Dopaminergic Modulation of NMDA-Induced Whole Cell Currents in Neostriatal Neurons in Slices: Contribution of Calcium Conductances

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Cepeda, Carlos, Christopher S. Colwell, Jason N. Itri, Scott H. Chandler, and Michael S. Levine. Dopaminergic modulation of NMDA-induced whole cell currents in neostriatal neurons in slices: contribution of calcium conductances. *J. Neurophysiol.* 79: 82–94, 1998. The present experiments were designed to examine dopamine (DA) modulation of whole cell currents mediated by activation of *N*-methyl-D-aspartate (NMDA) receptors in visualized neostriatal neurons in slices. First, we assessed the ability of DA, D_1 and D_2 receptor agonists to modulate membrane currents induced by activation of NMDA receptors. The results of these experiments demonstrated that DA potentiated NMDA-induced currents in medium-sized neostriatal neurons. Potentiation of NMDA currents occurred at three different holding potentials, although it was more pronounced at −30 mV. It was mediated by D_1 receptors, because it was mimicked by D_1 agonists and blocked by exposure to a D_1 antagonist. Activation of D_2 receptors produced inconsistent effects on NMDA-induced membrane currents. Either decreases, increases, or no effects on NMDA currents occurred. Second, we examined the contributions of intrinsic, voltage-dependent conductances to DA potentiation of NMDA currents. Blockade of K^+ conductances did not prevent DA enhancement of NMDA currents. However, voltage-activated Ca^{2+} conductances provided a major contribution to DA modulation. The dihydropyridine L-type Ca^{2+} channel blockers, nifedipine, and methoxyverapamil (D−600), markedly reduced but did not totally eliminate the ability of DA to modulate NMDA currents. The D_1 receptor agonist SKF 38393 also enhanced Ba^{2+} currents in neostriatal neurons. Together, these findings provide evidence for a complex interplay between DA, NMDA receptor activation and dihydropyridine-sensitive Ca^{2+} conductances in controlling responsiveness of neostriatal medium-sized neurons.

INTRODUCTION

Excitatory amino acids (EAAs) and dopamine (DA) interact in the neostriatum to provide a major driving force to modulate the activity of the medium-sized spiny neuron. Clinically, altered interactions between EAAs and DA are important and were implicated in the deficits associated with Parkinson's Disease (Schneider and Roeltger 1993) and drugs that alter EAA-mediated neurotransmission were used to improve classical dopaminergic therapy in this disorder (Blandini et al. 1996). Factors underlying DA-EAA interactions were the focus of recent studies. At the morphological level, a close association exists between the glutamate-containing and DA-containing inputs (Bouyer et al. 1984; Freund et al. 1984; Smith and Bolam 1990). The glutamate-containing inputs make synaptic contacts on the heads of spines, whereas the DA-containing inputs synapse on spine necks, dendritic shafts, and on cell bodies (Seasack et al. 1994; Smith and Bolam 1990). Additionally, there is a high degree of colocalization of subtypes of DA receptors and subunits of EAA receptors on neostriatal neurons (Ariano et al. 1997).

The electrophysiological effects of glutamate and DA were studied extensively. Activation of neocortical (and probably thalamic) inputs evokes responses in neostriatal medium-sized spiny cells that are mediated primarily by non-*N*-methyl-D-aspartate (NMDA) glutamate receptors (Cherubini et al. 1988; Herrling 1985). Responses mediated by NMDA receptors can be evoked by direct application of NMDA onto neostriatal neurons (Cepeda et al. 1991, 1993). Synaptic responses mediated by activation of NMDA receptors are typically small at resting membrane potentials, but become significantly larger when the membrane is depolarized (Kita 1996; Levine et al. 1995, 1996b; Nisenbaum et al. 1993). The effects of DA on the electrophysiology of neostriatal cells are complex. They are mediated by multiple receptor subtypes, involve several transduction systems, and can be excitatory or inhibitory (Abercrombie and Jacobs 1985; Akaike et al. 1987; Calabresi et al. 1987; Chiodo and Berger 1986; Herrling and Hull 1980; Rutherford et al. 1988).

Our laboratory has assessed some of the factors involved in determining how DA and EAAs functionally interact in the neostriatum (Altemus and Levine 1996; Cepeda et al. 1993; Levine et al. 1995 1996, 1996a). We have examined the hypothesis that the combination of subtypes of EAA and DA receptors activated determines the direction of DA modulation. We have shown that DA potentiates responses mediated by activation of NMDA receptors, but attenuates responses mediated by activation of nonNMDA receptors (Cepeda et al. 1993; Levine et al. 1995, 1996b). The effects of DA on responses mediated by NMDA receptor activation are mimicked by application of D_1 receptor agonists and are blocked by a D_1 antagonist. The ability of DA and D_1 agonists to potentiate responses mediated by activation of NMDA receptors also is reduced significantly in mutant mice lacking D_{1A} dopamine receptors (Levine et al. 1996a). These studies used current-clamp techniques and it was unclear whether or not effects were the result of DA's documented ability to alter intrinsic, voltage-gated, membrane conductances (Cepeda et al. 1995; Schiffman et al. 1995; Surmeier et al. 1992, 1995; Surmeier and Kitai 1993), or direct effects on ligand gated currents (Calabresi et al. 1995; Fraser and

MacVicar 1994; Hsu et al. 1995). In these current-clamp studies, membrane voltage responses were the result of activation of ligand-gated conductances and intrinsic, voltage-gated conductances to produce the membrane potential changes of the cell (Nisenbaum et al. 1994; Wilson 1993). DA and its receptor agonists have a complex set of effects on K^+, Na^+, and Ca^{2+} conductances (Cepeda et al. 1995; Grief et al. 1995; Schiffman et al. 1995; Surmeier et al. 1992, 1995; Surmeier and Kitai 1993) and these effects will interact with alterations in ligand-gated conductances to produce the resulting modulation of NMDA-induced voltage responses.

The present experiments were specifically designed to determine the relative contributions of voltage- and ligand-gated conductances to DA modulation of NMDA currents. These experiments had two purposes. First, they determined whether or not DA, D_1, and D_2 receptor agonists could modulate NMDA-evoked currents under voltage-clamp conditions by using whole cell techniques in visually-identified cells to measure directly ligand-gated currents. Second, they examined potential ionic mechanisms involved in this modulation.

The results indicated that there is a complex interaction between ligand- and voltage-gated conductances during DA-induced potentiation of NMDA currents. An important contribution of dihydropyridine-sensitive Ca^{2+} conductances occurred. This contribution may have resulted from space-clamp limitations in cells with extensive dendritic processes. However interactions with other factors, such as phosphorylation of NMDA receptor subunits could also be involved in DA-induced potentiation.

METHODS

Animals

All procedures were carried out in accordance with the United States Public Health Services Guide for Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee at UCLA. Sprague-Dawley rat pups (12- to 18-days old, $n = 40$) were used in these experiments. The choice of this age range was based on our experience with electrophysiological recordings from visualized cells with infrared videomicroscopy and differential interference contrast optics (IR-DIC) in neostriatal slices (Cepeda et al. 1995, 1996). Within this age range the cells are easily distinguished and viable and they respond to application of NMDA, DA and its receptor agonists.

Whole cell voltage clamp

After sacrificing, brains were dissected and placed in cold oxygenated artificial cerebrospinal fluid (ACSF) containing (in mM) 130 NaCl, 26 $NaHCO_3$, 3 KCl, 5 $MgCl_2$, 1.25 NaH_2PO_4, 1.0 $CaCl_2$, and 10 glucose (pH 7.2–7.4). After cutting (Microslicer, DSK Model 1500E), transverse neostriatal slices (350 μm) were placed in ACSF (25–27°C) for at least 1 h [in this solution $CaCl_2$ was increased to 2 mM, $MgCl_2$ was decreased to 2 mM, and 4 mM lactate was added (Schurr et al. 1988)]. Slices were constantly oxygenated with 95% O_2-5% CO_2 [pH 7.2–7.4, osmolality 290–300 mOsm, 32 ± 2°C (SE)]. For experiments examining Ba^{2+} currents, the external solution contained 120 NaCl, 3 KCl, 1.25 KH_2PO_4, 20 $NaHCO_3$, 1.3 $MgCl_2$, 10 glucose, 2.4 $BaCl_2$, and 10 tetraethylammonium (TEA; pH 7.2–7.4, osmolality 290–300 mOsm, 32 ± 2°C). After incubation, individual tissue sections were transferred to a custom-designed glass-bottomed perfusion chamber attached to the stage of a fixed-stage upright microscope. The slice was held down with thin nylon threads glued to a platinum wire (Edwards et al. 1989) and submerged in continuously flowing oxygenated ACSF (25°C) at 4 ml/min. Lactate was removed from this ACSF. The slice was continuously perfused at room temperature with various solutions of different compositions depending on the purpose of the experiment.

