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Dopamine D_2 Receptor Modulation of K^+ Channel Activity Regulates Excitability of Nucleus Accumbens Neurons at Different Membrane Potentials

Mariela F. Perez, Francis J. White, and Xiu-Ti Hu
Department of Cellular and Molecular Pharmacology, Chicago Medical School, Rosalind Franklin University of Medicine and Science, North Chicago, Illinois

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Perez, Mariela F., Francis J. White, and Xiu-Ti Hu. Dopamine D_2 receptor modulation of K^+ channel activity regulates excitability of nucleus accumbens neurons at different membrane potentials. *J Neurophysiol* 96: 2217–2228, 2006. First published August 2, 2006; doi:10.1152/jn.00254.2006. The nucleus accumbens (NAc) is a forebrain area in the mesocorticolimbic dopamine (DA) system that regulates many aspects of drug addiction. Neuronal activity in the NAc is modulated by different subtypes of DA receptors. Although DA signaling has received considerable attention, the mechanisms underlying D_2-class receptor (D_2R) modulation of firing in medium spiny neurons (MSNs) localized within the NAc remain ambiguous. In the present study, we performed whole cell current-clamp recordings in rat brain slices to determine whether and how D_2R modulation of K^+ channel activity regulates the intrinsic excitability of NAc neurons in the core region. D_2R stimulation by quinpirole or DA significantly and dose-dependently decreased evoked Na^+ spikes. This D_2R effect on inhibiting evoked firing was abolished by antagonism of D_2Rs, reversed by blockade of voltage-sensitive, slowly inactivating A-type K^+ currents (I_{As}), or eliminated by holding membrane potentials at levels in which I_{As} was inactivated. It was also mimicked by inhibition of cAMP-dependent protein kinase (PKA) activity, but not phosphatidylinositol-specific phospholipase C (PI-PLC) activity. Moreover, D_2R stimulation also reduced the inward rectification and depolarized the resting membrane potentials (RMPs) by decreasing "leak" K^+ currents. However, the D_2R effects on inward rectification and RMP were blocked by inhibition of PI-PLC, but not PKA activity. These findings indicate that, with facilitated intracellular Ca^{2+} release and activation of the $D_2R/G_q/PLC/PIP_2$ pathway, the D_2R-modulated changes in the NAc excitability are dynamically regulated and integrated by multiple K^+ currents, including but are not limited to I_{As}, inwardly rectifying K^+ currents (I_{Kir}), and "leak" currents ($I_{K\text{-}2P}$).

INTRODUCTION

The nucleus accumbens (NAc) is a limbic structure innervated by the dopaminergic (DAergic) input from the ventral tegmental area of the midbrain and the glutamatergic input from the medial prefrontal cortex (mPFC). This brain region is involved in control of cognitive tasks (Kalivas et al. 2005; Nicola et al. 2000; Pennartz et al. 1994). Activity of NAc neurons is regulated by both DA D_1- and D_2-class receptors (D_1Rs and D_2Rs, respectively) (Hu and White 1997; Hu et al. 2005; White and Wang 1986). Dysfunction in the mesocorticolimbic DA system has been implicated in certain neuropsychiatric disorders, including drug addiction (Hyman 2005; Hyman and Malenka 2001; Kalivas and Hu 2006; Kalivas et al. 2005; White and Kalivas 1998; Wise 1998). Previous findings indicate that D_2R activation modulates the intrinsic excitability of DA-innervated neurons, usually causing a decrease in evoked action potentials (Cepeda et al. 2001; Gulledge and Jaffe 1998; Hu and Wang 1988; Tseng and O'Donnell 2004; West and Grace 2002). This inhibitory effect of D_2R modulation on evoked Na^+ spike firing has been related to activation of a variety of K^+ currents (Congar et al. 2002; Greif et al. 1995; Ljungstrom et al. 2003). However, the mechanisms underlying activation of these K^+ currents remain unknown.

However, previous findings also revealed some excitatory effects of D_2R stimulation on evoked neuronal activity. For instance, coactivation of D_2Rs and D_1Rs can depolarize cell membrane in dorsal striatal cells and increase evoked Na^+ spike firing in NAc shell cells by inhibition of Na^+-K^+ ATPase and combination of $G_\beta\gamma$ subunits released from $D_2R/G_{i/o}$ coupling and $G_{\alpha s}$-like subunits from D_1R/G_s coupling, respectively (Bertorello et al. 1990; Hopf et al. 2003). Moreover, D_2R stimulation also facilitates Ca^{2+} mobilization (Greengard et al. 1999; Parikh et al. 1996), leading to an enhancement in voltage-sensitive sodium currents by facilitating dephosphorylation of the Na^+ channel by calcineurin (Hu et al. 2005). In addition, stimulation of D_2Rs also reduces the inward rectification in freshly dissociated mPFC pyramidal neurons by inactivating the cAMP/PKA cascade (Dong et al. 2004), which may induce membrane depolarization from the resting levels if the "leak" K^+ currents are suppressed. In spite of the apparent discrepancies in the D_2R regulation of ion channel/pump activity that have caused many controversies in the past with respect to the inhibitory or excitatory effects of D_2R stimulation, these findings actually indicate that the D_2R is functionally involved in modulation of the intrinsic excitability of DA-innervated neurons by dynamically integrating activity of voltage-gated ion channels, including various types of K^+ channels at different membrane potentials.

Given the above, we hypothesized that D_2R stimulation would significantly change the excitability of medium spiny NAc neurons by modulating the function of a variety of K^+ channel subtypes. The present study was performed to determine whether and how D_2R stimulation changes the evoked Na^+ spike firing, the inward rectification, and the RMP by modulating the activity of voltage-gated potassium channels (VGKCs, mainly I_A), I_{Kir}, and the "leak" K^+ currents ($I_{K\text{-}2P}$) in medium spiny neurons located within the core NAc of rats.

Address for reprint requests and other correspondence: X.-T. Hu, Department of Cellular and Molecular Pharmacology, Chicago Medical School, Rosalind Franklin University of Medicine and Science, 3333 Green Bay Road, North Chicago, IL 60064-3095 (E-mail: xiu-ti.hu@rosalindfranklin.edu).

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METHODS

Animals

Adolescent male Sprague–Dawley rats (Harlan, Indianapolis, IN), 4–5 wk old (Spear 2000), were group housed in a temperature- and humidity-controlled vivarium under a 12-h light/dark cycle. Food and water were freely available. After ≥3 days acclimation to the vivarium, rats were used for acute experiments examining the D_2R modulation of the membrane activity of medium spiny neurons (MSNs).

Preparation of brain slices

All procedures were in strict accordance with the Guide for the Care and Use of Laboratory Animals (1996) and were approved by our Institutional Animal Care and Use Committee. Rats were decapitated under halothane anesthesia and the brain was immediately excised and immersed in ice-cold artificial cerebrospinal fluid (aCSF) containing (in mM): NaCl 124, KCl 2.5, $NaHCO_3$ 26, $MgCl_2$ 2, $CaCl_2$ 2, and glucose 10; pH 7.4; 310 mOsm/l. Coronal slices (300 μm) containing the NAc were cut with a vibratome (Leica VT1000S) and incubated in oxygenated (95% O_2-5% CO_2) aCSF for 1 h at room temperature before recording.

Whole cell current-clamp recordings in brain slices

Brain slices were anchored in a recording chamber and perfused by gravity-fed oxygenated aCSF (34°C) with the γ-aminobutyric acid type A ($GABA_A$) receptor blocker SR-95531 (4 mM) and the glutamatergic receptor blocker kynurenic acid (2.5 mM) at a flow rate of 2–3 ml/min. Patch recording pipettes (3–5 MΩ) were pulled from Corning 7056 (Corning, NY) glass capillaries with horizontal pipette puller (Flaming/Brawn P-97, Sutter Instruments, Novato, CA) and filled with internal recording solution (in mM): K^+-gluconate 120, HEPES 10, KCl 20, $MgCl_2$ 2, Na_2ATP 3, Na_2GTP 0.3, and biocytin 0.1%. Recordings were initiated in visually identified MSNs within the core of the NAc using differential interference contrast (DIC) microscopy (Stuart et al. 1993) and an Axopatch 200B amplifier (Axon Instruments, Union City, CA). After a whole cell configuration was formed, voltage-clamp mode was converted to current-clamp recording. Voltage signals were amplified in bridge mode and digitized by a DigiData 1200 Series (Axon Instruments) and distributed to a computer running pCLAMP 9 software (Axon Instruments).

Na^+-dependent action potentials were generated by injection of step-depolarizing current pulses with 0.05-nA increments, ranging from 0 to 0.5 nA. Characteristics of the action potentials were obtained from the initial spike evoked by the minimal depolarizing current pulse (rheobase) in each MSN recorded. In all cases Na^+ spikes were evoked from the resting membrane potential (RMP). The amplitude of action potential (in millivolts) was measured from spike threshold to peak level. The deepness of afterhyperpolarization (AHP amplitude, mV) was measured from the equipotential point of the spike threshold to the maximum deflection of the membrane hyperpolarization after the end of the action potential. The half-action potential duration was measured at half-amplitude level.

