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Dopamine D1/5 Receptor-Mediated Long-Term Potentiation of Intrinsic Excitability in Rat Prefrontal Cortical Neurons: Ca^{2+}-Dependent Intracellular Signaling

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Chen L, Bohanick JD, Nishihara M, Seamans JK, Yang CR. Dopamine D1/5 receptor-mediated long-term potentiation of intrinsic excitability in rat prefrontal cortical neurons: Ca^{2+}-dependent intracellular signaling. *J Neurophysiol* 97: 2448–2464, 2007. First published January 17, 2007; doi:10.1152/jn.00317.2006. Prefrontal cortex (PFC) dopamine D1/5 receptors modulate long- and short-term neuronal plasticity that may contribute to cognitive functions. Synergistic to synaptic strength modulation, direct postsynaptic D1/5 receptor activation also modulates voltage-dependent ionic currents that regulate spike firing, thus altering the neuronal input–output relationships in a process called long-term potentiation of intrinsic excitability (LTP-IE). Here, the intracellular signals that mediate this D1/5 receptor-dependent LTP-IE were determined using whole cell current-clamp recordings in layer V/VI rat pyramidal neurons from PFC slices. After blockade of all major amino acid receptors ($V_{hold} = -65$ mV) brief tetanic stimulation (20 Hz) of local afferents or application of the D1 agonist SKF81297 (0.2–50 μM) induced LTP-IE, as shown by a prolonged (>40 min) increase in depolarizing pulse-evoked spike firing. Pretreatment with the D1/5 antagonist SCH23390 (1 μM) blocked both the tetani- and D1/5 agonist-induced LTP-IE, suggesting a D1/5 receptor-mediated mechanism. The SKF81297-induced LTP-IE was significantly attenuated by Cd^{2+}, $[Ca^{2+}]_i$ chelation, by inhibition of phospholipase C, protein kinase-C, and Ca^{2+}/calmodulin kinase-II, but *not* by inhibition of adenylate cyclase, protein kinase-A, MAP kinase, or L-type Ca^{2+} channels. Thus this form of D1/5 receptor-mediated LTP-IE relied on Ca^{2+} influx via non-L-type Ca^{2+} channels, activation of PLC, intracellular Ca^{2+} elevation, activation of Ca^{2+}-dependent CaMKII, and PKC to mediate modulation of voltage-dependent ion channel(s). This D1/5 receptor-mediated modulation by PKC coexists with the previously described PKA-dependent modulation of K^+ and Ca^{2+} currents to dynamically regulate overall excitability of PFC neurons.

INTRODUCTION

Dopaminergic innervation from the ventral tegmental area to the prefrontal cortex (PFC) constitutes a major modulatory system that regulates higher cognitive processes (Goldman-Rakic 1998; Seamans and Yang 2004). Dopamine modulates both long-term and short-term synaptic plasticity in the PFC (Gurden et al. 2000; Huang et al. 2004; Kawashima et al. 2006; Otani et al. 2003; Young and Yang 2005) and a major portion of this D1/5 modulation is mediated by the cAMP/PKA/DARPP32/PP1 signaling cascade that leads to receptor phosphorylation, inhibition of dephosphorylation, receptor trafficking, gene transcription, and protein synthesis (Greengard 2001; Jay et al. 1998; Seamans and Yang 2004). These intracellular events may underlie the late maintenance, protein synthesis-dependent phase of long-term synaptic plasticity (Huang and Kandel 1995; Huang et al. 2004; Smith et al. 2005; also see Taylor et al. 1999).

Additional forms of neural plasticity may also contribute significantly to the cellular correlates of memory mechanisms (Calabresi et al. 1997; Daoudal and Debanne 2003; Debanne et al. 2003; Desai 2003). A second major form of plasticity, synergistic to synaptic plasticity, occurs by modulation of postsynaptic soma-dendritic ion channels that regulate neuronal excitability, thus allowing coupling of excitatory postsynaptic potential (EPSP) (input) to spike firing (output), to promote (or suppress) communications by "privileged" pathways (Daoudal and Debanne 2003). The resultant prolonged changes in spike firing are now termed long-term potentiation (or depression) of intrinsic excitability (LTP/D-IE) (Daoudal and Debanne 2003; Debanne et al. 2003; Desai 2003; Zhang and Linden 2003).