Slices were viewed with an upright compound microscope (Zeiss Axioskop) using a ×40 water immersion lens (Zeiss, achroplan, numerical aperture 0.75; MacVicar 1984; Dodt and Zieglgansberger 1990, 1994). They were illuminated with near IR light by placing an IR band-pass filter (790 nm, Ealing Optics, Hollston, MA) in the light path. This filter permitted passage of light between 750–1050 nm and thus, the longer wavelengths of IR radiation, which could heat the tissue were cutoff. The image was detected with an IR-sensitive CCD camera (Hamamatsu C2400, Tokyo) and displayed on a video monitor. Analog contrast enhancement and gain control were provided by the camera controller. Digital images were stored on computer/optical disk for subsequent analysis and additional digital contrast adjustment when necessary. Cells were typically visualized from 30–100 μm below the surface of the slice.

Patch electrodes (3–6 MΩ) were filled with one of the following internal solutions depending on the experiment (in mM): 140 K-gluconate, 10 *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES) 2 $MgCl_2$, 0.1 $CaCl_2$, 1.1 ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*,*N*′*N*′-tetraacetic acid (EGTA), and 2 K_2ATP or 125 Cs-methanesulfonate, 4 NaCl, 3 KCl, 1 $MgCl_2$, 5 MgATP, 9 EGTA, 8 HEPES, 1 guanosine 5′-triphosphate (GTP), 10 phosphocreatine, and 0.1 Leupeptin (pH 7.25–7.3, osmolality 280–290 mOsm). Tight seals (2–10 GΩ) from visualized medium-sized cells were obtained by applying negative pressure. The membrane was disrupted with additional suction and the whole cell configuration was obtained. The access resistances ranged from 8–15 MΩ. Cells were held at −70 mV, which closely corresponded to the resting membrane potential of neostriatal neurons in the slice. In some cases the membrane was clamped at −30 mV to remove the Mg^{2+} block of NMDA receptors, or at 30 mV to reduce the contribution of selected inward voltage-dependent currents. Axopatch 200A or 1D amplifiers were used for voltage-clamp recordings. Access resistance was compensated 60–85%. A 3 M KCl agar bridge was inserted between the extracellular solution and the Ag-AgCl indifferent electrode. In all experiments, tetrodotoxin (TTX, 1 μM) was added to the bath to block Na^+ currents after the whole cell configuration was obtained.

To examine the potential contribution of voltage-dependent conductances to DA modulation of NMDA currents, K^+ and Ca^{2+} channels were blocked with selective antagonists. K^+ currents were blocked with a combination of Cs^+ in the pipette and tetraethylammonium (TEA, 10–20 mM) in the bath and selected Ca^{2+} currents were blocked with methoxyverapamil (100 μM) or nifedipine (10–20 μM). Evoked currents were analyzed both on- and off-line (pClamp version 6.0.1). Whole cell voltage-clamp data, especially quantitative estimates of currents from neurons in slices, should be interpreted with caution because the currents measured at the soma are undoubtedly distorted as a result of the nonisopotentiality over the neuronal surface because of space clamp limitations (Armstrong and Gilly 1992).

Drugs were applied in the bath or iontophoretically through a five-barreled pipette (tip diameter 6–8 μm) placed close to the recorded cell (Fig. 1, *A*, *D*, and *E*). The distance of the iontophoretic pipette from the soma of the recorded cell varied from <15 μm at the closest to ~80 μm at the furthest. Distance did not seem to be a crucial variable as similar effects occurred whether pipettes were very close or further from the soma. The pipette contained NMDA (0.1 M, pH 8), DA (0.2 M, pH 4.5), and saline for current

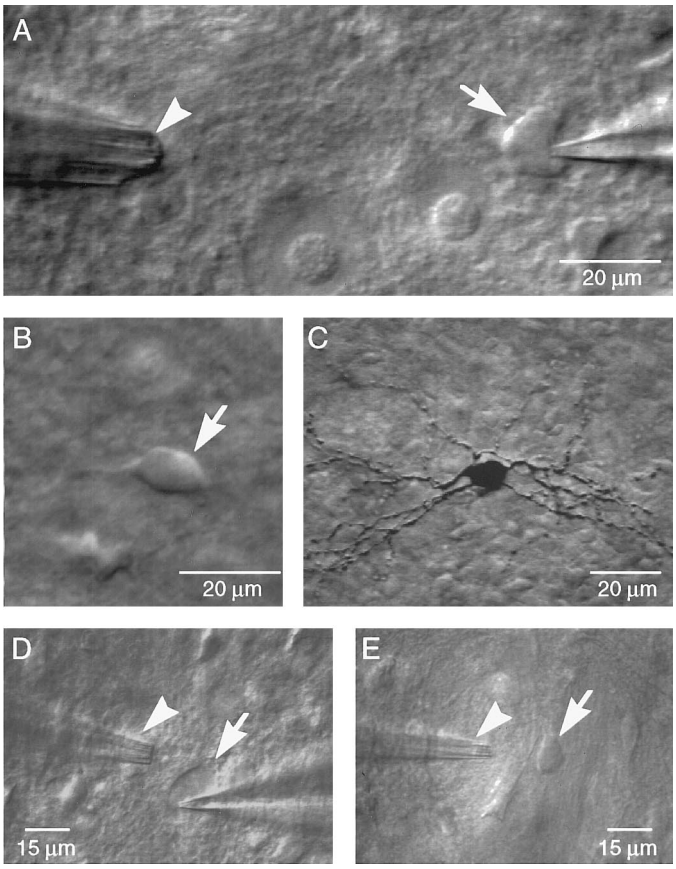


FIG. 1. *A*: example of a medium-sized neostriatal neuron selected for recording. Multibarreled iontophoretic ejection pipette (▽) was positioned 60–80 μm from recorded somata (⇒). Patch electrode is attached to cell. *B*: medium-sized neostriatal neuron (⇒) from a 14 day old rat that was subsequently recorded and filled with biocytin (*C*). *C*: neuron from *B* filled with biocytin. This cell had multiple dendrites that contained numerous varicosities. *D* and *E*: examples showing multibarreled iontophoretic pipettes (▽) ~10–15 μm from recorded cells (⇒). *D*: patch pipette is attached. *D* and *E*: cells are from 16 day old pups.

balancing and control. Holding currents of appropriate polarity ranged between 15–20 nA. NMDA was iontophoretically ejected in an ascending series of current pulse intensities until a threshold for inducing inward currents was obtained. Ejection pulse duration varied from 3 to 5 s, but was held constant for each cell. The interval between ejection pulses was at least 2 min to avoid cumulative effects of the drugs. Applications of saline (of similar polarity and of equal or greater amplitude than those required to produce effects with NMDA or with DA) never produced changes in membrane currents (data not shown). To test the effects of DA, D_1, or D_2 agonists, a single NMDA ejection intensity was chosen (50% above the threshold for inducing inward currents). After a stable baseline response was obtained (2–3 applications of NMDA), DA (applied iontophoretically 30–60 s before and during NMDA application or 50 μM for 10 min in the bath), D_1 (SKF 38393, 10–20 μM, 10-min bath application; A77636, 3 μM, 10-min bath application), or D_2 (quinpirole; 20 μM, 10-min bath application) receptor agonists were applied and responses to NMDA reassessed. Responses to iontophoretically applied NMDA were assessed after application of DA, D_1, or D_2 agonists ceased (5–10 min after iontophoretic application of DA and 10–15 min after bath application of DA, D_1, or D_2 agonists). For bath application, drugs were freshly prepared and dissolved in standard ACSF. Na-metabisulfite (50 μM) was added to solutions of DA and SKF 38393 to prevent oxidation. Concentrations for iontophoretic or bath application of DA, SKF 38393 (10–20 μM), and quinpirole (20 μM) were based on our previous work demonstrating modulation of NMDA- and nonNMDA-induced responses in neostriatum (Cepeda et al. 1993; Levine et al. 1996a,b). The concentration of the isochrom derivative A 77636 (3 μM), was based on the literature (Acquas et al. 1994; Kebabian et al. 1992) and our experience in the mouse neostriatum (Levine et al. 1996). To demonstrate specificity, SCH 23390 (10 μM) was also used to block the effects of SKF 38393. Although several concentrations of some agonists were used, in the electrophysiological experiments we did not attempt to examine systematically concentration-response relationships between cells or within the same cell (e.g., by performing cumulative response studies).

