Dopamine D2 Receptor Modulation of K+ Channel Activity Regulates Excitability of Nucleus Accumbens Neurons at Different Membrane Potentials

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Perez, Mariela F., Francis J. White, and Xiu-Ti Hu. Dopamine D2 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 D2-class receptor (D2R) modulation of firing in medium spiny neurons (MSNs) located 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 D2R modulation of K+ channel activity regulates the intrinsic excitability of NAc neurons at the core region. D2R stimulation by quinpirole or DA significantly and dose-dependently decreased evoked Na+ spikes. This D2R effect on inhibiting evoked firing was abolished by antagonism of D2Rs, reversed by blockade of voltage-sensitive, slowly inactivating A-type K+ currents (I_KA), or eliminated by holding membrane potentials at levels in which I_KA 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, D2R stimulation also reduced the inward rectification and depolarized the resting membrane potentials (RMPs) by decreasing “leak” K+ currents. However, the D2R effects on inward rectification and RMP were blocked by inhibition of PI-PLC, but not PKA activity. These findings indicate that, with facilitated intracellular Ca2+ release and activation of the D2R/GalphaPLC/PIF2 pathway, the D2R-modulated changes in the NAc excitability are dynamically regulated and integrated by multiple K+ currents, including but not limited to I_KA, inwardly rectifying K+ currents (I_Kir), and “leak” currents (I_Kleak).

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 D1- and D2-class receptors (D1Rs and D2Rs, respectively) (Hu and White 1997; Hu et al. 2005; White and Wang 1986). Dysfunctions in the mesocorticolimbic DA system have 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 D2R activation modulates the intrinsic excitability of DA-innervated neurons, usually causing a decrease in evoked action potentials (Cepeda et al. 2001; Gulludge and Jaffe 1998; Hu and Wang 1988; Tseng and O’Donnell 2004; West and Grace 2002). This inhibitory effect of D2R 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 D2R stimulation on evoked neuronal activity. For instance, coactivation of D2Rs and D1Rs 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 Gbeta-gamma subunits released from D2R/GalphaPLC/Ca2+ coupling and GalphaPLC-like subunits from D2R/GalphaPLC coupling, respectively (Bertorello et al. 1999; Hopf et al. 2003). Moreover, D2R stimulation also facilitates Ca2+ 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 D2Rs 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 D2R regulation of ion channel/pump activity that have caused many controversies in the past with respect to the inhibitory or excitatory effects of D2R stimulation, these findings actually indicate that the D2R 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 D2R 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 D2R 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_KA, I_Kleak, and the “leak” K+ currents (I_Kleak) in medium spiny neurons located within the core NAc of rats.

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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 D2R 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, NaHCO3 26, MgCl2 2, CaCl2 2, and glucose 10; pH 7.4, 310 mMosl. Coronal slices (300 μm) containing the NAc were cut with a vibrotome (Leica VT1000S) and incubated in oxygenated (95% O2-5% CO2) 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-amino butyric acid type A (GABA_A) receptor blocker SR-95531 (4 mM) and the glutamate-matricergic 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/Brown P-97, Sutter Instruments, Novato, CA) and filled with internal recording solution (in mM): KCl 10, HEPES 10, KCl 2, NaATP 3, NaGTP 0.3, and bicytin 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 steepness 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 D2R 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 (Cd2+, 200 μM). Five minutes after TTX and Cd2+ 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 IK,Na (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 (Rin, 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 D2Rs (quinpirole, 10 μM, and eticlopride, 10 μM, respectively) were used to determine whether D2R stimulation affects the evoked action potentials, the inward rectification during membrane hyperpolarization, and RMP. Moreover, the effects of D2R 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 D2R-mediated inhibition of firing, the D2R 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-18-OCH3, 1-O-octadecyl-2-O-methyl-sn-glycerol-3-phosphorylcholine, 500 μM) was externally applied in the aCSF medium. These inhibitors were used to determine whether the D₂R-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 D₂R-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

**D₂R 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 D₂R 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. 1A). 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. 1B). 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. 1C). Because greater reduction in firing was observed with 10 μM of the D₂R 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, \( F_{(7,803)} = 4.75, P < 0.05 \); 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 t-test, \( *P < 0.05 \)) (Fig. 2, A and B). To further determine whether the quinpirole-induced decrease in evoked firing was mediated by D₂Rs, DA (40 μM) was concurrently applied with SCH-23390 (10 μM), a selective D₂R antagonist. Similar to quinpirole, combined application of DA and SCH-23390 induced a significant and reversible decrease in evoked Na⁺ spikes.
eticsloride, 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\(_2\)R.