To determine the effects of applied drugs on evoked Na^+ spikes as well as on hyperpolarized membrane potentials, the majority of NAc neurons were recorded under a condition in which the RMP was held at −80 mV. This approach gave each individual NAc neuron the same basal (control) potential level and therefore made the results obtained from different cells comparable (Hu et al. 2004). In MSN, it was previously shown that secondary dendrites are a crucial cellular compartment for postsynaptic D_2 modulation of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) currents (Hernandez-Echeagaray et al. 2004). To avoid influences from depolarization/hyperpolarization of dendritic membrane in the soma, AMPA and *N*-methyl-D-aspartate (NMDA) currents were blocked by kynurenic acid applied in the bath medium (see above). In another group of cells, the drug effects on evoked Na^+ spikes were studied by holding the RMP at −50 mV. In these cells, Na^+-dependent action potentials were generated by injection of step-depolarizing current pulses with 0.04-nA increments ranging from 0 to 0.2 nA. Injection of higher currents caused artificial distortions in the form of Na^+ spikes, which made analysis of evoked action potentials very difficult. Under this depolarized RMP, certain VGKCs were inactivated and their effects on action potentials were eliminated. In addition, to determine the effects of applied drugs on RMP, a group of NAc neurons was recorded at their resting status without any membrane potential holding. To calculate the percentage change in spiking for the time-course experiments, a current pulse was selected to evoke six or seven spikes as basal activity. This current pulse was then used at different time points in all cells. Firing rates of evoked spikes were averaged from a 2-min time period before drug application in each cell. Such values were then normalized to 100% as control and used to compare with that during drug application. To calculate the percentage change in the dose–response curve, the firing rate of evoked spikes was averaged from the last 2 min of quinpirole perfusion (the third to fifth minute) in which quinpirole achieved its maximal effect and then compared with the normalized control (100%).

The current–voltage relationship (*I–V* curve) was studied with perfusion of the specific Na^+ channel blocker tetrodotoxin (TTX, 1 μM) and the calcium channel blocker cadmium (Cd^{2+}, 200 μM). Five minutes after TTX and Cd^{2+} application, the cell membrane was hyperpolarized by injecting negative current pulses (200-ms duration, −0.8 to 0 nA). Under these conditions, recorded changes in the *I–V* curve would reflect activation or inactivation of I_{Kir} (Nisenbaum and Wilson 1995). Membrane properties were studied in the following manners: RMP was measured in the absence of injected current and the input resistance (R_{in}, MΩ) was determined from linear regression in the linear range (±0.1-nA range) of the *I–V* curve established by plotting the steady-state potential change in response to hyperpolarizing current pulses. Time constants were determined by the fit function of pCLAMP software. The whole cell pipette series resistance was <20 MΩ and bridge was compensated. Only NAc cells that had a stable RMP at or more negative than −75 mV with evoked spikes that overshot across 0-mV membrane potentials were used for analysis of membrane properties and further drug treatment.

Drug application

Separate subgroups of NAc neurons were recorded with application of different drugs and ion channel blockers. Selective agonist and antagonist for D_2Rs (quinpirole, 10 μM, and eticlopride, 10 μM, respectively) were used to determine whether D_2R stimulation affects the evoked action potentials, the inward rectification during membrane hyperpolarization, and RMP. Moreover, the effects of D_2R agonists on evoked Na^+ spikes were also studied when the membrane potentials were held at a more depolarized membrane potential level (about −50 mV). In addition, the effects of DA on evoked firing were studied with different concentrations (0, 20, 40, and 80 μM). DA at a concentration of 40 μM was used in the time-course experiment. To minimize oxidation, all experiments with DA were conducted in the dark. To confirm the effects of quinpirole on D_2R-mediated inhibition of firing, the D_1R antagonist SCH-23390 (10 μM) was concurrently applied with DA in the time-course experiments. The selective PKA inhibitor H-89 (10 μM) was applied in bath solution, whereas the Rp isomer of adenosine-3′,5′-cyclic monophosphorothioate (Rp-cAMPs, 500 μM), another selective PKA inhibitor, was dialyzed to the cytosol by the recording pipette. Because internally applied Rp-cAMPs might affect RMP during formation and stabilization of whole cell configuration, thereby causing inaccurate measurement of control RMP, data from other experimental groups were pooled to form the control group. Measurements of RMP from this group were then compared with that affected by Rp-cAMPs and quinpirole plus Rp-cAMPs, respectively. The selective phosphatidylinositol-specific PLC (PI-

PLC) inhibitor ($ET\text{-}18\text{-}OCH_3$, 1-*O*-octadecyl-2-*O*-methyl-*sn*-glycero-3-phosphorylcholine, 500 μM) was externally applied in the aCSF medium. These inhibitors were used to determine whether the D_2R-mediated changes in NAc neurons were modulated by the cAMP/PKA cascade or through other signaling pathway(s) associated with activation of PLC.

The relatively selective A-type K^+ channel blocker 4-aminopyridine (4-AP, at a low concentration of 10 μM) was used to determine whether and how I_A was involved in the D_2R-mediated changes in evoked action potentials. All drugs were prepared according to manufacturer's specifications (Sigma Chemicals, St. Louis, MO). Bath solutions with such drugs and ion channel blockers were made with aCSF immediately before use.

Statistical analysis

Unpaired Student's *t*-tests were used to estimate the significance of the difference (*$P < 0.05$ and **$P < 0.01$) in the membrane properties between control and drug-treated groups of NAc neurons. Repeated-measures ANOVA was used for comparison of the drug-induced changes in the current–response (evoked spikes) curves as well as in the inward rectification curves between control and drug-treated groups. Comparisons of the drug-induced alterations in evoked action potentials in the time-course experiments were carried out with ANOVA. In addition, post hoc comparisons were carried out using the Newman–Keuls test.

RESULTS

D_2R stimulation decreased evoked Na^+ spikes in NAc neurons

All NAc neurons were MSNs, as evidenced by differential interference contrast (DIC) microscopy, and recorded within the core region (Hu et al. 2004; O'Donnell and Grace 1993). The majority of neurons showed a slow, repetitive spike-firing pattern reported for typical MSNs (Mahon et al. 2000; Nisenbaum et al. 1994). To study the effects of D_2R stimulation on the properties of evoked Na^+ spikes, a series of current pulses (200 ms) was delivered to NAc neurons, at an interval of 20 s between each pulse. These current pulses ranged from 0 to +0.5 nA, in 0.05-nA steps (Fig. 1*A*). Current-evoked spike-response curves showed that bath application of quinpirole (10 μM) significantly and reversibly decreased evoked action potentials in the majority of MSNs (13 of 20 cells recorded, 65%) [control vs. quinpirole, $n = 20$ cells, repeated-measures ANOVA, $F_{(1,38)} = 6.89$, $P < 0.05$; post hoc Newman–Keuls test, *$P < 0.05$] (Fig. 1*B*). Quinpirole dose-dependently decreased evoked Na^+ spikes at all doses tested (2, 4, 6, and 10 μM) ($n = 10$ cells for each concentration, unpaired *t*-test, *$P < 0.05$) (Fig. 1*C*). Because greater reduction in firing was observed with 10 μM of the D_2R agonist (30.6 ± 3.1%), this concentration was used for later experiments in this study. In time-course experiments, quinpirole-induced reduction in firing occurred quickly and achieved its maximal levels 3–5 min with application of the agonist [$n = 13$ cells; ANOVA, $F_{(77,803)} = 4.75$, $P < 0.05$; post hoc Newman–Keuls test, $P < 0.05$] (Fig. 1, *D–F*).

The effects of DA (10, 20, 40, and 80 μM) on evoked firing were also studied in NAc cells. Bath application of DA significantly and reversibly decreased evoked action potentials at 40 and 80 μM (control vs. DA 40 μM: 100.3 ± 0.29 vs. 87.2 ± 0.5%; control vs. DA 80 μM: 100.3 ± 0.29 vs. 86.6 ± 0.7%, respectively; $n = 10$ cells for each concentration, unpaired