In some experiments, electrodes were filled with 0.2% Biocytin (Sigma, St Louis, MO) in the internal solution to label dendritic and axonal processes of visualized cells. After the experiment, the slice was fixed in 4% paraformaldehyde overnight, then processed according to published protocols (Horikawa and Armstrong 1988).

Data quantification and statistics

Three measures were recorded: maximum response amplitude, response duration at half-maximum amplitude, and response area (amplitude × duration). Area was used as a response measure because subpopulations of cells displayed changes in amplitude or duration but not in both measures; thus the area measurement incorporated information from both measures (Levine et al. 1996a,b). To compare data across experimental conditions, differences in mean response parameters (amplitudes, durations, and areas) in the presence of DA, D_1, or D_2 agonists, were assessed with appropriate one-way analyses of variance for repeated measures followed by multiple comparisons with the Newman-Keuls method or the appropriate *t*-test when mean values from two groups were compared. To provide a more comprehensive analysis of distributional shifts, differences between median values were also analyzed with Wilcoxon signed-rank tests.

In the text and tables, values are presented as means ± SE or medians ± interquartile ranges. Differences between means and medians for experimental and control conditions were considered statistically significant when $P < 0.05$.

RESULTS

Visually identified cells

All recordings were obtained from cells with medium-sized somata (maximum diameter <15 μm; cross-section area = 99 ± 6 μm^2; Fig. 1, *A–E*). Although cells with large somata (maximum diam >20 μm) could also be identified in the slices, these were not sampled in the present experiments. Dendritic spines could not be resolved by using IR-DIC optics. When biocytin was placed in recording pipettes, neurons displayed dendritic fields characteristic of medium-sized spiny neurons (Fig. 1*C*). Spines were occasionally present on dendrites of biocytin-filled cells, but they were not abundant because of the young age of the rats. More frequently dendritic processes displayed numerous varicosities (Fig. 1*C*).

NMDA-induced currents

Whole cell voltage-clamp recordings were obtained from 65 cells. The effects of SKF 38393 and nifedipine on high-voltage–activated (HVA) Ba^{2+} currents were examined in 15 cells. In 46 cells, inward currents were evoked by iontophoretic

application of NMDA (Fig. 2). Increasing iontophoretic ejection current intensity produced greater amplitude and duration inward currents (Fig. 2*A*). At 50% above threshold iontophoretic ejection current (−96 ± 11 nA) and at V_h −70 mV, NMDA-induced inward currents ranged from −20 to −220 pA in maximum amplitude and 5–28 s in duration for all cells tested. The peak inward current occurred within 3–5 s of the start of the ejection pulse. Changing the distance between the iontophoretic electrode and the cell did not change the characteristics of the inward currents. These inward currents were voltage dependent, displaying maximum amplitudes at holding potentials of −20 to −40 mV (Fig. 2*D*). NMDA-induced currents were enhanced in Mg^{2+}-free ACSF (Fig. 2*B*) and were reduced by bath application of 2-amino-5-phosphonovalerate (AP5), a selective NMDA receptor antagonist (Fig. 2*C*). The slow time course of the activation of NMDA-induced current at the more negative holding potentials (−70 to −30 mV) was primarily a result of the presence of Mg^{2+}. When experiments were run in Mg^{2+}-free ACSF (Fig. 2*B*) or at positive holding potentials (Figs. 2*D* and 6), time courses for activation induced currents were considerably more rapid. Within the age-range studied, there were no consistent age-induced differences in NMDA-induced currents. However when recordings were performed in cells from younger rats (postnatal days 7–10), NMDA-induced currents were substantially smaller (Cepeda et al. 1996). Therefore the present analysis restricted the age-range examined to minimize differences because of the maturation of NMDA-induced currents in neostriatal neurons.

MODULATION OF NMDA-INDUCED CURRENTS BY DA, D_1, AND D_2 AGONISTS. DA enhanced NMDA-induced currents. In these first experiments, all quantitative measures of the effects of DA, D_1, and D_2 agonists were made at a V_h of −70 mV to minimize activation and DA-induced modulation of intrinsic, voltage-dependent K^+ and Ca^{2+} currents and in the presence of TTX to block most Na^+-dependent conductances. Application of DA either iontophoretically (n = 9; ejection currents ranged from 50 to 140 nA) or in the bath (n = 6; 50 μM) produced statistically significant, reversible increases in the mean maximum amplitude (14 ± 6%), the mean duration (19 ± 7%), and the mean area (38 ± 14%) of NMDA-evoked currents (Fig. 3*A*; Table 1). Alone, DA produced no change in current at −70 mV. To further examine changes in the population of cells, distributions of percentage change in response area were constructed (Fig. 4). As pointed out above, area was the most consistent measure. In the presence of DA, 1 response decreased in area, 4 were relatively unchanged (between 0–10% increase), and 10

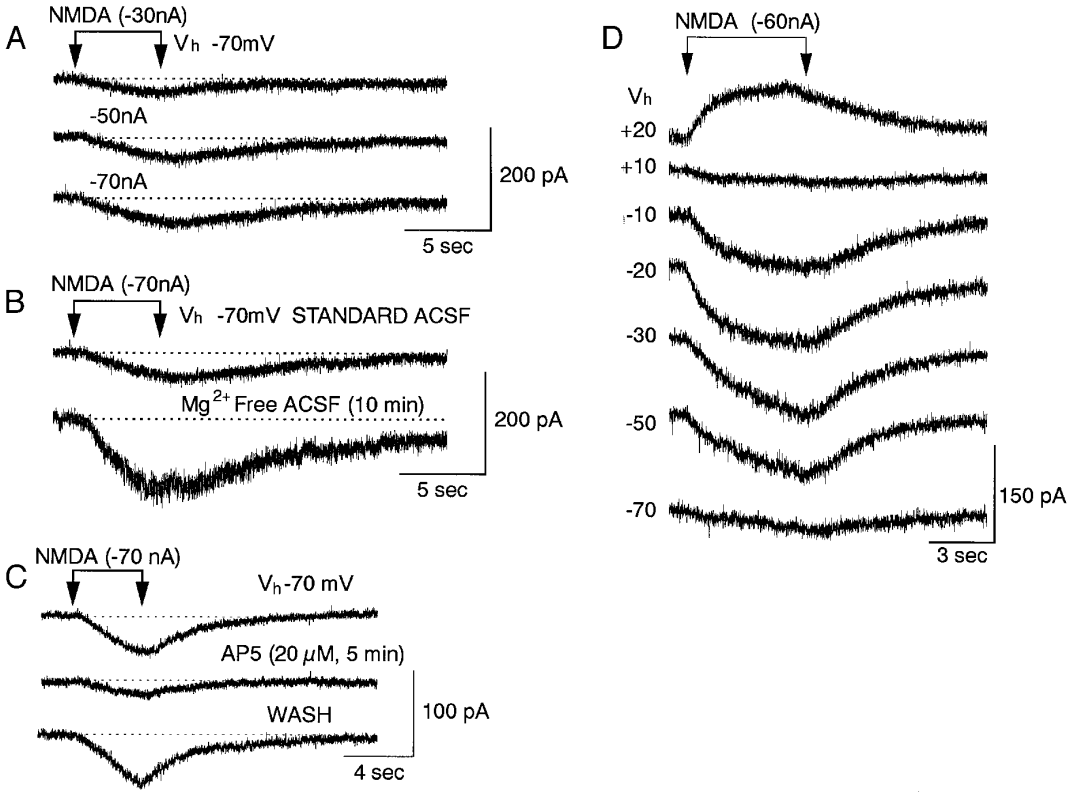


FIG. 2. Properties of currents evoked by iontophoretic application of *N*-methyl-D-aspartate (NMDA). *A*: inward currents evoked by 3 increasing intensities of iontophoretic ejection currents (−30, −50, and −70 nA). Increasing ejection current intensity evoked larger amplitude and longer duration inward currents. In this and other figures, NMDA is applied between arrows, numbers refer to iontophoretic ejection current polarities and intensities. V_h, holding potential. *B*: enhancement in Mg^{2+}-free artificial cerebrospinal fluid (ACSF). *Top*: control in standard ACSF at V_h −70 mV. *Bottom*: bathing cell in Mg^{2+}-free ACSF (10 min) produced an increase in amplitude and duration of NMDA-evoked current. *C*: attenuation of NMDA-induced current by 2-amino-5-phosphonovalerate (AP5). *Top*: control response. *Middle*: obtained 5 min after exposure to AP5 (20 μM). *Bottom*: obtained 10 min after wash in ACSF without AP5. *D*: voltage dependence of NMDA current. NMDA (−60 nA) was applied for 5 s at different holding potential steps (−70–20 mV). For this cell maximum amplitude of inward current occurred between V_h −30 to −20 mV.

