsal to the M cell. The stained axons send thin (0.1–0.5 μm) branches that have beaded varicosities toward the lateral dendrite, soma, axon cap, and ventral dendrite of this neuron (not shown). In the vicinity of the distal part of the lateral dendrite, the immunoreactive profiles (Fig. 4 *A* and *B*) run between the large myelinated club endings and the small vesicle boutons present in this region (8). However, when serial sections were studied with EM, no direct contacts were found on either the M-cell or the presynaptic terminals (Fig. 4*C*), although the varicosities might be sites of transmitter release, as they contain small clear vesicles (Fig. 4*C Inset*). Instead, tyrosine hydroxylase-stained fibers remain 0.5–5 μm from the dendritic membrane.

DISCUSSION

The present results support the concept that a dopaminergic system can mediate long-lasting modulation of a primary afferent input to the M-cell lateral dendrite. The observed structural pattern, with thin fibers that have many small interposed dopamine-containing varicosities meandering between the afferent terminals in the synaptic bed but apparently not contacting pre- or postsynaptic elements, is similar to that associated with a postulated "nonsynaptic" type of release of monoamines (18, 19).

Dopamine-induced enhancements of both components of synaptic transmission occurred within minutes, a latency comparable to that generally reported for modulators that utilize second messengers (1). Specific evidence supporting the role of cAMP includes the persistence of these effects and the fact that they do not occur after postsynaptic injection of PKI_{5-24}, which binds to the catalytic subunit of the cAMP-dependent protein kinase and prevents subsequent phosphorylations. Presumably, there are different target proteins involved in the regulation of the gap junctions and of chemical transmission, since the two were not always modified by the same proportions (Fig. 1*B*$_1$) or in parallel (Fig. 3 *Top*). Since PKI_{5-24} is a large molecule (molecular weight = 2222.4), it most likely cannot cross the gap junctions to act presynaptically, suggesting that at least the induction of these effects occurs at a postsynaptic site. The tentative identification of the dopamine receptor as being of the D_1 subfamily (17) is consistent with the implication of cAMP.