The D\(_2\)R-mediated reduction in evoked firing was accompanied by significant alterations in certain membrane properties, including increased rheobase (control vs. quinpirole: \( 0.23 \pm 0.01 \) vs. \( 0.27 \pm 0.02 \) nA, \( n = 20 \) cells, paired t-test, \( P < 0.05 \)) and reduced threshold of action potential (control vs. quinpirole: \(-40.25 \pm 0.68 \) vs. \(-42.06 \pm 0.92 \) 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: \( 113.34 \pm 10.68 \) vs. \( 109.41 \pm 9 \); \( 83.58 \pm 1.62 \) vs. \( 82.55 \pm 1.71 \); \( 0.95 \pm 0.03 \) vs. \( 0.97 \pm 0.04 \)); \( 13.92 \pm 0.59 \) vs. \( 13.15 \pm 0.61 \) mV, respectively, \( n = 20 \) cells, paired t-test, \( P > 0.05 \)).

D\(_2\)R-mediated inhibition in evoked firing was blocked by concurrent application of eticlopride, a selective D\(_2\)R antagonist. Nevertheless, because some MSNs did not show reduction in evoked spikes in response to D\(_2\)R stimulation, this antagonist experiment was performed only in spiny cells that showed agonist effect. The D\(_2\)R-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 \( \mu \)M). Under these conditions, quinpirole-induced reduction in evoked firing was completely blocked by eticlopride \( [n = 6 \) cells, ANOVA, \( F_{(52,340)} = 3.56, P < 0.05 \)] (Fig. 3, A and B).

**Inhibition of PKA, but not PLC, mimicked the D\(_2\)R-mediated decrease of evoked \( \text{Na}^+ \) spikes**

It is well established that activation of D\(_2\)Rs inhibits adenylyl cyclase (AC) activity, thereby reducing cytosolic cAMP levels and PKA activity (Sibley 1995; Stoof and Kebabian 1981, 1982). Therefore if the D\(_2\)R-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 \( \mu \)M) mimicked this effect of D\(_2\)R 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\(_2\)R 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. 4B). 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 \( \mu \)M), a different PKA inhibitor, also decreased evoked Na\(^+\) spikes and...
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 \(\mu\)M) failed to affect the ability of D\(_2\)R 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. 3.** D\(_2\)R-mediated inhibition of evoked firing was prevented by blockade of D\(_2\)Rs. A: representative traces showing that concurrently applied eticlopride (10 \(\mu\)M) 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\(_2\)R agonist on Na\(^+\) spikes was washed out and blocked by concurrent application of eticlopride (n = 6 cells, with post hoc test, \(P < 0.05\))

**FIG. 4.** Inhibition of cAMP-dependent protein kinase (PKA) mimicked D\(_2\)R-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 \(\mu\)M) produced a similar but more profound inhibition of evoked Na\(^+\) spikes than the D\(_2\)R agonist (quinpirole, 10 \(\mu\)M), 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. 1B (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 \(\mu\)M), 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, \(P > 0.05\)).
mediated reduction of Na$_2$R-mediated reduction in evoked Na$^+$ spikes (Fig. 6A).

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. 6B). 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. 6B).

To confirm the involvement of $I_{As}$ in inhibiting evoked spikes mediated by D$_2$Rs, 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 $I_{As}$, we determined its 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$_2$R stimulation increases $I_{As}$ in these cells (Surmeier and Kitai 1993).

To determine whether $I_{As}$ was functionally involved in the D$_2$R-mediated reduction in evoked Na$^+$ spikes in NAc MSNs, 4-AP (10 $\mu$M), a relatively selective inhibitor for $I_{As}$ at a concentration range of 5–60 $\mu$M (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. 6A).

Current–spike-response curves 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 $\mu$M). 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 D₂R stimulation, current pulses were applied from 0 to +0.2 nA with 0.04-nA increments. Under this circumstance, D₂R 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).

**D₂R 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 D₂R 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 D₂R stimulation. Furthermore, because D₂R stimulation not only can decrease activity of the cAMP/PKA cascade (Sibley 1995; Stoof and Kebabian 1981, 1982), but also can activate the PLCβ1/IP₃-calcineurin-signaling cascade in striatal MSNs (Hernandez-Lopez et al. 2000), we also investigated whether the potential changes in I_Kir mediated by D₂Rs were related to the two distinct signaling pathways.