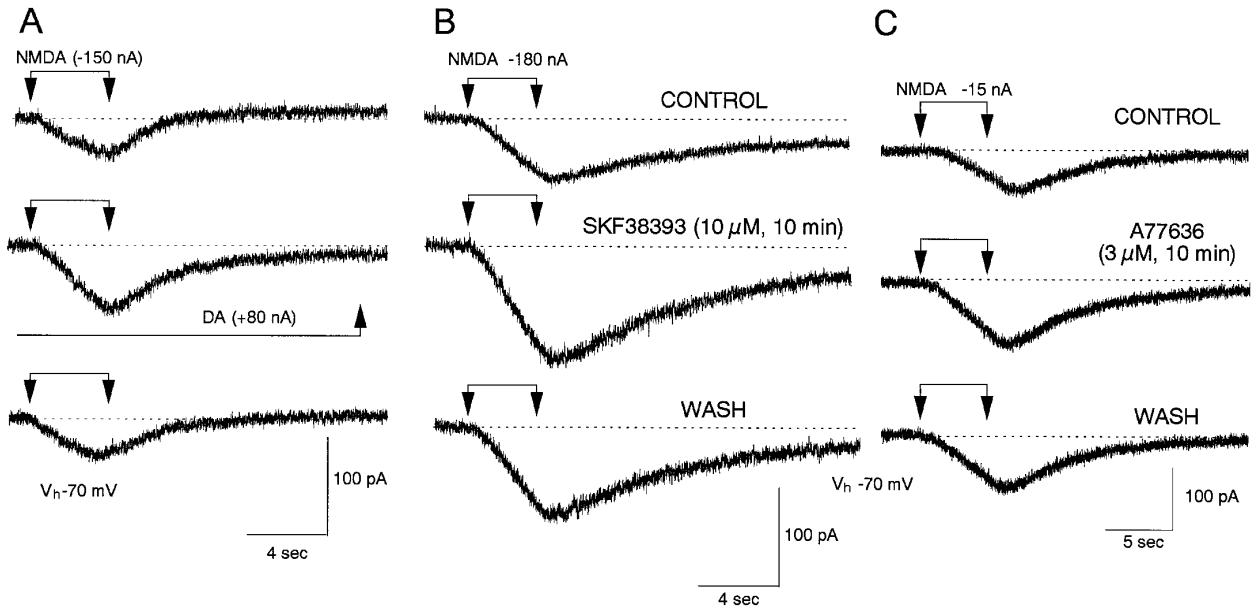


FIG. 3. Dopamine (DA) and D_1 agonists enhance NMDA-induced currents. *A*, *top*: a control response to NMDA. *Middle*: in presence of DA (80 nA, ejection began 1 min before trace and continued until upward arrow), response was increased in amplitude and duration. *Bottom*: response returned to control amplitude and duration 10 min after ejection of DA ceased. *B*, *top*: a control response to NMDA. *Middle*: bath application of SKF 38393 (10 μM, 10 min) increased amplitude and duration of response. *Bottom*: partial recovery 20 min after exposure to SKF 38393. *C*, *Top*: a control response. *Middle*: bath application of isochrom derivative, A 77636 (3 μM, 10 min), increased amplitude and duration of response. *Bottom*: partial recovery 20 min after exposure to A 77636.

displayed increases >10% (Fig. 4*A*). There was a statistically significant increase in the median value (median = 29% increase in area; interquartile range = 0–50%; P = 0.00684).

Application of D_1 receptor agonists, SKF 38393 (n = 8; 10–20 μM) and A 77636 (n = 3; 3 μM) also significantly and reversibly increased amplitude (26 ± 7%) and area (32 ± 9%) of the NMDA-evoked inward currents (Figs. 3,

TABLE 1. *Modulation of responses induced by NMDA*

	Response Parameters				Percent Change From Control		
	Amplitude (pA)	*n*	Duration (s)	Area	Amplitude	Duration	Area
			A. Dopamine				
Control	76 ± 11	15	13 ± 1	1032 ± 178			
Dopamine	88 ± 14*		16 ± 1*	1412 ± 262*	14 ± 6	19 ± 7	38 ± 14
Wash	68 ± 9		12 ± 1	903 ± 169	−2 ± 5	1 ± 4	0 ± 8
			B. D_1 agonists				
Control	102 ± 15	11	14 ± 2	1365 ± 186			
D_1	124 ± 16*		15 ± 2*	1798 ± 245*	26 ± 7	5 ± 2	32 ± 9
Wash	112 ± 15		14 ± 2	1464 ± 1177	8 ± 4	−4 ± 3	3 ± 5
			C. D_2 agonists				
Control	105 ± 17	6	15 ± 1	1640 ± 386			
D_2	122 ± 28		13 ± 1	1640 ± 448	10 ± 11	−11 ± 5	−3 ± 10
Wash	122 ± 27		13 ± 1	1595 ± 393	10 ± 10	−12 ± 5	−5 ± 7

Values are means ± SE; *n*, number of cells tested. *B:* data are pooled for responses modulated by both D_1 agonists. NMDA, *N*-methyl-D-aspartate.

* Differences between mean values for experimental and control treatments are statistically significant for each measure using one-way analyses of variance for repeated measures followed by Newman-Keuls multiple comparison tests. For DA: F = 5.10, df = 2/25, P = 0.014 for amplitude; F = 8.61, df = 2/25, P = 0.0014 for duration; and F = 6.40, df = 2/25, P = 0.0057 for area. For each analysis of variance, Newman-Keuls multiple comparisons revealed that experimental treatment was significantly different from control and wash. Wash and control treatments were not significantly different. For D_1 agonists: F = 16.4, df = 2/19, P = 0.00007 for amplitude; F = 3.91, df = 2/19, P = 0.0378 for duration; and F = 11.7, df = 2/19, P = 0.00048 for area. For amplitude and area, Newman-Keuls multiple comparisons revealed that experimental treatment was significantly different from control and wash. For duration, the experimental treatment was significantly different from wash only. Wash and control treatments were not significantly different for each comparison.

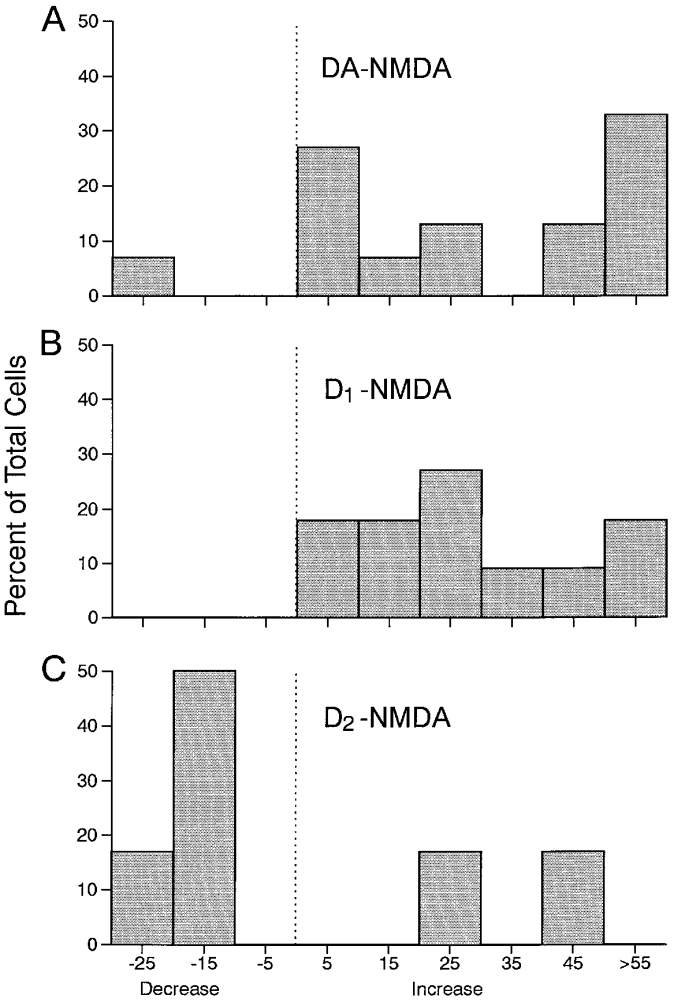


FIG. 4. Distributions of percentage change in response area. *A*: distribution of changes in NMDA-induced response area for all cells exposed to DA. Response area of all but one cell increased. *B*: distribution of changes in NMDA-induced response area for all cells exposed to D_1 agonists. Response area of all cells increased. *C*: distribution of changes in NMDA-induced response area for all cells exposed to D_2 agonist. Both increases and decreases in response area occurred. For all graphs, vertical axes are percentages of cells falling into each category. Horizontal axes are percentage changes in response area. Bin sizes are 10%, except for last bin, which represents all responses ≥50%. Note that both increases and decreases in response area are shown.