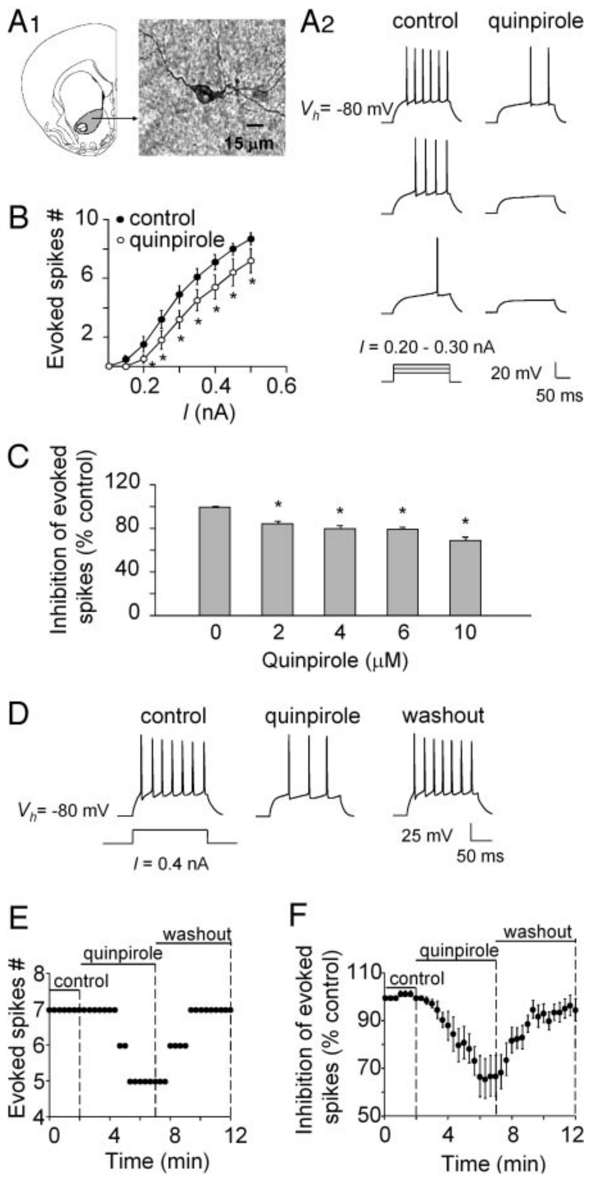

FIG. 1. D_2-class receptor (D_2R) stimulation decreased action potentials evoked in nucleus accumbens (NAc) neurons. *A_1*: a medium spiny neuron (MSN) in the core of NAc labeled by biocytin staining. *A_2*: representative traces showing that evoked action potentials in the NAc neuron were reduced by stimulation of D_2Rs with quinpirole (10 μM). Depolarizing currents required for generation of action potentials were also increased with application of quinpirole (*left*: control vs. *right*: quinpirole). *B*: current-evoked spike-response curves showing that evoked Na^+ spikes were significantly reduced in NAc neurons with quinpirole ($n = 20$ cells, with post hoc test, *$P < 0.05$). *C*: dose–response graphs showing that quinpirole-induced decrease in firing was significant at all concentrations studied (2–10 μM). A greater effect (≅30%) was achieved with 10 μM. Bars represent means ± SE. *D*: quinpirole-induced decrease in firing was reversible and washed out by fresh bath solution. *E* and *F*: time–response curves indicate that the D_2R-mediated inhibition in evoked Na^+ spikes achieved its maximal levels about 5 min after bath application of quinpirole (10 μM). It returned to control levels after 3–5 min of washout (*E*: single cell; *F*: $n = 12$ cells, with post hoc test, $P < 0.05$).

t-test, *$P < 0.05$) (Fig. 2, *A* and *B*). To further determine whether the quinpirole-induced decrease in evoked firing was mediated by D_2Rs, DA (40 μM) was concurrently applied with SCH-23390 (10 μM), a selective D_1R antagonist. Similar to quinpirole, combined application of DA and SCH-23390 induced a significant and reversible decrease in evoked Na^+

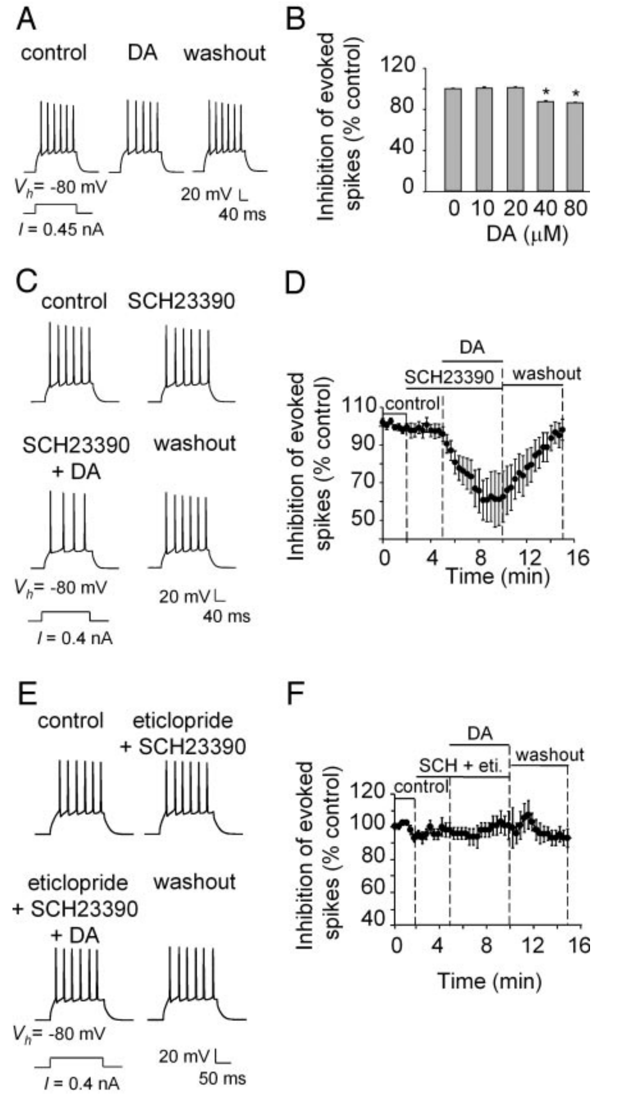

FIG. 2. Dopamine (DA) decreased evoked firing in NAc spiny cells in a dose-dependent manner. *A*: representative traces showing that application of DA (40 μM) reduced evoked action potentials. *B*: dose–response (10, 20, 40, 80 μM) graphs showing that DA at concentrations of 40 and 80 μM induced a significant decrease in evoked firing (n = 10 cells/concentration group, unpaired t-test, $*P < 0.05$). Bars represent means ± SE. *C*: representative traces showing that, when DA (40 μM) was applied with the selective D_1R antagonist SCH-23390 (10 μM), it produced a greater reduction in evoked firing as compared with its action along. *D*: time–response curve shows that evoked firing was significantly and reversibly decreased after concurrent application of DA and SCH-23390 (n = 11 cells, with post hoc test, $P < 0.05$). *E*: representative traces showing that concurrent application of DA (40 μM) with the selective D_1R antagonist SCH-23390 (10 μM) and the selective D_2R antagonist eticlopride (10 μM) did not produce a significant change in evoked firing. *F*: time–response curve shows that evoked firing was not significantly altered after concurrent application of DA, SCH-23390, and eticlopride (n = 8 cells, $P > 0.05$).

spikes [n = 11 cells, ANOVA, $F_{(45,460)} = 3.48$, $P < 0.05$; post hoc Newman–Keuls test, $P < 0.05$] (Fig. 2, *C* and *D*). Furthermore, this DA-mediated reduction in evoked Na^+ spikes was completely abolished when the D_2R antagonist eticlopride (10 μM) was applied concurrently with DA (40 μM) and SCH-23390 (10 μM). Under these experimental conditions there was no significant difference in evoked firing along the time course [n = 8 cells, control vs. DA plus SCH-23390 and eticlopride, ANOVA, $F_{(45,322)} = 0.49$, $P > 0.05$] (Fig. 2, *E* and *F*). This result confirms that the DA- or quinpirole-induced reduction in evoked Na^+ spikes is selectively mediated by activation of the D_2R.

The D_2R-mediated reduction in evoked firing was accompanied by significant alterations in certain membrane properties, including increased rheobase (control vs. quinpirole: 0.23 ± 0.01 vs. 0.27 ± 0.02 nA, n = 20 cells, paired t-test, $P <$ 0.05) and reduced threshold of action potential (control vs. quinpirole: −40.25 ± 0.68 vs. −42.06 ± 0.92 mV, n = 20 cells; paired t-test, $P < 0.05$). There were no significant changes in the input resistance, spike amplitude, duration of action potential measured at the half-amplitude level (half-AP duration), and amplitude of AHP (control vs. quinpirole: 113.34 ± 10.68 vs. 109.41 ± 9; 83.58 ± 1.62 vs. 82.55 ± 1.71; 0.95 ± 0.03 vs. 0.97 ± 0.04; 13.92 ± 0.59 vs. 13.15 ± 0.61 mV, respectively, n = 20 cells, paired t-test, $P > 0.05$).

D_2R-mediated inhibition in evoked firing was blocked by concurrent application of eticlopride, a selective D_2R antagonist. Nevertheless, because some MSNs did not show reduction in evoked spikes in response to D_2R stimulation, this antagonist experiment was performed only in spiny cells that showed agonist effect. The D_2R-mediated inhibition in spike firing was identified first and washed out. These cells were then recorded with concurrent application of quinpirole and eticlopride with the same concentration (10 μM). Under these conditions, quinpirole-induced reduction in evoked firing was completely blocked by eticlopride [n = 6 cells, ANOVA, $F_{(52,340)} = 3.56$, $P < 0.05$, post hoc Newman–Keuls test, $P < 0.05$] (Fig. 3, *A* and *B*).