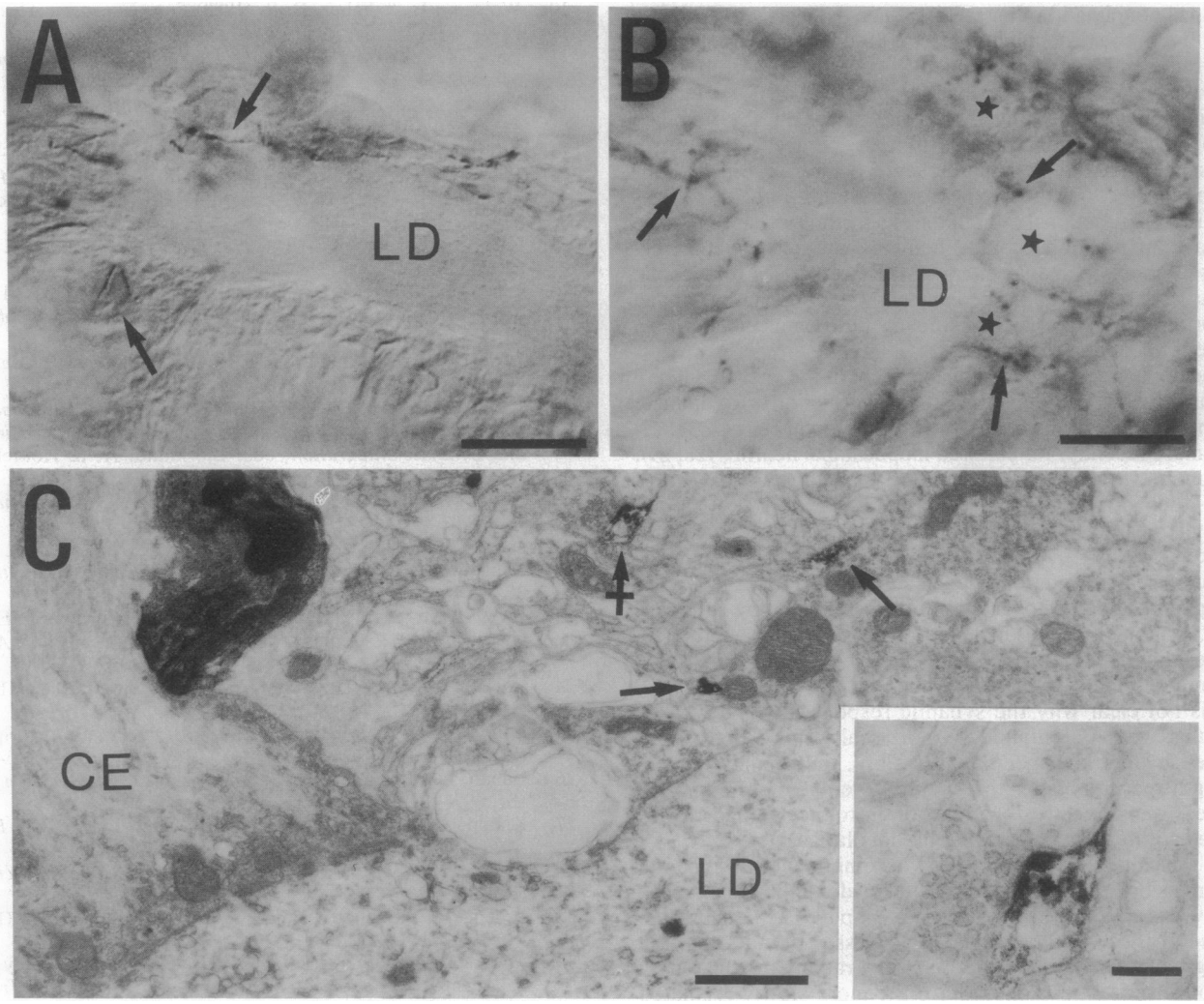

FIG. 4. Dopaminergic innervation of the M-cell lateral dendrite (LD). (*A*) Tyrosine hydroxylase immunoreactive profiles (arrows) at a short distance from the dendritic surface. (*B*) Staining with anti-dopamine immunoglobulins revealing the presence of varicose fibers (arrows) lying between the club endings, some of which appear in cross section (⋆). *A* and *B* were obtained with Nomarski optics. (*C*) Electron micrograph showing the presence of profiles (arrows) containing tyrosine hydroxylase in the vicinity of a large myelinated club ending (CE; left side). (*Inset*) One varicosity (crossed arrow), shown at higher magnification, contains small clear vesicles. (*A* and *B*, bar = 15 μm; *C*, bar = 1 μm; *Inset*, bar = 0.2 μm.)

The concentration of SCH-23390 used here, 50 μM, is somewhat higher than that used previously (5–20 μM) to block D_1 receptor activation in isolated preparations (20, 21). However, the effective concentration at the dendrite, 1.5 mm below the medullary surface in this *in vivo* preparation, is probably less, although the technique used does not allow us to specify it. Regardless, this antagonist is highly specific in all vertebrate species tested (17, 20), including the goldfish, whose D_1 receptor is pharmacologically similar to that of mammals (20). In particular, the gene for the goldfish D_1 receptor has been cloned recently, and its expressed gene product actually has a 5-fold higher affinity for this blocker than does the human receptor (22).

Since dopamine enhances coupling between the afferent endings and the dendrite, with an associated increase in the postsynaptic current, while decreasing input resistance, we conclude that it acts to increase junctional conductance and chemical transmission (see below). The evidence that these effects on synaptic transmission are blocked by PKI_{5-24} further suggests they involve phosphorylation mechanisms. This conclusion is consistent with evidence that only about 2% of the gap junction channels are normally conducting at these synapses (7) and that some connexins, the homologous proteins that form these channels, are good substrates for phosphorylation (23). Our results also complement findings that cyclic nucleotides enhance coupling in other tissues, such as heart (24) and liver (23). In contrast, dopamine decreases junctional conductance and reduces electrotonic and dye coupling between retinal horizontal cells (2, 4) by elevating cAMP levels. These contrasting effects may reflect (*i*) differences in the involved connexins, of which there is a large number (25), or (*ii*) cell-specific modulation of the same connexin. Finally, this distinction between the two neuronal systems probably reflects the different functional roles of the electrotonic synapses. In the retina, gap junctions couple homologous cells, so that decreased coupling should enhance their responses to synaptic inputs, whereas in the M-cell system, the coupling itself is an important component of the monosynaptic connection between afferent fibers and their target.

The dopamine-mediated enhancement of the chemical EPSP may involve a cAMP-dependent phosphorylation of a glutamate receptor similar to that proposed for horizontal cells (5, 26, 27) and, more recently, for cultured hippocampal neurons (28, 29). In contrast to the modifications of synaptic transmission, a cAMP-dependent phosphorylation is not implicated in the additional dopamine effect on input conductance since that is not blocked by PKI_{5-24}. Rather, an alternative intracellular pathway may be involved [for example, one related to the increased phosphoinositide turnover that can be stimulated by D_1 receptor activation (30)]. This increased input conductance probably is due to an action on K^+ channels, which are modulated by dopamine in many tissues (31).

It is not clear why the synaptic responses to the weaker stimulus were potentiated proportionally more than those evoked by the stronger one. Possibly, increasing stimulus strength recruits higher threshold afferents whose synapses are less sensitive to dopamine or introduce nonlinearities related to the larger postsynaptic depolarizations.