B and *C*; Table 1). Duration only increased slightly (5 ± 2%). Data were pooled for cells tested with the two D_1 agonists because there were no differences in their effects. Alone, these D_1 agonists produced no change in current. All cells displayed increases in response area (Fig. 4*B*). There was a significant increase in the median value (median = 28% increase in area; interquartile range = 11–38%; P = 0.00195). The effects of SKF 38393 were blocked when cells were exposed first to SCH 23390 (n = 3; 10 μM), a D_1 receptor antagonist (Fig. 5). Mean percentage changes in amplitude (4.5 ± 4.5%), duration (0 ± 0%), and area (4.5 ± 4.5%) were small when cells were exposed to SCH 23390 before and during SKF 38393. In this group, one cell displayed an increase in amplitude with no change in duration though NMDA-induced current responses in the other two cells were unchanged.

Application of the D_2 receptor agonist, quinpirole (20 μM; n = 6) produced inconsistent effects from cell to cell (Table 1). Mean changes in amplitude (10 ± 11%), duration (−11 ± 5%), and area (−3 ± 10%) were small. Three cells displayed increases in the area of the response and three cells displayed decreases (Fig. 4*C*). There was no statistically significant change in median values (median = −13% decrease in area; interquartile range = −17–18%).

Contribution of K^+ and CA^{2+} conductances to DA modulation of NMDA-induced currents

To more systematically analyze the contributions of voltage-dependent K^+ and Ca^{2+} conductances to DA modulation, the compositions of the internal and external solutions were altered. When Cs^+ was used in the internal solution and TEA (10–20 mM) was included in the external solution to block the majority of voltage-dependent K^+ conductances, DA enhanced NMDA currents (Fig. 6*A*). Under these conditions, DA-induced increases were present at −70 mV holding potential, maximized at holding potentials of −30 mV but

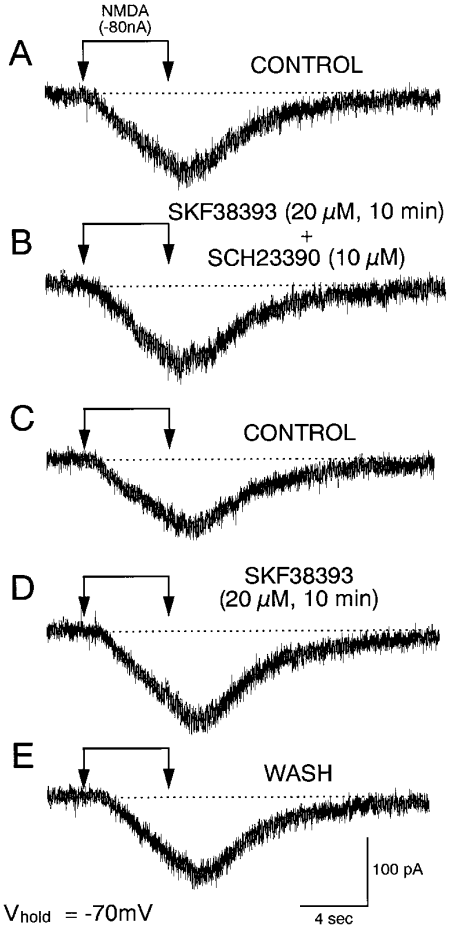


FIG. 5. Effects of SKF 38393 are blocked by D_1 antagonist SCH 23390. *A*: control response to NMDA. *B*: slice was first bathed in SCH 23390 (10 μM) for 5 min and then bathed in SKF 38393 (20 μM) and SCH 23390 for 10 min. Amplitude and duration of NMDA-evoked current were similar to control values. *C*: subsequent control response in same cell. *D*: bath application of SKF 38393 (20 μM) alone increased amplitude and duration of NMDA-evoked current. *E*: recovery of NMDA-evoked response after 15-min wash in absence of SKF 38393.