Inhibition of PKA, but not PLC, mimicked the D_2R-mediated decrease of evoked Na^+ spikes

It is well established that activation of D_2Rs inhibits adenylyl cyclase (AC) activity, thereby reducing cytosolic cAMP levels and PKA activity (Sibley 1995; Stoof and Kebabian 1981, 1982). Therefore if the D_2R-mediated decrease in evoked Na^+ spikes was regulated by the cAMP/PKA cascade, inhibition of PKA activity should resemble the effects of quinpirole on suppressing evoked action potentials. Indeed, direct inhibition of PKA activity by internally dialyzed Rp-cAMPs (500 μM) mimicked this effect of D_2R stimulation on decreasing Na^+ spikes with greater potency [(control vs. Rp-cAMPs: n = 13 cells, repeated-measures ANOVA, $F_{(1,22)}$ = 60.41, $P < 0.01$; post hoc Newman–Keuls test, $*P < 0.05$] (Fig. 4, *A* and *B*). Under this condition, the inhibitory effect of D_2R stimulation on firing was occluded and quinpirole was no longer able to produce further reduction in the evoked spikes. Thus there was no significant difference in the current–spike-response curves between NAc neurons recorded with application of Rp-cAMPs alone and those treated with the PKA inhibitor plus quinpirole [Rp-cAMPs vs. Rp-cAMPs + quinpirole: n = 11 cells, repeated-measures ANOVA, $F_{(1,20)} = 0.2$, $P > 0.05$] (Fig. 4*B*). Increasing the depolarizing currents to levels >0.5 nA did not induce further increases in evoked Na^+ spikes in either Rp-cAMPs–treated or Rp-cAMPs plus quinpirole–treated cells, indicating that a maximal effect on blocking PKA activity to suppress action potential had been achieved (data not shown). Moreover, bath-applied H-89 (10 μM), a different PKA inhibitor, also decreased evoked Na^+ spikes and

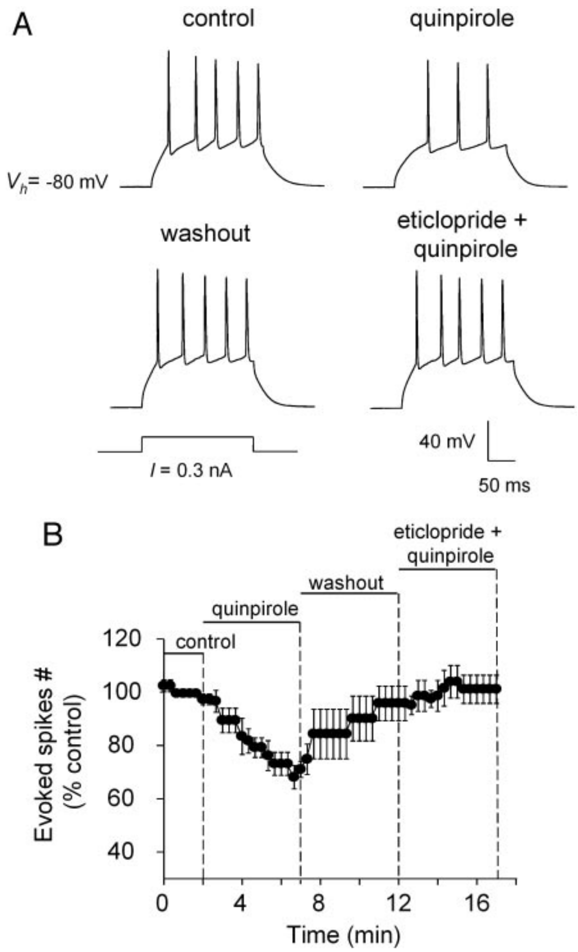

FIG. 3. D_2R-mediated inhibition of evoked firing was prevented by blockade of D_2Rs. *A*: representative traces showing that concurrently applied eticlopride (10 μM) blocked quinpirole-induced suppression in evoked Na^+ spikes. *B*: time–response curve shows that evoked firing was decreased after application of quinpirole. This inhibitory effect of D_2R agonist on Na^+ spikes was washed out and blocked by concurrent application of eticlopride (n = 6 cells, with post hoc test, $P < 0.05$).

occluded the inhibitory effects of quinpirole on evoked firing [control vs. H-89: n = 6 cells, repeated-measures ANOVA, $F_{(1,10)} = 7.9$, $P < 0.05$, post hoc Newman–Keuls test, *$P < 0.05$; H-89 vs. H-89 + quinpirole: n = 6 cells, repeated-measures ANOVA, $F_{(1,10)} = 0.2$, $P > 0.05$] (Fig. 4, *C* and *D*).

In contrast, inhibition of PI-specific PLC by externally applied ET-18-OCH_3 (500 μM) failed to affect the ability of D_2R stimulation in decreasing evoked Na^+ spikes. Under this condition, quinpirole-induced reduction in evoked firing was not affected by inhibition of PI-PLC [control vs. ET-18-OCH_3 + quinpirole: n = 12 cells, repeated-measures ANOVA, $F_{(1,22)} = 5.06$, $P < 0.05$; post hoc Newman–Keuls test, *$P < 0.05$] (Fig. 5). Nevertheless, there was still a significant difference in evoked spikes between MSNs treated with ET-18-OCH_3 alone and ET-18-OCH_3 plus quinpirole [ET-18-OCH_3 vs. ET-18-OCH_3 + quinpirole: n = 12 cells, repeated-measures ANOVA, $F_{(1,22)} = 4.93$, $P < 0.05$; post hoc Newman–Keuls test, *$P < 0.05$] (Fig. 5). In addition, ET-18-OCH_3 by itself did not cause any significant changes in evoked Na^+ spikes [control vs. ET-18-OCH_3, n = 12 cells; repeated-measures ANOVA, $F_{(1,22)} = 0.0035$, $P > 0.05$] (Fig. 5).

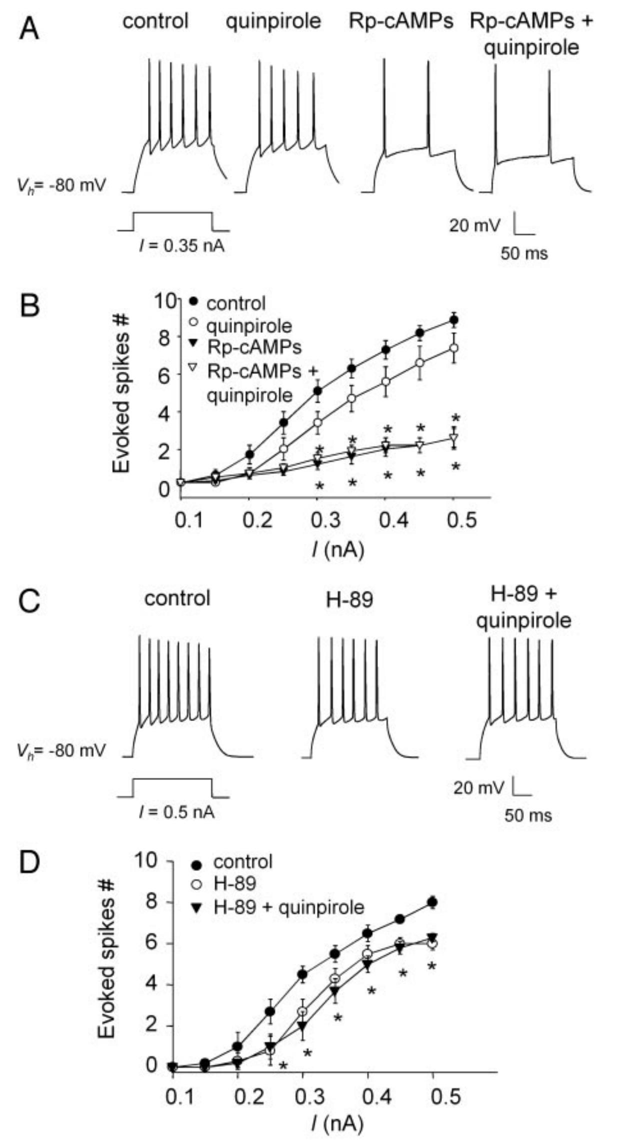

FIG. 4. Inhibition of cAMP-dependent protein kinase (PKA) mimicked D_2R-mediated reduction in evoked Na^+ spikes. *A*: representative traces showing that cytosolic application of the Rp isomer of adenosine-3′,5′-cyclic monophosphorothioate (Rp-cAMPs, 500 μM) produced a similar but more profound inhibition of evoked Na^+ spikes than the D_2R agonist (quinpirole, 10 μM), in response to the same intensity of depolarizing current pulse (0.35 nA). *B*: current-evoked spike-response curves showing a significant decrease in evoked spikes when Rp-cAMPs was dialyzed (control vs. Rp-cAMPs: n = 13, $P < 0.01$; with post hoc test, *$P < 0.05$) and with Rp-cAMPs + quinpirole perfusion (control vs. Rp-cAMPs + quinpirole: n = 13 cells, *$P < 0.05$) as compared with control. In addition, no significant difference in evoked firing was found between cells treated with Rp-cAMPs and with Rp-cAMPs + quinpirole (n = 11 cells, $P > 0.05$). Note that plots from Fig. 1*B* (control vs. quinpirole) were included to compare the effects of Rp-cAMPs and quinpirole on evoked spikes. *C*: representative traces show that perfusion of H-89 (10 μM), another selective PKA inhibitor, not mimicked but also occluded the inhibitory effect of quinpirole on evoked Na^+ spikes. *D*: current-evoked spike-response curves indicate a significant decrease in evoked Na^+ spikes after application of either H-89 alone (control vs. H-89: n = 6 cells, $P < 0.05$; with post hoc test, *$P < 0.05$) or H-89 + quinpirole (control vs. H-89 + quinpirole: n = 6 cells, *$P < 0.05$), as compared with control. There was no significant difference in evoked firing between cells recorded with H-89 alone and H-89 + quinpirole (n = 6 cells, $P > 0.05$).