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 D₂R action on reducing the inward rectification was completely blocked by concurrent bath application of the PI-PLC inhibitor ET-18-OCH₃ (500 μM) (Fig. 8A). There was a significant difference in the I–V curves between NAc neurons recorded with quinpirole alone and cells recorded with ET-18-OCH₃ plus quinpirole [quinpirole vs. ET-18-OCH₃ + quinpirole: n = 10 cells, repeated-measures ANOVA, F(1,18) = 10.07, P < 0.01; post hoc Newman–Keuls test, *P < 0.05] (Fig. 8B). There was no significant difference between control NAc cells and neurons treated with ET-18-OCH₃ plus quinpirole [control vs. ET-18-OCH₃ + quinpirole: n = 10 cells, repeated-measures ANOVA, F(1,18) = 0.15, P > 0.05] (Fig. 8B). In addition, inhibition of PI-PLC by ET-18-OCH₃ alone caused no significant change in the inward rectification [control vs. ET-18-OCH₃: 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 D₂R-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).

**D₂R 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 D₂R stimulation (see above), we also studied whether the RMP of NAc neurons was modulated by D₂Rs. 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-
DISCUSSION

The present study has demonstrated that the D2R-mediated reduction in evoked action potentials was receptor specific, which involves inhibition of PKA activity and activation of I_AK. We also determined that D2R stimulation attenuated the inward rectification in response to membrane hyperpolarization, indicating a decreased I_Kir. In addition, D2R stimulation induced a small but significant RMP depolarization, revealing a reduction in "leak" K^+ currents. These findings indicate that D2Rs modulate the intrinsic excitability of NAc cells with integrated regulation of different K^+ channel types by multiple signaling pathways.

D2R-mediated I_AK activation decreases evoked Na^+ spikes

The major finding of this study is that D2R stimulation, either by DA or the selective D2R agonist quinpirole, suppressed evoked Na^+ spikes. The predominant mechanism underlying the D2R-modulated suppression of evoked Na^+ spikes should be attributed to activation of I_AK 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). D2R stimulation usually enhances I_AK, although D3R stimulation decreases the current in striatal MSNs (Surmeier and Kitai 1993). Associated with D2R stimulation, evoked action potentials are decreased in dorsal striatal cells, not only with increased I_AK but also with decreased Ca^{2+} influx through L-type Ca^{2+} channels, which is mediated by a novel G_{PLC/ calcium} pathway (Hernandez-Lopez et al. 2000).

Accordingly, findings from the present study further indicate that D2R-modulated reduction in evoked firing recorded in...
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 D₂R and neuronal Ca²⁺ sensor proteins (see following text), increased firing in the shell NAc cells is mediated by combined Gαᵢ and Gαₛ subunits released from D₁R/Gₛ coupling and Gαᵢ-like subunits from D₂R/Gᵢ/oS coupling (Hopf et al. 2003). In addition, increased firing in striatal cells can also be induced by coactivation of the D₁R and D₂R by a synergistic inhibition of Na⁺⁺⁻K⁺ ATPase (Bertorello et al. 1990).

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 D₂R modulation of ion channel activity. It is established that stimulation of D₁Rs leads to activation of L-type Ca²⁺ channels in medium spiny striatal cells (Hernandez-Lopez et al. 1997). This specific effect of D₁R modulation on the L-channel activity ought actually to increase the intrinsic excitability of these neurons in response to membrane depolarization. Thus blockade of D₁Rs with enhanced stimulation of D₂Rs 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 D₂R-coupled A-type K⁺ channels are predominant.

The mechanism underlying activation of I₄₄₅

Despite numerous previous findings demonstrating a D₂R-modulated reduction in Na⁺ spikes in striatal MSNs, the exact mechanism underlying this D₂R action is unknown. The present study reveals that the D₂R-modulated increase in I₄₅ 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 I₄₅ 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 (I₄₅), including I₄₅ channels (Hille 2001; Koh et al. 1996), this scenario is unlikely. The other one could be related to a D₂R-facilitated increase in Ca²⁺ mobilization, which elevates cytosolic free Ca²⁺ levels ([Ca²⁺]ᵢ) in striatal MSNs (Nishi et al. 1997, 1999). We previously determined that the D₂R-mediated increase in intracellular Ca²⁺ release is regulated by inhibition of IP₃ receptors after inhibition of PKA activity in NAc neurons (Hu et al. 2005). Based on these findings, we propose that D₂R-mediated activation of I₄₅ in core NAc neurons is regulated by a signaling pathway involving inhibition of PKA activity and facilitation of Ca²⁺ mobilization.