Activation of the Gs-coupled D1 receptor is classically known to stimulate adenylate cyclase-catalyzed cAMP formation, which then activates protein kinase A (PKA)–dependent intracellular signaling cascade to modulate synaptic plasticity (Greengard 2001). More recently, supporting evidence for a brain D1-like receptor that couples to PLC/IP3 pathway is accumulating (Bergson et al. 2003; Felder et al. 1989; Friedman et al. 1997; Jin et al. 2001; O'Dowd et al. 2005; Rashid et al. 2007; Undie et al. 1994; Yu et al. 1996; Zhen et al. 2004). There is also evidence that the D1 receptor alone, or D1/D2 receptor oligomer, can couple to a Gq protein, provided that there is a priming elevation of the intracellular concentration of Ca^{2+} ($[Ca^{2+}]_i$), (Bergson et al. 2003; Friedman et al. 1997; Jin et al. 2001; Lezcano et al. 2000; Rashid et al. 2007; Undie et al. 1994; Yu et al. 1996). Downstream, differential activation by dopamine (DA) of either PKA or protein kinase C (PKC) may phosphorylate Na^+, K^+, and/or Ca^{2+} channels that regulate spike threshold and regenerative spike firing for a given depolarizing postsynaptic input (Cantrell et al. 1999; Dong and

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White 2003; Dong et al. 2004; Franceschetti et al. 2000; Gorelova and Yang 2000; Maurice et al. 2001; Penit-Soria et al. 1987; Seamans et al. 1997; Yang and Seamans 1996; Young and Yang 2004).

Dopamine-mediated changes in neuronal excitability appear to be regionally specific. In hippocampal, entorhinal cortex, and striatal neurons there is a general suppression of neuronal excitability (Onn et al. 2003; Rosenkranz and Johnston 2006; Schiffmann et al. 1995, 1998; Stanzione et al. 1984; but see Pedarzani and Storm 1995). In pyramidal PFC neurons recorded in slices, a brief exposure of DA or D1 agonists, but not D2 agonists, led to a prolonged overall increase in neuronal excitability. This is characterized by a slow onset but long-lasting increase in the number of spikes evoked by the same depolarizing pulses irrespective of whether the recordings were made using sharp electrodes (with little or no dialysis of intracellular milieu) or patch electrodes in whole cell mode (with rapid dialysis of intracellular milieu) (Ceci et al. 1999; Gorelova and Yang 2000; Gulledge and Jaffe 2001; Henze et al. 2000; Lavin and Grace 2001; Lavin et al. 2005; Penit-Soria et al. 1987; Shi et al. 1997; Tseng and O'Donnell 2004; Yang and Seamans 1996). Furthermore, D1/5 receptor-mediated increases in PFC neuronal excitability were also observed in vivo after tetanic stimulation of the ventral tegmental area (VTA) dopamine inputs to the PFC neurons (Lavin et al. 2005). These changes may involve D1/5 receptor modulation of several ionic currents, such as the persistent Na^+ and K^+ currents or voltage-activated Ca^{2+} current that regulates excitability (Dong and White 2003; Dong et al. 2004; Gorelova and Yang 2000; Maurice et al. 2001; Yang and Seamans 1996; Young and Yang 2004). In this study, we determined the intracellular signaling cascades that mediate D1/5 receptor modulation of a prolonged increase in neuronal excitability (LTP-IE) in PFC pyramidal layer V/VI neurons. We report here that the D1/5 receptor-mediated LTP-IE is not dependent on adenylate cyclase and PKA activation, but is dependent on Ca^{2+} influx, intracellular Ca^{2+} elevation, activation of phospholipase C (PLC), PKC, as well as Ca^{2+}/calmodulin kinase II. A preliminary report of these findings was communicated in abstract form (Chen L et al. 2004).

METHODS

Rat brain slice preparations

Brains of young adult [postnatal day (P) 27–P36] male Sprague–Dawley rats were used to make brain slices. After decapitation by a guillotine (using a plastic decapicone rat restrainer; Braintree Scientific, Tampa, FL), the brain was quickly removed and immersed for nearly 1 min in ice-cold (about 4°C) oxygenated (95% O_2-5% CO_2) artificial cerebrospinal fluid (ACSF) containing (in mM): 124 NaCl, 26 $NaHCO_3$, 2.5 KCl, 0.5 $CaCl_2$, 4 $MgCl_2$, 0.4 ascorbic acid, and 10 glucose. Coronal bilateral brain slices (350 μM thick) that included the prelimbic and infralimbic prefrontal cortex (PFC) (corresponding to anterior–posterior = 2.2–3.5 mm anterior to the bregma: dorsal–ventral = 3–5 mm from the cortical surface; medial–lateral = 0.8–0.9 mm from the midline in Paxinos and Watson 1998) were cut on a vibratome (Vibroslice, World Precision Instruments, Sarasota, FL). The cut slices were placed immediately in warm (35°C) continuously oxygenated ACSF, containing (in mM) 124 NaCl, 26 $NaHCO_3$, 3 KCl, 2.3 $CaCl_2$, 1.3 $MgCl_2$, and 10 glucose. After 30 min of incubation, the slices were cooled to room temperature (22–23°C) in the same ACSF for the rest of the day. After ≥1 h in the latter incubation, a single slice was transferred to a submersion-type recording chamber (Warner Instruments, Hamden, CT) where electrophysiological recordings were made. The submerged slices were perfused with gravity-fed ACSF that passed through an in-line heater (SH7B in-line heater; Warner Instruments) and the bath temperature was maintained at 33°C by a feedback temperature controller (TC-344B heat controller, Warner Instruments) throughout the experiments.