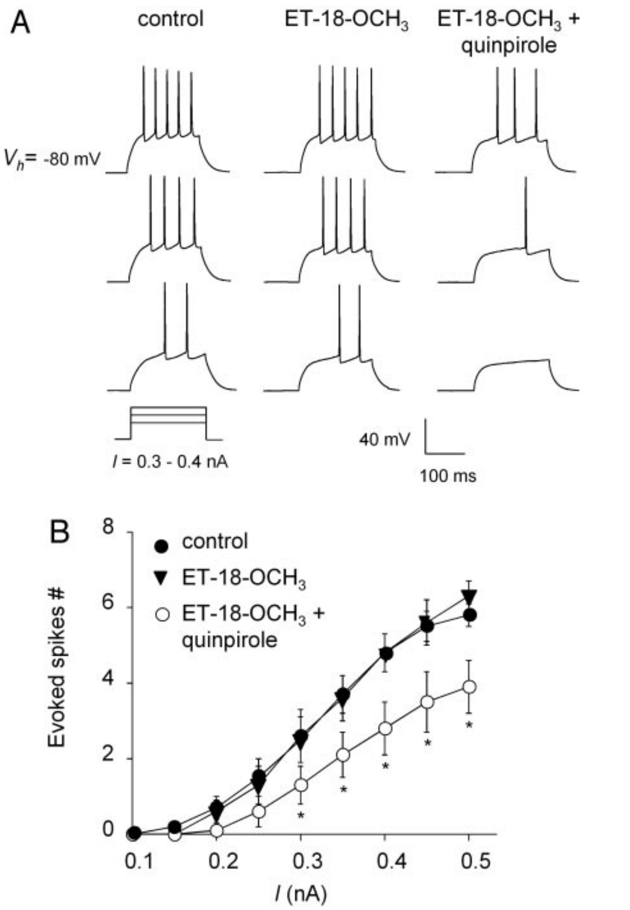

FIG. 5. Inhibition of phosphatidylinositol-specific phospholipase (PI-PLC) failed to affect D_2R-mediated reduction in evoked Na^+ spikes. *A*: representative traces showing that application of the selective PI-PLC inhibitor ET-18-OCH_3 (500 μM) did not induce significant changes in evoked Na^+ spikes either in the presence or the absence of quinpirole. *B*: current-evoked spike-response curves showing that the effects of quinpirole on suppressing evoked action potentials were not affected by ET-18-OCH_3 (n = 12 cells, $P > 0.05$). Therefore there was a significant difference in evoked spikes between neurons recorded from control group and those treated with ET-18-OCH_3 + quinpirole (n = 12 cells, with post hoc test, $^*P < 0.05$).

Participation of slow-inactivating A-type K^+ current in D_2R-mediated reduction of Na^+ spikes

Previous studies indicate that one of the major determinants of neuronal firing are VGKCs (or delayed rectifiers) (Hille 2001). Neostriatal MSNs possess at least three types of VGKCs, including two types of A-currents [e.g., slow-inactivating A-type K^+ current (I_{As}) and fast-inactivating A-type K^+ current (I_{Af})] and a noninactivating K^+ current (Surmeier et al. 1991, 1992). Although I_{As} makes a relatively minor contribution to the total amount of K^+ currents generated by VGKCs (Hopf et al. 2003; Surmeier and Kitai 1993), it plays an important role in controlling spike firing because inhibition of I_{As} increases evoked Na^+ spike firing (Mahon et al. 2000; Nisenbaum et al. 1994; Wickens and Wilson 1998). In addition, most striatal MSNs show I_{As} with the absence of I_{Af} (Surmeier and Kitai 1993), whereas D_2R stimulation increases I_{As} in these cells (Surmeier and Kitai 1993).

To determine whether I_{As} was functionally involved in the D_2R-mediated reduction in evoked Na^+ spikes in NAc MSNs, 4-AP (10 μM), a relatively selective inhibitor for I_{As} at a concentration range of 5–60 μM (Surmeier et al. 1991), was used in our experiments. Bath application of 4-AP appreciably increased evoked action potentials in all MSNs recorded (n = 13 cells). Under this condition, quinpirole failed to inhibit evoked Na^+ spikes (Fig. 6*A*). Current–spike-response curves indicate that there was a significant increase in the number of evoked action potentials in 4-AP–treated cells compared with that in the control group [control vs. 4-AP: n = 13 cells, repeated-measures ANOVA, $F_{(1,24)} = 4.5$, $P < 0.05$; post hoc Newman–Keuls test, $^*P < 0.05$] (Fig. 6*B*). There also was a significant difference in evoked spikes between NAc neurons treated with 4-AP plus quinpirole and control cells without drug treatment [control vs. 4-AP + quinpirole: n = 13 cells, repeated-measures ANOVA, $F_{(1,24)} = 4.42$, $P < 0.05$; post hoc Newman–Keuls test, $^*P < 0.05$]. In contrast, there was no significant difference in the number of evoked spikes between NAc neurons treated with 4-AP alone and 4-AP plus quinpirole [4-AP vs. 4-AP + quinpirole: n = 13 cells, repeated-measures ANOVA, $F_{(1,24)} = 0.02$, $P > 0.05$] (Fig. 6*B*).

To confirm the involvement of I_{As} in inhibiting evoked spikes mediated by D_2Rs, we took advantage of the different response characteristics of I_{As} and I_{Af} to voltage-dependent inactivation. In a group of cells, RMP was held at −50 mV, which resulted in inactivation of I_{As} (Gabel and Nisenbaum 1998; Surmeier et al. 1991, 1994). To study the properties of

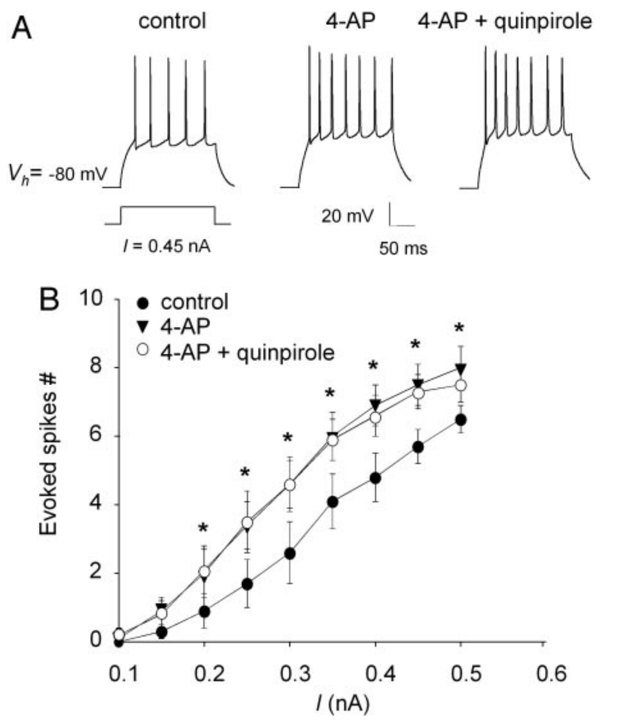

FIG. 6. Blockade of A-type K^+ current (I_A) increased evoked Na^+ spikes and abolished D_2R-mediated reduction of firing. *A*: representative traces showing that the number of action potentials evoked by depolarizing current pulses was dramatically increased in NAc neurons after blockade of A-type K^+ channels with bath application of 4-aminopyridine (4-AP) at a relatively low concentration (10 μM). Moreover, 4-AP also reversed the effects of quinpirole on suppressing firing activity. *B*: current–spike-response curves indicating that there was a significant difference in the number of evoked action potentials between control NAc cells and those treated with 4-AP alone (n = 13 cells, with post hoc test, $^*P < 0.05$) or 4-AP + quinpirole (n = 13 cells, with post hoc test, $^*P < 0.05$). In addition, there was no significant difference in evoked firing between NAc neurons treated with 4-AP alone and 4-AP + quinpirole (n = 13 cells, $P > 0.05$).

evoked Na^+ spikes either with or without D_2R stimulation, current pulses were applied from 0 to +0.2 nA with 0.04-nA increments. Under this circumstance, D_2R stimulation by quinpirole did not cause any significant changes in evoked firing with application of different current intensities [control vs. quinpirole: $n = 10$ cells, repeated-measures ANOVA, $F_{(1,6)} = 5.93$, $P > 0.05$] (Fig. 7, *A* and *B*).