At this mixed synapse, the two components of synaptic transmission are modified in parallel by dopamine to increase the overall efficacy of the excitatory input. The similar time course of these two modifications stresses the fact that the strength of an electrical synapse can be rapidly modulated by neurotransmitters, in addition to the more slowly regulated turnover of gap junction proteins (25). Finally, there now is evidence for two mechanisms for inducing long-lasting enhancements of these mixed synapses—namely, those produced by dopamine and by activity (11). Possibly, both plasticities converge at the molecular level, as may also be the case in the hippocampus, where it has been suggested that dopamine is necessary for the maintenance of long-term potentiation (32) and that a cAMP-dependent pathway participates in the induction of persistent changes in synaptic efficacy (33).

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1. Levitan, I. & Kaczmareck, L. (1991) *The Neuron, Cell and Molecular Biology* (Oxford Univ. Press, New York), pp. 247–272.
2. Piccolino, M., Neyton, J. & Gerschenfeld, H. (1982) *J. Neurosci.* **4**, 2477–2488.
3. Teranishi, T., Negishi, K. & Kato, S. (1983) *Nature (London)* **301**, 243–246.
4. Lasater, E. & Dowling, J. E. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 4299–3029.
5. Knapp, A. G. & Dowling, J. E. (1987) *Nature (London)* **325**, 437–439.
6. Martin, A. R. & Pilar, G. (1963) *J. Physiol. (London)* **168**, 443–463.
7. Lin, J. W. & Faber, D. S. (1988) *J. Neurosci.* **8**, 1302–1312.
8. Nakajima, Y. (1974) *J. Comp. Neurol.* **156**, 375–402.
9. Tuttle, R., Masuko, S. & Nakajima, Y. (1986) *J. Comp. Neurol.* **246**, 202–211.
10. Wolszon, L. & Faber, D. S. (1988) *Soc. Neurosci. Abstr.* **14**, 939.
11. Yang, X. D., Korn, H. & Faber, D. S. (1990) *Nature (London)* **348**, 542–545.
12. Yang, X. D. & Faber, D. S. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 4299–4303.
13. Wolszon, L. & Faber, D. S. (1988) *J. Neurosci.* **9**, 784–797.
14. Faber, D. S. & Korn, H. (1978) in *Neurobiology of the Mauthner Cell*, eds. Faber, D. S. & Korn, H. (Raven, New York), pp. 47–132.
15. Klein, M. & Kandel, E. R. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 3512–3516.
16. Lin, J. W. & Faber, D. S. (1988) *J. Neurosci.* **8**, 1313–1325.
17. Sibley, D. & Monsma, F. J., Jr. (1992) *Trends Pharmacol. Sci.* **13**, 61–69.
18. Descarries, L., Beaudet, A. & Watkins, F. (1975) *Brain Res.* **100**, 563–588.
19. Korn, H., Faber, D. S. & Triller, A. (1990) in *Handbook of Chemical Neuroanatomy: Analysis of Neuronal Microcircuits and Synaptic Integration*, eds. Björklund, A. & Hökfelt, T. (Elsevier, New York), Vol. 8, pp. 403–480.
20. Wong, A. O., Chang, J. P. & Peter, R. E. (1992) *Endocrinology* **130**, 1201–1210.
21. Jensen, R. (1992) *Vis. Neurosci.* **8**, 463–467.
22. Manelli, A. M. & Frail, D. E. (1991) *Soc. Neurosci. Abstr.* **17**, 85.
23. Saez, J. C., Spray, D. C., Nairn, A. C., Hertzberg, E. L., Greengard, P. & Bennett, M. V. L. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 2473–2477.
24. Burt, J. & Spray, D. C. (1988) *Am. J. Physiol.* **254**, H1206–H1210.
25. Bennett, M. V. L., Barrio, L. C., Bargiello, T. A., Spray, D. C., Hertzberg, E. & Saéz, J. C. (1991) *Neuron* **6**, 305–320.
26. Liman, E. R., Knapp, A. G. & Dowling, J. (1989) *Brain Res.* **481**, 399–402.
27. Knapp, A. G., Schmidt, K. F. & Dowling, J. E. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 767–771.
28. Wang, L. Y., Salter, M. W. & Mac Donald, J. F. (1991) *Science* **253**, 1132–1135.
29. Greengard, P., Jen, J., Nairn, A. C. & Stevens, C. F. (1991) *Science* **253**, 1135–1138.
30. Andersen, P. H., Gingrich, J. A., Bates, M. D., Dearry, A., Falardeau, P., Senogles, S. E. & Caron, M. (1990) *Trends Pharmacol. Sci.* **11**, 61–69.
31. Lacey, M. G. (1989) *Semin. Neurosci.* **1**, 137–143.
32. Frey, U., Schroeder, H. & Matthies, H. (1990) *Brain Res.* **522**, 69–75.
33. Sarvey, J. M., Burgard, E. C. & Decker, G. (1989) *J. Neurosci. Methods* **28**, 109–124.