Recent findings reveal that the neuronal Ca²⁺ sensor (NCS) proteins [e.g., NCS-1 and K⁺ channel-interacting proteins (KChIPs)] modulate I₄₅, leading to an increase in I₄₅ density and prolonged I₄₅ deactivation (An et al. 2000). More important, because some of these NCS proteins are functionally and conformationally coupled to D₂Rs (for review see Bergson et
al. 2003; Burgoyne et al. 2004), whereas a D2R-mediated increase in free \([\text{Ca}^{2+}]_\text{in}\) effectively activates them (Kabbani et al. 2002), these NCS proteins may play a critical role in D2R modulation of \(I_\text{A}\) and inhibition of firing. Thus it is most likely that the D2R-mediated reduction of evoked \(\text{Na}^+\) spikes in the core NAc cells results from a consequence of inhibition of PKA activity, disinhibition of IP3 receptors, facilitation of \(\text{Ca}^{2+}\) release, and activation of the NCS proteins, which eventually increases \(I_\text{As}\).

**D2R-mediated attenuation of inward rectification is modulated by activated PLC**

Another important finding in this study is that D2R stimulation decreases the inward rectification in the core NAc cells. Previous investigations found that several subtypes of \(K_r\) channels, including the classic inwardly rectifying \(K^+\) channels (IRK1–3 or \(K_\text{ir}\), 2.1–3) and G-protein–activated inward rectifiers (GIRK1 and 3, or \(K_\text{ir}\), 3.1 and \(K_\text{ir}\), 3.3) are located in NAc neurons (Karschin et al. 1996). It is also well established that \(K_r\) 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 D2R-modulated decrease in the inward rectification was regulated by a D2R-coupled PI-PLC pathway because D2R-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 \(K_r\) channels (e.g., both IRKs and GIRKs) (Leaney et al. 2001; Sharon et al. 1997; Takano et al. 1995), the mechanism underlying D2R-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 cytotoxic levels of phosphatidylinositol-4,5-bisphosphate (PIP2). On D1R-coupled activation, PLC hydrolyzes PIP2 and decreases local levels of PIP2 in cells (Stauffer et al. 1998; van der Wal et al. 2001). This decrease in cellular PIP2 levels could reduce \(K_r\) activity by two pathways. First, because binding of PIP2 to certain sites of \(K_r\) channels increases activity of the channel (Du et al. 2004; Huang et al. 1998; Kobrinsky et al. 2000), reduced PIP2 availability would diminish activity of IRK and GIRK (Meyer et al. 2001), thereby decreasing the inward rectification.

Second, PIP2 is hydrolyzed by PLC to form IP3 and diacylglycerol (DAG). DAG activates protein kinase C (PKC), which can also inhibit \(K_r\) channel activity (Mao et al. 2004; Stevens et al. 1999) that should also decrease the inward rectification. Given the above, we propose that the D2R-mediated reduction in the inward rectification (representing decreased \(I_\text{K}^\text{ir}\)) should be attributed to activation of PI-PLC and a consequent reduction of intracellular PIP2 levels.

**D2R-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 (K2P) and serves as a molecular determinant of several "leak" \(K^+\) currents (Goldstein et al. 2001; Kang and Kim 2006; Lesage and Lazdunski 2000). These K2P channels allow \(K^+\) currents (\(I_{K2P}\)) outflow, helping to set and stabilize RMP at levels slightly above the \(K^+\) equilibrium potential. These K2P 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 D2R-modulated membrane depolarization from RMP, but also unmask its mechanism in which activation of PI-PLC was responsible for this D2R action on decreasing "leak" \(K^+\) currents. This result from our study is in agreement with a recent finding that demonstrates that activity of K2P channels is decreased by agonist-activated PLC and hydrolysis of PIP2 (Lopes et al. 2005). Taken together, these findings suggest that the D2R-mediated RMP depolarization is regulated by activation of PLC and depletion of PIP2, which lead to a decreased K2P channel activity.

In conclusion, in this study we determined that D2R modulation inhibits evoked \(\text{Na}^+\) spike firing by activating \(I_\text{As}\), reduces the inward rectification by diminishing \(I_\text{K}^\text{ir}\), and depolarizes RMP likely by decreasing the "leak" currents (\(I_{K2P}\)) in MSNs located in the core NAc. We also reveal that the integrated D2R actions are regulated by multiple signaling pathways, including but not limited to the cAMP/PKA cascade, D2R-coupled intracellular \(\text{Ca}^{2+}\) release, and D2R-associated Gq/PI-PLC/PIP2 pathway. By modulating \(I_\text{As}\), \(I_{K}^\text{ir}\), and \(I_{K2P}\) along with voltage-sensitive \(I_\text{Na}\) and \(I_{Ca}\) (see above) at different membrane potential levels, the D2R dynamically and integratively regulates the intrinsic excitability of NAc spiny neurons. Given that D2Rs 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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