D_2R stimulation reduced inward rectification during membrane hyperpolarization

One of the defining electrophysiological properties of striatal MSNs is a pronounced inward rectification, which mainly represents activation of inwardly rectifying K^+ currents (I_{Kir}) evoked by membrane hyperpolarization (Nisenbaum and Wilson 1995). To determine whether D_2R stimulation affected the activity of I_{Kir}, the inward rectification was studied by comparing the *I–V* curves across cells during membrane hyperpolarization between NAc neurons with or without D_2R stimulation. Furthermore, because D_2R stimulation not only can decrease activity of the cAMP/PKA cascade (Sibley 1995; Stoof and Kebabian 1981, 1982), but also can activate the PLCβ1-IP_3-calcineurin-signaling cascade in striatal MSNs (Hernandez-Lopez et al. 2000), we also investigated whether the potential changes in I_{Kir} mediated by D_2Rs were related to the two distinct signaling pathways.

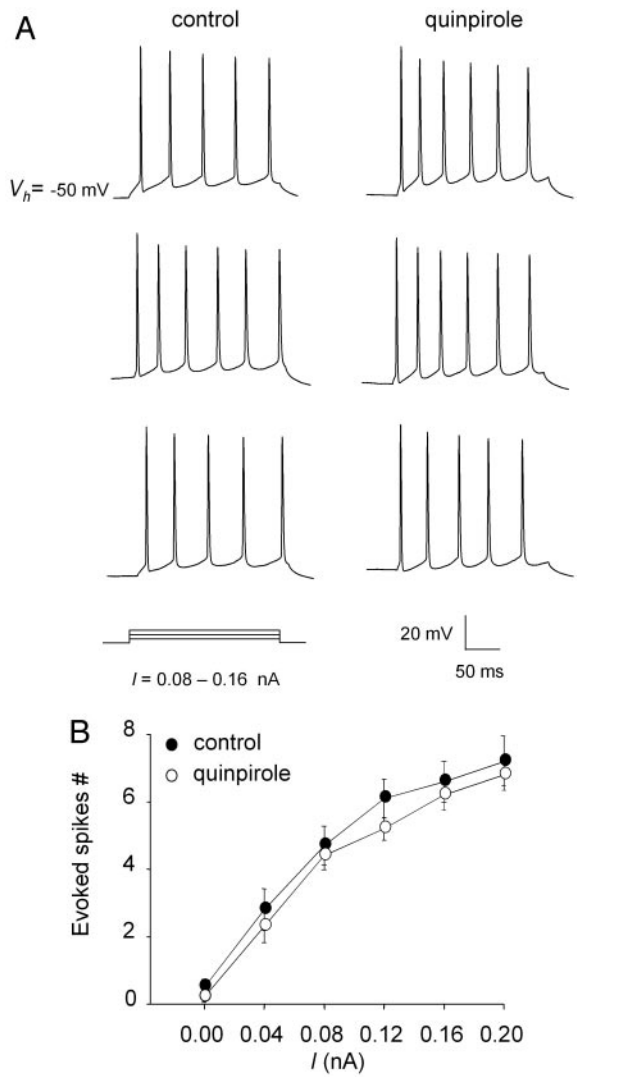

FIG. 7. D_2R-mediated reduction in evoked Na^+ spikes was eliminated at more depolarized membrane potential levels. *A*: representative traces showing action potentials evoked from a holding potential (V_h) at approximately −50 mV, either with or without D_2R stimulation. At this depolarized membrane potential, quinpirole (10 μM) failed to suppress evoked Na^+ spikes in NAc neurons. *B*: current–spike-response curves showing that evoked action potentials were not significantly affected by D_2R stimulation when the membrane potentials were held at the −50 mV level ($n = 10$ cells, $P > 0.05$).

With injection of negative current pulses (0 to −0.8 nA), membrane potentials of control neurons were hyperpolarized from the initial level of −80 mV to about −125 mV. An apparent inward rectification was induced, indicating activation of I_{Kir} (and other hyperpolarization-activated currents). Quinpirole significantly attenuated the inward rectification, leading the membrane potential to a more hyperpolarized level (shifting the *I–V* plot downward and inducing greater linearity) [control vs. quinpirole: $n = 13$ cells, repeated-measures ANOVA, $F_{(1,21)} = 7.4$, $P < 0.02$; post hoc Newman–Keuls test, $^*P < 0.05$] (Fig. 8, *A* and *B*). However, this D_2R action on reducing the inward rectification was completely blocked by concurrent bath application of the PI-PLC inhibitor ET-18-OCH_3 (500 μM) (Fig. 8*A*). There was a significant difference in the *I–V* curves between NAc neurons recorded with quinpirole alone and cells recorded with ET-18-OCH_3 plus quinpirole [quinpirole vs. ET-18-OCH_3 + quinpirole: $n = 10$ cells, repeated-measures ANOVA, $F_{(1,18)} = 10.07$, $P < 0.01$; post hoc Newman–Keuls test, $^*P < 0.05$] (Fig. 8*B*). There was no significant difference between control NAc cells and neurons treated with ET-18-OCH_3 plus quinpirole [control vs. ET-18-OCH_3 + quinpirole: $n = 10$ cells, repeated-measures ANOVA, $F_{(1,18)} = 0.15$, $P > 0.05$] (Fig. 8*B*). In addition, inhibition of PI-PLC by ET-18-OCH_3 alone caused no significant change in the inward rectification [control vs. ET-18-OCH_3: $n = 10$ cells, repeated-measures ANOVA, $F_{(1,17)} = 0.13$, $P > 0.05$] (Fig. 8, *A* and *B*).

Unlike inhibition of PI-PLC, inhibition of PKA activity by internally dialyzed Rp-cAMPs (500 μM) did not block the D_2R-mediated reduction in the inward rectification [Rp-cAMPs vs. Rp-cAMPs + quinpirole: $n = 13$ cells, repeated-measures ANOVA, $F_{(1,24)} = 8.62$, $P < 0.01$; post hoc Newman–Keuls test, $^*P < 0.05$]. In addition, cytosolic application of Rp-cAMPs alone produced no significant change in the inward rectification of NAc neurons [control vs. Rp-cAMPs: $n = 13$ cells, repeated-measures ANOVA, $F_{(1,24)} = 1.61$, $P > 0.05$] (Fig. 8, *A* and *C*).

D_2R stimulation depolarized RMP

It is well known that, despite their name, certain inward rectifiers carry *outward* K^+ currents to maintain the RMP of neurons (Hille 2001). Blockade of these outflowing K^+ currents leads to depolarization from RMP (Nasif et al. 2005). Because these K^+ channels belong to the superfamily of the inward rectifier and activity of inwardly rectifying K^+ channels is reduced with D_2R stimulation (see above), we also studied whether the RMP of NAc neurons was modulated by D_2Rs. In this experiment, RMP was not clamped during recording. Quinpirole induced a small but significant membrane depolarization in all NAc neurons recorded (control vs. quin-