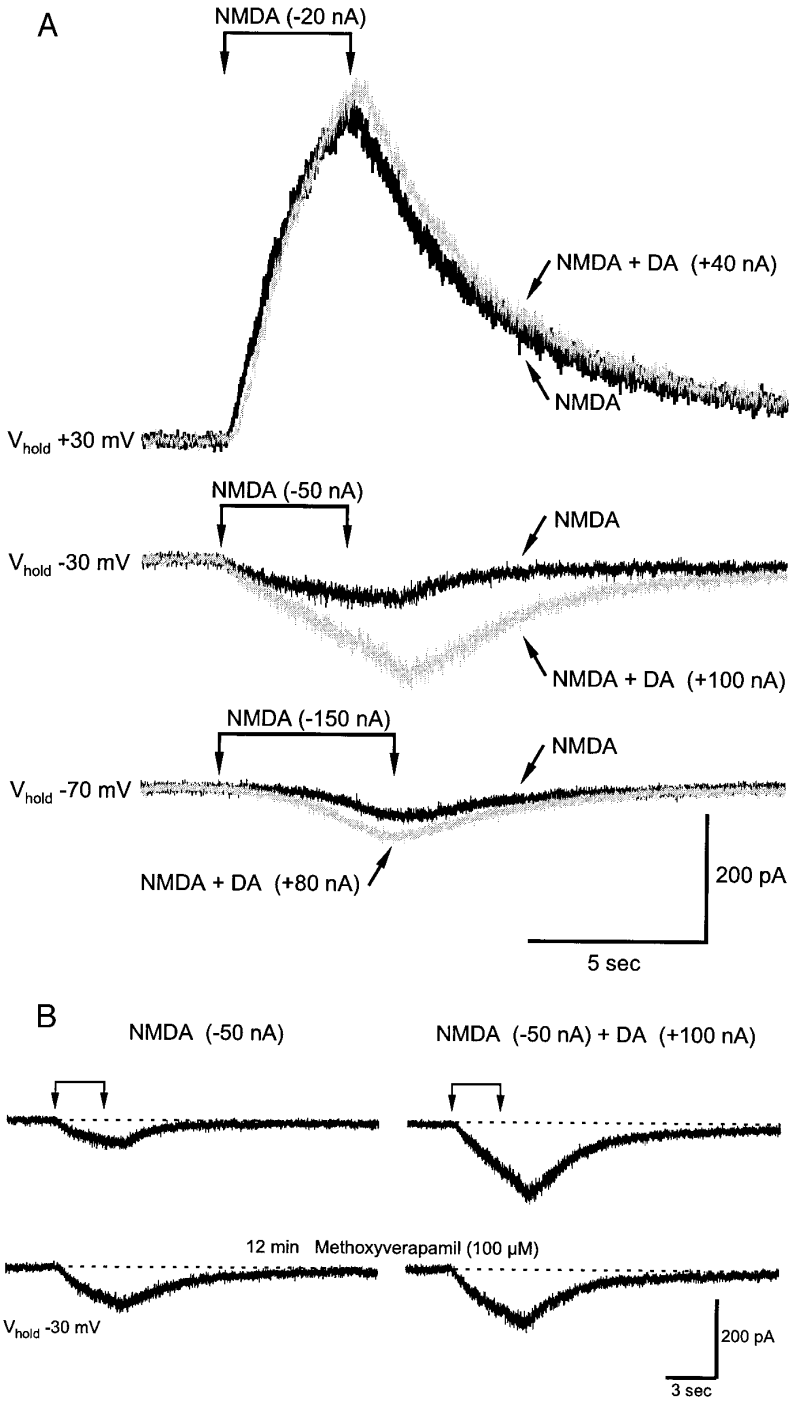


FIG. 6. *A*: DA enhances NMDA-induced currents in presence of K^+ channel blockers, Cs, and tetraethylammonium (TEA) at 3 different holding potentials. Black traces show NMDA-induced currents at 3 different holding potentials (30, −30, and −70 mV) in presence of TEA (20 mM) and with Cs in patch pipette. NMDA applied between arrows. Gray traces show that DA (applied before and during NMDA application) enhanced each response. Each pair of traces is from a different cell. NMDA and DA ejection currents are shown for each trace in parentheses. *B*: DA enhancement of NMDA-induced currents is reduced in presence of Ca^{2+} channel blocker, methoxyverapamil (D-600, 100 μM). *Left*: NMDA-induced currents at −30 mV holding potential, in presence of TEA (20 mM) and with Cs in patch pipette before (*top*) and 12 min after (*bottom*) exposure to bath application of methoxyverapamil. *Right*: DA potentiation of NMDA current (*top*) and its reduction after 12 min exposure to D-600 (*bottom*). Top 2 traces are same traces superimposed at −30 mV holding potential in *A*.

also occurred at holding potentials of 30 mV (Fig. 6*A*, Table 2*A*). At −70 mV holding potentials, DA induced statistically significant reversible increases in maximum response amplitude, response duration, and response area. At −30 mV holding potentials, DA significantly enhanced response duration and area (Table 2*A*). The increase in amplitude was not statistically significant (Table 2*A*). At 30 mV holding potentials, DA continued to produce enhancements, but only the increase in area was statistically significant (Table 2*A*). At 30 mV, 5 cells displayed increases in response area whereas the responses of two cells did not change markedly in the presence of DA.

As a further procedure to reduce the effects of unclamped voltages on dendrites, in some experiments we placed the iontophoretic pipette very close to the soma (10–20 μm; Fig. 1, *D* and *E*), the region of the cell that is clamped best. Under these conditions DA enhancements of NMDA currents were also observed. These enhancements were greater in magnitude than enhancements induced when iontophoretic pipettes were further apart (at −70 mV holding potentials response area potentiation was 71 ± 21 vs. 38 ± 14% for close and distant electrode placements, respectively).

Because blocking Na^+ and K^+ channels did not alter the

TABLE 2. *Contribution of Ca^{2+} and K^+ conductances to DA modulation of responses induced by NMDA*

Holding potential	Percent Change		
	Amplitude	Duration	Area
A. K^+ Conductance block (Cs and TEA)			
−70 mV	30 ± 8[a,b]	31 ± 13[b]	71 ± 21[b]
−30 mV	41 ± 36	54 ± 22[c]	139 ± 96[c]
+30 mV	12 ± 15	31 ± 14	55 ± 38[e]
B. Ca^{2+} and K^+ Conductance block (D-600, Cs, and TEA)			
−30 mV	8 ± 11[d]	29 ± 12	38 ± 14
C. K^+ Conductance block (Cs and TEA)			
−30 mV	11 ± 4[f]	34 ± 8[f]	48 ± 8[f]
D. Ca^{2+} and K^+ Conductance block (Nifedipine, Cs, and TEA)			
−30 mV	0.2 ± 2[g]	15 ± 6	16 ± 7

DA, dopamine; NMDA, *N*-methyl-D-aspartate; TEA, tetraethylammonium. [a] Mean ± SE. Data are based on recordings from 8 neurons. Not all neurons were tested at each holding current under each condition. Data are percentage changes for each measure. [b] Increases in mean values are statistically significant for each measure using paired *t*-tests. $t = 4.5$, df = 6, $P < 0.004$; $t = 2.25$, df = 6, $P < 0.05$; $t = 4.24$, df = 6, $P < 0.005$ for amplitude, duration, and area, respectively. [c] Increases in mean values are statistically significant for duration and area only using paired *t*-tests. $t = 2.90$, df = 4, $P < 0.05$; $t = 4.86$, df = 4, $P < 0.01$ for duration and area, respectively. [d] Increases in amplitude, duration, and area were not statistically significant. [e] Increases in mean values are statistically significant for area only using paired *t*-tests. $t = 1.95$, df = 6, $P < 0.05$. [g] Increases in mean values are statistically significant for each measure using paired *t*-tests. $t = 4.89$, df = 3, $P < 0.05$; $t = 2.54$, df = 3, $P < 0.05$, $t = 2.35$, df = 3, $P < 0.05$ for amplitude, duration, and area, respectively. [g] Increases in amplitude, duration, and area were not statistically significant.

ability of DA to enhance NMDA currents, we explored next the potential contribution of Ca^{2+} conductances. Specific Ca^{2+} conductances (L-type) were shown to be enhanced by D_1 receptor activation in a subpopulation of acutely isolated neostriatal cells (Surmeier et al. 1995). It is possible that such conductances could contribute to DA modulation. Slices were bathed in a solution containing TTX; TEA and Cs was used in the internal solution. Either D-600 (methoxyverapamil) or nifedipine was added after DA enhancement of NMDA-induced currents was demonstrated. In this condition, DA facilitation of NMDA currents was markedly reduced but not totally eliminated (Fig. 6*B* and Table 2, *B* and *D*). Data were quantified at −30 mV holding potentials because DA-induced enhancements in area were maximal. D-600 (100 μM) markedly reduced the DA-induced potentiation of response amplitude, but had less effect on response duration (Table 2*B*). Response area increased but the increase in the presence of D-600 was not as great compared with the increase in the absence of D-600 (38 ± 14 vs. 139 ± 96%). None of the mean increases in the presence of D-600 were statistically significant (Table 2*B*). Before D-600 was added to the bath 4 of 5 cells displayed increases in response amplitude and all cells displayed increases in response duration and area. After 20 min in D-600 only one cell displayed an increase in amplitude, all cells continued to display increases in duration, although they were smaller, and all cells displayed increases in area, but again they were smaller compared with increases in the absence of D-600. A similar pattern of reduction in DA-induced potentiation of NMDA responses occurred at 30 mV holding potentials in the presence of D-600. The potentiation in amplitude was reduced but the DA-induced increases in response duration and area were less affected.

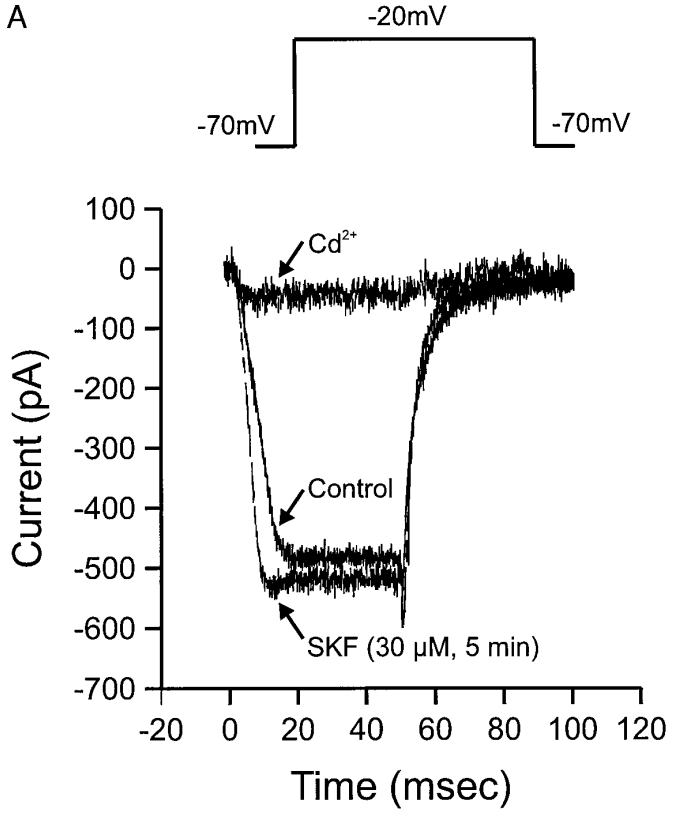
Nifedipine (10–20 μM, 5–10 min, $n = 4$) also reduced but did not totally eliminate DA or SKF 38393-induced potentiation. Before nifedipine, DA induced statistically significant increases in response amplitude, duration, and area (Table 2*C*). After exposure to nifedipine only average duration and area increased and the increases were no longer statistically significant (Table 2*D*). These findings indicate that dihydropyridine-sensitive (probably L-type) voltage-dependent Ca^{2+} conductances contribute to DA-induced modulation.