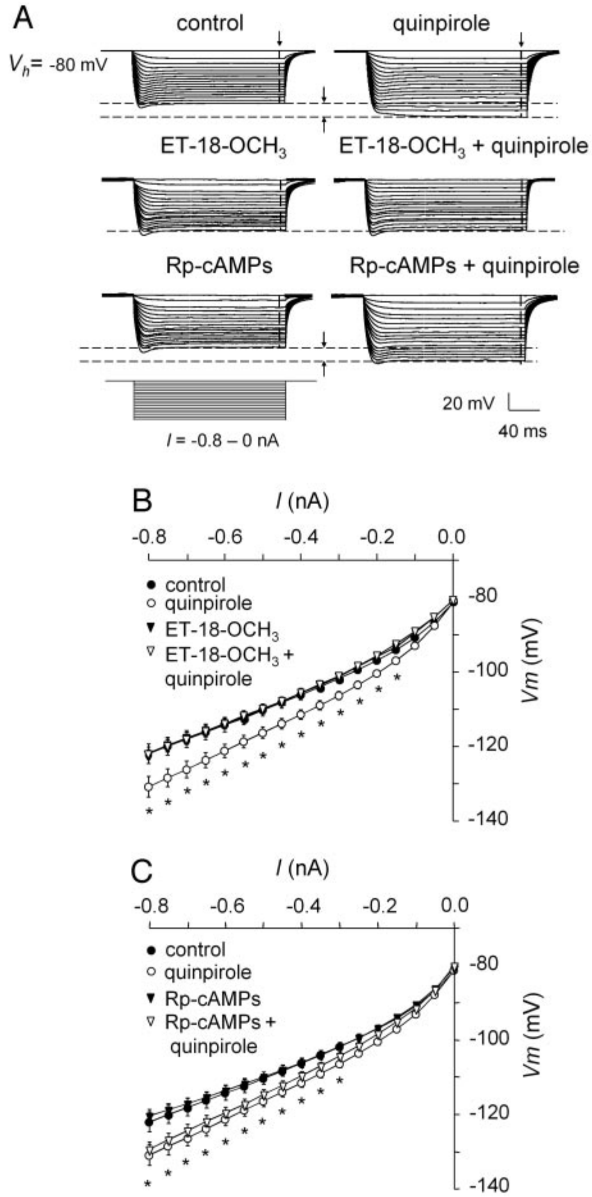

FIG. 8. D_2R stimulation attenuated inward rectification during membrane hyperpolarization. *A*: representative traces show the potential responsiveness of NAc neurons to injection of hyperpolarizing current pulses (-0.8 to 0 nA, 200-ms duration) in the control group and other compound-treated groups (quinpirole, 10 μM; ET-18-OCH$_3$, 500 μM; ET-18-OCH$_3$ + quinpirole; Rp-cAMPs, 500 μM; or Rp-cAMPs + quinpirole, respectively). Resting membrane potential (RMP) was held at -80 mV (V_h). Arrows indicate the time points at which the hyperpolarized membrane potentials were measured. *B*: *I–V* curves indicate the changes in inward rectification induced by quinpirole and/or ET-18-OCH$_3$. D_2R stimulation by quinpirole significantly attenuated inward rectification as compared with control (n = 13 cells, with post hoc test, *$P < 0.05$). This D_2R action on inward rectification was completely blocked by ET-18-OCH$_3$ (n = 10 cells, with post hoc test, *$P < 0.05$), whereas application of this PI-PLC inhibitor alone did not significantly change inward rectification (control vs. ET-18-OCH$_3$: n = 10 cells, $P > 0.05$). *C*: *I–V* curves indicate the changes in inward rectification induced by quinpirole and/or Rp-cAMPs. Quinpirole produced a significant reduction in the inward rectification, either with or without the presence of Rp-cAMPs (control vs. quinpirole: n = 13 cells; control vs. quinpirole + Rp-cAMPs: n = 13 cells, with post hoc test, *$P < 0.05$). PKA inhibitor Rp-cAMPs alone did not induce any significant changes in inward rectification as compared with control (control vs. Rp-cAMPs: n = 13 cells, $P > 0.05$).

pirole: -82.92 ± 0.63 vs. -80.73 ± 0.85 mV, n = 14 cells, paired t-test, *$P < 0.05$). This effect of quinpirole on RMP was washed out and returned to more hyperpolarized levels (Fig. 9*A*). There was no significant difference in RMP recorded from cells in control group versus washout group (-82.92 ± 0.63 vs. -82.47 ± 1.27 mV, n = 14 cells, paired t-test, $P > 0.05$) (Fig. 9*B*). However, concurrent bath application of the selective PI-PLC inhibitor ET-18-OCH$_3$ blocked this effect of D_2R stimulation on RMP (control vs. quinpirole + ET-18-OCH$_3$: -80.73 ± 0.87 vs. -80.15 ± 0.77 mV, n = 12 cells; paired t-test; $P > 0.05$), without inducing any significant changes in RMP (control vs. ET-18-OCH$_3$: -80.73 ± 0.87 vs. -80.66 ± 1.08 mV, n = 12 cells, paired t-test, $P > 0.05$) (Fig. 9*C*). In contrast, coapplication of Rp-cAMPs failed to block quinpirole-induced depolarization in RMP (Rp-cAMPs vs. Rp-cAMPs + quinpirole: -80.01 ± 0.7 vs. -78.01 ± 0.74 mV; n = 11 cells, paired t-test, *$P < 0.05$; and control vs. Rp-cAMPs + quinpirole: -80.6 ± 0.6 vs. -78.01 ± 0.74 mV, n = 11 cells, unpaired t-test; **$P < 0.01$) (Fig. 9*D*). Rp-cAMPs alone did not produce a significant change in RMP compared with control (control vs. Rp-AMPs: -80.6 ± 0.6 vs. -80.01 ± 0.7 mV, n = 11 cells, unpaired t-test, $P > 0.05$).

DISCUSSION

The present study has demonstrated that the D_2R-mediated reduction in evoked action potentials was receptor specific, which involves inhibition of PKA activity and activation of I_{As}. We also determined that D_2R stimulation attenuated the inward rectification in response to membrane hyperpolarization, indicating a decreased I_{Kir}. In addition, D_2R stimulation induced a small but significant RMP depolarization, revealing a reduction in "leak" K^+ currents. These findings indicate that D_2Rs modulate the intrinsic excitability of NAc cells with integrated regulation of different K^+ channel types by multiple signaling pathways.

D_2R-mediated I_{As} activation decreases evoked Na^+ spikes

The major finding of this study is that D_2R stimulation, either by DA or the selective D_2R agonist quinpirole, suppressed evoked Na^+ spikes. The predominant mechanism underlying the D_2R-modulated suppression of evoked Na^+ spikes should be attributed to activation of I_{As} in the core NAc cells. K^+ channels are one of the key regulators of the intrinsic excitability in both dorsal and ventral striatal MSNs (Hu et al. 2004; Surmeier and Kitai 1993, 1997; Wickens and Wilson 1998). There are at least three major K^+ currents activated by membrane depolarization in striatal MSNs (Nisenbaum and Wilson 1995; Surmeier et al. 1991). Among them, I_{As} plays an important role in regulating firing (Mahon et al. 2000; Nisenbaum et al. 1994; Wickens and Wilson 1998). D_2R stimulation usually enhances I_{As}, although D_1R stimulation decreases the current in striatal MSNs (Surmeier and Kitai 1993). Associated with D_2R stimulation, evoked action potentials are decreased in dorsal striatal cells, not only with increased I_{As} but also with decreased Ca^{2+} influx through L-type Ca^{2+} channels, which is mediated by a novel $G_{\beta\gamma}$/$PLC_{\beta 1}$/IP_3/Ca^{2+}/calcineurin pathway (Hernandez-Lopez et al. 2000).

Accordingly, findings from the present study further indicate that D_2R-modulated reduction in evoked firing recorded in

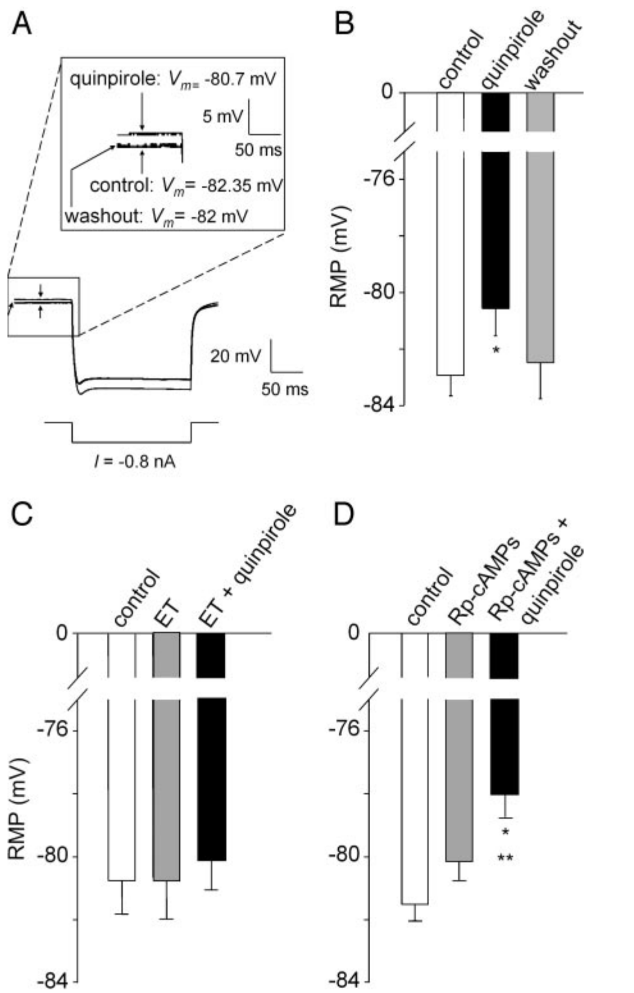

FIG. 9. D_2R stimulation depolarized RMP by activation of PLC. *A*: representative traces showing that RMP of a single NAc neuron was slightly depolarized with application of quinpirole and returned to control levels after washout. *B*: bar graph showing that the D_2R-mediated small depolarization of RMP was significant as compared with control or washout (n = 14 cells, *P < 0.05). Furthermore, there is no significant difference in the RMP between control cells and neurons with washout of quinpirole (n = 14, P > 0.05). *C*: bar graph showing that, although the PI-PLC inhibitor ET-18-OCH_3 (500 μM) alone did not induce significant changes in RMP as compared with control, it blocked the ability of quinpirole to depolarize RMP (control vs. ET-18-OCH_3 and control vs. ET-18-OCH_3 +quinpirole, n = 12 cells, P > 0.05). *D*: bar graph showing that the PKA inhibitor Rp-cAMPs (500 μM) failed to block the quinpirole-induced depolarization of RMP. Even with concurrent application of Rp-cAMPs, quinpirole was still able to induce a small but significant depolarization in RMP as compared with control (control vs. Rp-cAMPs: n = 11cells, P > 0.05; control vs. Rp-cAMPs +quinpirole: n = 11 cells, **P < 0.01; and Rp-cAMPs vs. Rp-cAMPs + quinpirole, n = 11 cells, *P < 0.05). Bars represent means ± SE.

MSNs of the core NAc should be attributed primarily to activation of A-type K^+ channels because either blockade or inactivation of A-type K^+ channels abolishes the D_2R-mediated inhibition in evoked Na^+ spikes. These results are consistent with and supportive of previous findings regarding the inhibitory effects of A-type K^+ channels on evoked action potentials in striatal MSNs. Interestingly, I_{As} is also found to be inhibited by coactivation of D_1Rs and D_2Rs in NAc cells located within the shell region, leading to an increase in evoked firing (Hopf et al. 2003). However, unlike activation of A-type K^+ channels in the core NAc cells, which is regulated most likely by the D_2R and neuronal Ca^{2+} sensor proteins (see following text), increased firing in the shell NAc cells is mediated by combined $G_\beta\gamma$ subunits released from $D_2R/G_{i/o}$ coupling and $G_{\alpha s}$-like subunits from D_1R/Gs coupling (Hopf et al. 2003). In addition, increased firing in striatal cells can also be induced by coactivation of the D_1R and D_2R by a synergistic inhibition of Na^+-K^+ ATPase (Bertorello et al. 1990). Given the above, we propose that *1*) activation of the D_2R in different NAc circuitries modulates activity of various types of ion channels by regulating multiple signaling pathways, which leads to an integrated change in neuronal intrinsic excitability; and *2*) MSNs located in the core and shell region of the NAc have distinct characteristics that may be related to their unique functions in the reward pathway.

Interestingly, some differences were observed between DA-induced inhibition in evoked firing (roughly 80% of baseline) and that induced by coapplication of DA and SCH-23390 (roughly 60% of baseline). The mechanism of this phenomenon should be attributed to involvement of D_1R modulation of ion channel activity. It is established that stimulation of D_1Rs leads to activation of L-type Ca^{2+} channels in medium spiny striatal cells (Hernandez-Lopez et al. 1997). This specific effect of D_1R modulation on the L-channel activity ought actually to increase the intrinsic excitability of these neurons in response to membrane depolarization. Thus blockade of D_1Rs with enhanced stimulation of D_2Rs could induce an integrated regulation that leads to a greater suppression in evoked action potentials than DA does. It should be particularly true when the effects of D_2R-coupled A-type K^+ channels are predominant.

The mechanism underlying activation of I_{As}

Despite numerous previous findings demonstrating a D_2R-modulated reduction in Na^+ spikes in striatal MSNs, the exact mechanism underlying this D_2R action is unknown. The present study reveals that the D_2R-modulated increase in I_{As} is regulated by inhibition of PKA activity, suggesting that at least two PKA-related mechanisms may be involved. The first one seems to be associated with decreased phosphorylation of I_A channels by PKA because this kinase is inhibited by quinpirole, Rp-cAMPs, or H-89. However, because PKA-induced phosphorylation usually increases activity of the delayed rectifier (I_K), including I_A channels (Hille 2001; Koh et al. 1996), this scenario is unlikely. The other one could be related to a D_2R-facilitated increase in Ca^{2+} mobilization, which elevates cytosolic free Ca^{2+} levels ($[Ca^{2+}]_{in}$) in striatal MSNs (Nishi et al. 1997, 1999). We previously determined that the D_2R-mediated increase in intracellular Ca^{2+} release is regulated by disinhibition of IP_3 receptors after inhibition of PKA activity in NAc neurons (Hu et al. 2005). Based on these findings, we propose that D_2R-mediated activation of I_{As} in core NAc neurons is regulated by a signaling pathway involving inhibition of PKA activity and facilitation of Ca^{2+} mobilization.

Recent findings reveal that the neuronal Ca^{2+}-sensor (NCS) proteins [e.g., NCS-1 and K^+ channel-interacting proteins (KChIPs)] modulate I_A, leading to an increase in I_A density and prolonged I_A deactivation (An et al. 2000). More important, because some of these NCS proteins are functionally and conformationally coupled to D_2Rs (for review see Bergson et al. 2003; Burgoyne et al. 2004), whereas a D_2R-mediated increase in free $[Ca^{2+}]_{in}$ effectively activates them (Kabbani et al. 2002), these NCS proteins may play a critical role in D_2R modulation of I_A and inhibition of firing. Thus it is most likely that the D_2R-mediated reduction of evoked Na^+ spikes in the core NAc cells results from a consequence of inhibition of PKA activity, disinhibition of IP_3 receptors, facilitation of Ca^{2+} release, and activation of the NCS proteins, which eventually increases I_{As}.

D_2R-mediated attenuation of inward rectification is modulated by activated PLC

Another important finding in this study is that D_2R stimulation decreases the inward rectification in the core NAc cells. Previous investigations found that several subtypes of K_{ir} channels, including the classic inwardly rectifying K^+ channels (IRK1–3 or K_{ir} 2.1–3) and G-protein–activated inward rectifiers (GIRK1 and 3, or K_{ir} 3.1 and K_{ir} 3.3) are located in NAc neurons (Karschin et al. 1996). It is also well established that K_{ir} channels, which are activated in response to membrane hyperpolarization and inactivated during depolarization, exert their role in diverse cellular functions, especially in regulating K^+ homeostasis, synaptic inhibition, neuronal firing, and resting conductance (D'Ambrosio et al. 2002; Hille 2001; Wang 1995).

Findings from the present study indicate that the D_2R-modulated decrease in the inward rectification was regulated by a D_2R-coupled PI-PLC pathway because D_2R-mediated reduction in the inward rectification was abolished by inhibition of PI-PLC activity, but not of PKA. Although this finding is supported by considerable evidence that indicates that activation of PLC is associated with reduced activity of K_{ir} channels (e.g., both IRK_s and $GIRK_s$) (Leaney et al. 2001; Sharon et al. 1997; Takano et al. 1995), the mechanism underlying D_2R-modulated decrease in the inward rectification during membrane hyperpolarization in NAc cells remains unknown. However, it is possible that the reduced inward rectification is related to a decrease in the cytosolic levels of phosphatidylinositol-4,5-bisphosphate (PIP_2). On D_2R-coupled activation, PLC hydrolyzes PIP_2 and decreases local levels of PIP_2 in cells (Stauffer et al. 1998; van der Wal et al. 2001). This decrease in cellular PIP_2 levels could reduce K_{ir} activity by two pathways. First, because binding of PIP_2 to certain sites of K_{ir} channels increases activity of the channel (Du et al. 2004; Huang et al. 1998; Kobrinsky et al. 2000), reduced PIP_2 availability would diminish activity of IRK_s and $GIRK_s$ (Meyer et al. 2001), thereby decreasing the inward rectification. Second, PIP_2 is hydrolyzed by PLC to form IP_3 and diacylglycerol (DAG). DAG activates protein kinase C (PKC), which can also inhibit K_{ir} channel activity (Mao et al. 2004; Stevens et al. 1999) that should also decrease the inward rectification. Given the above, we propose that the D_2R-mediated reduction in the inward rectification (representing decreased I_{Kir}) should be attributed to activation of PI-PLC and a consequent reduction of intracellular PIP_2 levels.

D_2R-mediated depolarization of RMP is also modulated by activation of PLC

Despite their name, certain inward rectifiers carry outward ("background" or "leak") K^+ currents that are activated at the RMP (Hille 2001). This type of K^+ channel consists of the two-pore domain (K_{2P}) and serves as a molecular determinant of several "leak" K^+ currents (Goldstein et al. 2001; Kang and Kim 2006; Lesage and Lazdunski 2000). These K_{2P} channels allow K^+ currents ($I_{K\text{-}2P}$) outflow, helping to set and stabilize RMP at levels slightly above the K^+ equilibrium potential. These K^+ channels are highly expressed in the NAc (Karschin et al. 1996; Talley et al. 2001). Blockade of this K^+ efflux depolarizes RMP in neurons (Nasif et al. 2005). Our findings not only show a D_2R-modulated membrane depolarization from RMP, but also unmask its mechanism in which activation of PI-PLC was responsible for this D_2R action on decreasing "leak" K^+ currents. This result from our study is in agreement with a recent finding that demonstrates that activity of K_{2P} channels is decreased by agonist-activated PLC and hydrolysis of PIP_2 (Lopes et al. 2005). Taken together, these findings suggest that the D_2R-mediated RMP depolarization is regulated by activation of PLC and depletion of PIP_2, which lead to a decreased K_{2P} channel activity.

In conclusion, in this study we determined that D_2R modulation inhibits evoked Na^+ spike firing by activating I_A, reduces the inward rectification by diminishing I_{Kir}, and depolarizes RMP likely by decreasing the "leak" currents ($I_{K\text{-}2P}$) in MSNs located in the core NAc. We also reveal that the integrated D_2R actions are regulated by multiple signaling pathways, including but not limited to the cAMP/PKA cascade, D_2R-coupled intracellular Ca^{2+} release, and D_2R-associated G_q/PI-PLC/PIP_2 pathway. By modulating I_{As}, I_{Kir}, and $I_{K\text{-}2P}$ along with voltage-sensitive I_{Na} and I_{Ca} (see above) at different membrane potential levels, the D_2R dynamically and integratively regulates the intrinsic excitability of NAc spiny neurons. Given that D_2Rs play an important role in the pathophysiology and treatment of neurodegenerative diseases, attention-deficit–hyperactivity disorder, schizophrenia, and drug addiction, novel findings from the present study should assist future investigations focusing on elucidation of the mechanisms underlying these diseases.

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