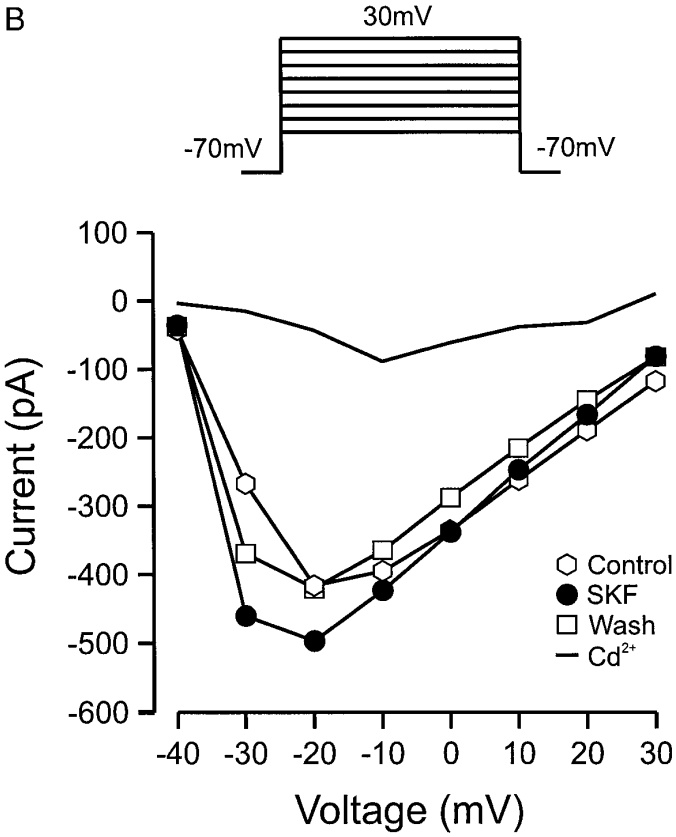
Modulation of BA^{2+} currents by the D_1 receptor agonist SKF 38393

To provide a more direct test of D_1 modulation of Ca^{2+} currents, Ba^{2+} was substituted as the charge carrier (Surmeier et al. 1995) and the effects of SKF 38393 on Ba^{2+} currents were examined. Ba^{2+} currents were evoked by a series of voltage steps before and during exposure to SKF 38393 (30 μM, 5 min). Cells were held at −70 mV and stepped to test voltages (−60–30 mV, 10 mV per step) for 50 ms. This protocol evokes primarily HVA conductances in neostriatal cells, as there is little evidence for low voltage-activated (LVA) currents (Surmeier et al. 1995). The internal solution contained Cs^+ and the external solution contained TEA and TTX as described above. Baseline currents were recorded for 10 min followed by 5-min applications of SKF 38393 and then wash. Ba^{2+} currents remained stable for up to 40 min (data not shown). In control conditions average peak current was 803 ± 135 pA ($n = 7$) and occurred at voltage steps of −20 to −10 mV. Cd^{2+} (100 μM, 5 min) reduced the peak current by 86 ± 3% ($n = 6$; Fig. 7). In the presence of SKF 38393, average peak currents increased significantly (15 ± 4%, $t = 2.64$, df = 5, $P < 0.05$; Fig. 7). In contrast, in the presence of nifedipine (10 μM) SKF 38393 produced an average decrease in peak Ba^{2+} currents that was statistically significant (−7 ± 3%, $t = 2.40$, df = 7, $P < 0.05$). These findings provide confirmatory evidence that L-type HVA currents are enhanced by SKF 38393.

DISCUSSION

Several new observations have emerged from the present experiments. An important finding was that DA potentiated NMDA-induced currents in medium-sized neostriatal neurons. Potentiation appeared to be mediated by D_1 receptors, as it was mimicked by D_1 agonists and blocked by a D_1 antagonist. Activation of D_2 receptors produced inconsistent effects on NMDA currents. Another major outcome was that DA-induced potentiation of NMDA currents was mediated, at least in part, by enhancement of dihydropyridine-sensitive Ca^{2+} conductances. In contrast, DA modulation appeared to be independent of pre- and postsynaptic TTX-sensitive Na^+ conductances and Cs^+ and TEA-sensitive K^+ conductances.





Modulation in developing neostriatum

The use of developing animals is an issue that could influence interpretation of these findings. We limited the age range to 12–18 postnatal days. This age represents a compromise between the presence of NMDA responses, the effects of DA, and the ability to clearly visualize cells. By postnatal day 14 most, but not all, of the functional properties of neostriatal neurons have developed (Tepper and Trent 1993; Walsh et al. 1989). We have found that NMDA-induced membrane currents develop primarily over the first 14 postnatal days in the rat neostriatum (Cepeda et al. 1996). The ability of DA and its receptor agonists to modulate these responses appears to develop in parallel (unpublished observations). Although we cannot rule out additional maturation of NMDA-induced currents, nor additional maturation of DA-induced modulation, the present findings support our previously published current-clamp work in adults.

A further developmental issue that contributes to the present studies (see below) is the maturation of Ca^{2+} conductances in neostriatal cells. There is little published work on maturation of different types of Ca^{2+} conductances in neostriatum. In cultured neostriatal cells, both HVA and LVA Ca^{2+} conductances are present at birth and at least up to 2 wk of age (Bargas et al. 1991). A more recent study suggests that HVA Ca^{2+} currents develop during the first postnatal week in medium-sized neostriatal cells (DeFazio and Walsh 1995), although it is unclear whether these are L-type currents. Thus Ca^{2+} conductances appear to be present before DA modulation of NMDA-induced currents matures.

DA-EAA interactions

Electrophysiological evidence indicates that DA and EAAs interact to alter the responsiveness of neurons in a number of brain areas. In vivo, DA modulates glutamate-induced excitatory responses in neostriatal cells in awake unrestrained rats (Kiyatkin and Rebec 1996; Pierce and Rebec 1995) and in anesthetized preparations (Hu and White 1997). In vitro, DA and EAAs in the neostriatum also were implicated in use-dependent synaptic changes (Calabresi et al. 1992a–c; Walsh 1993; Walsh and Dunia 1993; Wickens et al. 1996). There is now considerable support for a role for activation of D_1 receptors enhancing NMDA-induced responses in nucleus accumbens and hippocampus (Gonon and Sundström 1996; Harvey and Lacey 1997; Hsu 1996) in addition to the neostriatum (Cepeda et al. 1993; Fraser and MacVicar 1994; Gonon 1997; Levine et al. 1996a,b). In nucleus accumbens, DA via D_1 receptors also can attenuate synaptic responses, possibly via a presynaptic mechanism (Harvey and Lacey 1996; Nicola et al. 1996; Nicola and Malenka 1997). Responses of basal forebrain neurons are decreased by DA via D_1 receptors (Momiyama and Sim

FIG. 7. Effects of SKF 38393 on Ba^{2+} currents. *A*: example of a Ba^{2+} current induced by voltage steps from −70 to −20 mV (*inset*). Inward current was blocked by Cd^{2+}. In presence of SKF 38393 (30 μM, 5 min), current was enhanced. *B*: current-voltage plot for cell shown in *A*. Peak current was plotted for each voltage step (*inset*). In presence of SKF 38393, peak current was increased at −30 and −20 mV voltage steps. This effect was reversible. Inward current was markedly decreased in presence of Cd^{2+}.

1996; Momiyama et al. 1996). In the neocortex, a number of different interactions between DA and responses mediated by EAAs occur (Cepeda et al. 1992; Fraser and MacVicar 1994; Law-Tho et al. 1994). DA modulates EAA currents in Mauthner cells (Pereda et al. 1992, 1994). In cultured chick spinal cord motoneurons, DA and activation of D_1 receptors enhances glutamate-induced currents (Smith et al. 1995). In perch horizontal cells, DA decreases the desensitizing component of currents induced by glutamate and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) but not kainate, producing a greater steady-state current (Knapp et al. 1990; Schmidt et al. 1994). DA via D_1 receptors enhances a glutamate-gated current in bipolar cell dendrites of the retina of the tiger salamander as well (Maguire and Werblin 1994). It would appear, then, that the actions of DA on EAA receptors may be expressed differently depending on the brain area and the type of neuron studied.

Intrinsic, voltage-gated conductances and mechanisms underlying potentiation

In voltage-clamp experiments in neurons in slices, currents recorded at the soma are distorted as a result of space clamp limitations. This is a major problem especially when the cells have extensive dendritic trees and it becomes most problematic when contributions of intrinsic, voltage-sensitive currents have to be ruled out. In the present study, specific Na^+ and K^+ conductances did not appear to be involved in this aspect of DA modulation. All experiments were conducted in the presence of TTX to rule out contributions of pre- and postsynaptic TTX-sensitive Na^+ conductances and many experiments were performed in the presence of Cs^+ and TEA to rule out contributions of specific K^+ conductances. DA potentiation of NMDA currents occurred in virtually all cases. Previously we demonstrated that blockade of K^+ conductances had minimal effects on DA modulation of synaptically-evoked responses in neostriatal cells (Altemus and Levine 1996). These responses were mediated primarily by activation of non-NMDA receptors.

In contrast with Na^+ and K^+ conductances, it is clear that unclamped voltages, because of activation of voltage-dependent, intrinsic Ca^{2+} conductances on dendrites, contributed to DA potentiation of NMDA currents. The experiments provided three major pieces of evidence for Ca^{2+} conductance involvement. First, pharmacological blockade of Ca^{2+} conductances markedly decreased DA-induced potentiation. Application of dihydropyridine L-type Ca^{2+} channel blockers significantly reduced but did not entirely eliminate DA potentiation. Second, the magnitude of the potentiation was voltage dependent. Enhancements in response area occurred at -70 mV, were maximized at more depolarized holding potentials (-30 mV), and were greatly reduced at 30 mV. Third, D_1 receptor activation enhanced Ba^{2+} conductances in neostriatal neurons in slices. Although not described in the present results, we also have demonstrated, by using imaging techniques, that application of DA or a D_1 agonist increases NMDA-induced intracellular Ca^{2+} in cultured cortical and acutely dissociated neostriatal cells (Levine et al. 1997). Together this evidence indicates that DA modulation of NMDA currents appears to be partially dependent on activation of Ca^{2+} conductances, probably generated by unclamped voltages in dendrites. This fact may be thought of as a major drawback in the more intact preparation as in the present study. Of potentially more importance, it underscores the active role of voltage-dependent conductances in dendrites in the integration of synaptic signals (Eilers and Konnerth 1997; Magee and Johnston 1995).

In the neostriatum, most medium-sized neurons express HVA Ca^{2+} currents exclusively (Bargas et al. 1994), although LVA currents also were reported (Hoehn et al. 1993). D_1 receptor activation produces different effects on Ca^{2+} currents, reversibly reducing N- and P-type but enhancing L-type conductances. These actions are mediated by the adenosine 3′,5′-cyclic monophosphate (cAMP) protein kinase A (PKA) transduction cascade (Surmeier et al. 1995). In current-clamp recordings, D_1 receptor activation increases depolarization-induced plateau potentials (Hernandez-Lopez et al. 1997), which are mediated principally by L-type Ca^{2+} conductances (Cherubini and Lanfumey 1987). When membrane potentials are depolarized (more positive than -60 mV), D_1 receptor activation enhances excitation in all neostriatal neurons (Hernandez-Lopez et al. 1997). Because L-type Ca^{2+} conductances are the only inward current enhanced by D_1 receptor agonists, the authors suggest that facilitation of this current is the principal mechanism used by DA to potentiate excitatory events (Hernandez-Lopez et al. 1997). Interestingly, L-types channels also display voltage-dependent facilitation in neostriatal neurons (Song and Surmeier 1996). Together with the present findings, these data provide converging evidence that DA-induced potentiation of NMDA responses involves enhancement of L-type Ca^{2+} conductances.

Although it is likely that unclamped voltages were responsible for activation of voltage-dependent Ca^{2+} conductances in dendrites, it is also conceivable that specific types of Ca^{2+} conductances can be activated at resting membrane potentials (-70 mV). There is evidence that a population of dihydropyridine-sensitive channels are active at resting membrane potentials. These channels appear present throughout the neuron and are concentrated in the proximal dendrites (Magee et al. 1996). There also is evidence for a novel dihydropyridine-sensitive Ca^{2+} LVA conductance that operates at negative potentials (Ferroni et al. 1996). Finally, it is possible that D_1 receptor activation recruits dihydropyridine-sensitive Ca^{2+} channels as described in chromaffin cells (Artalejo et al. 1990). These channels are quiescent but can be activated by repetitive depolarizations, depolarizing prepulses, or by agents that raise cAMP, such as D_1 receptor agonists.

Enhancement of NMDA currents by DA may involve other potential mechanisms besides facilitation of voltage-dependent Ca^{2+} conductances. First, if one accepts that L-type Ca^{2+} channels, activated because of unclamped voltages in distal processes, are the main contributors to DA modulation, these should be localized more exclusively to these processes. However there is some evidence that L-type channels are more densely located in the soma and proximal dendrites (Westenbroek et al. 1992), areas that would be better clamped in our study. Assuming this is the case, then the increase in NMDA responses may not be accounted for solely by unclamped distal processes. Second, a study in

hippocampus has demonstrated a differential modulatory effect of DA in slices and in dissociated cells (Hsu et al. 1995). Low concentrations of DA (0.3 μM) inhibited synaptic responses in slices but had no effect on NMDA-mediated responses in dissociated cells. A higher concentration (10 μM) enhanced NMDA currents in dissociated cells (K.-S. Hsu, personal communication). Influences from unclamped voltages in more distal dendritic segments are minimized in acutely dissociated cells, suggesting an effect that may be independent of Ca^{2+} conductances in dendrites. Finally, in some experiments, we applied NMDA and DA very close to the soma (10–20 μm), a region that is better clamped. In all cases, DA potentiated NMDA currents. However it should be pointed out that diffusion of the NMDA and DA may still have been sufficient to affect more distal dendritic segments that are less well-clamped.

There is a growing body of evidence that the cAMP-dependent PKA and the protein kinase C (PKC) cascades modulate voltage-gated Ca^{2+}, as well as EAA ligand-gated conductances (Artalejo et al. 1990; Blank et al. 1996; Greengard et al. 1991; Lukyanetz and Kostyuk 1996; Markram and Segal 1991; Pfeiffer-Linn and Lasater 1993; Smart 1997). In a previous study with current-clamp recordings, we showed that forskolin potentiates NMDA responses (Colwell and Levine 1995). There is also evidence for direct regulation of NMDA receptor phosphorylation by DA. In nucleus accumbens slices, forskolin, DA, or D_1 receptor agonists (but not D_2 agonists) increase phosphorylation of the NMDA-R1 subunit. DA-induced phosphorylation of NMDA-R1 was blocked by an inhibitor of PKA. Moreover, the ability of DA to phosphorylate NMDA-R1 was attenuated in mice lacking the gene for DARPP-32 (Snyder et al. 1996). Both PKA and PKC potentiate NMDA-mediated responses in the neostriatum but only PKC in the hippocampus (Blank et al. 1996). It is thus becoming increasingly clear that activation of D_1 DA receptors converges on the same transduction systems that phosphorylate NMDA receptors (Konradi et al. 1996). This mechanism does not exclude interactions with facilitation of Ca^{2+} conductances and both mechanisms may simultaneously contribute to the enhancement of NMDA currents.

Conclusions

Interactions between DA and EAA transmitter systems are important in controlling responsiveness of neostriatal neurons. The present experiments demonstrate that DA potentiates NMDA-induced currents and that one important mechanism underlying this effect is DA's ability to potentiate L-type Ca^{2+} conductances. It is possible that an interaction between D_1 receptor activation, Ca^{2+} channels, cAMP production, and NMDA-R1 phosphorylation occurs to form the basis of a positive feedback loop. From a functional perspective, this interaction can have multiple consequences. As we pointed out previously (Cepeda et al. 1993; Levine et al. 1996) and as pointed out by others more recently (Hernandez-Lopez et al. 1997), strong, maintained inputs to medium-sized neostriatal cells that activate NMDA receptors will be enhanced by this mechanism. Depression of synaptic responses can become potentiation when the cell is depolarized (Wickens et al. 1996). Moreover these findings underscore the diversity of actions that can occur as a consequence of DA receptor activation.

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