Electrophysiological recording

An Olympus BX50WI upright microscope equipped with differential interference contrast optics and infrared videoimaging system (DIC-IR, Hamamatsu C2400-07ER) was used to visualize neurons in slices. Layer V/VI PFC pyramidal neurons were easily recognizable by a ×40 water-immersion lens by the pyramidal shape of their cell bodies and the presence of long apical dendrite extending toward the superficial layers.

Whole cell patch-clamp recordings in current-clamp mode were used to study neuronal excitability changes of PFC pyramidal neurons in response to intracellularly injected depolarizing pulses. Patch pipettes (3–5 MΩ) were fabricated from borosilicate tubing (1.5 mm OD, 1.1 mm ID; Sutter Instrument, Novato, CA) on a horizontal microelectrode puller (P-97; Sutter Instrument). The internal pipette solution contained (in mM): 100 K^+ methyl sulfate, 60 sucrose, 10 HEPES, 1 EGTA, 2 Na_2ATP, 0.5 Tris-GTP, 2 $MgCl_2$, and 10 di-Na^+ phosphocreatine; pH was adjusted to 7.3 by KOH and had an osmolality of 285–295 mOsm. In some neurons a modification of the internal pipette solution was also used. This consists of (in mM): 110 K^+ methyl sulfate, 10 HEPES, 20 KCl, 1 EGTA, 4 Na_2ATP, 0.4 Tris-GTP, 2 $MgCl_2$, and 10 di-Na^+ phosphocreatine. Results obtained using either pipette solution were identical.

Current-clamp recordings were made using an AxoPatch 200B amplifier (Molecular Devices, Sunnyvale, CA). The spike activation protocol (pClamp 9, Molecular Devices) consisted of a 200-ms hyperpolarizing prepulse that was separated by 250–350 ms [depending on the complete return of the hyperpolarizing response to the holding potential (V_{hold}) = −65 mV, before the delivery of a 500-ms depolarizing pulse]. This sequence of pulses was delivered to the recorded neuron every 20 s (0.05 Hz). The magnitude of the hyperpolarizaing prepulse and the depolarizing pulses varied between cells and were adjusted accordingly. To monitor the input resistance of each neuron, the hyperpolarizing prepulse (−30 to −100 pA) was adjusted to evoke a nearly 10-mV deflection. The depolarizing pulses were adjusted to evoke three to four spikes in the baseline and monitored for ≥20 min before acquisition of usable baseline readings. The holding potential was maintained continuously at −65 mV by manual DC injection or removal throughout the entire experiment. Series resistance (10–20 MΩ after "break-in") was 90% compensated and was monitored constantly during the entire experiment by "bridge"-balancing of the instantaneous voltage responses to the hyperpolarizing current prepulse before each depolarizing stimulus delivery. Recordings were terminated and the data were discarded if the series resistance changed by >6 MΩ. Steady-state baseline-evoked spike activity was recorded 20–30 min after the slice had been exposed to the appropriate cocktail of antagonists or intrapipette perfusion of chelators or blockers.

Drug applications

All drugs were bath-applied by gravity. Complete exchange of the bathing solution took about 1.5 min. In all experiments, 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) disodium salt (10 μM), bicuculline (Bic, 10 μM), (−)-(*R*)-5,5-dimethylmorpholinyl-2-acetic acid ethyl ester hydrochloride (SCH50911, 10 μM), and 2-amino-5-phosphonovaleric acid (APV, 50 μM; Tocris Cookson, Bristol, UK) were bath-applied continuously to block α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), γ-aminobutyric acid types A and B

($GABA_A$ and $GABA_B$), and *N*-methyl-D-aspartate (NMDA) channels, respectively. Stock solutions (1,000-fold concentrated) of the amino acid receptor antagonists were prepared in deionized water and stored as frozen aliquots at −20°C. A concentrated stock solution of (±)-6-chloro-7,8-dihydroxy-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine (SKF81297, Sigma, St. Louis, MO) in DMSO was made up in ascorbic acid and stored as frozen aliquots until use (<2 mo) when it was diluted to the appropriate final concentration in the perfusate (final concentration of ascorbic acid was 10 μM). (*R*)-(+)-SKF81297 was bath-applied for 10 min.

Stock solutions of the PKC inhibitory peptides [19–36] (10 μM Calbiochem, San Diego, CA) and PKA inhibitory peptides [5–24] (10 μM, Calbiochem) were dissolved in deionized water, whereas the Ca^{2+} chelator BAPTA tetra potassium salt (5–10 mM, Sigma–Aldrich, St. Louis, MO) or the adenylate cyclase inhibitor 2′,5′-dideoxyadenosine 3′-diphosphate trisodium salt (2′,5′,d,d-3′-ADP, 200 μM, Sigma–Aldrich) were separately dissolved directly into patch pipette solution for internal perfusion. On the other hand, another PKA inhibitor, KT-5720 (10 μM, Sigma–Aldrich), was bath-applied. To facilitate cell penetration of other enzyme inhibitors such as mitogen-activated protein kinase (MAPK) and CaMKII inhibitors (Sigma) U-0126 (20 μM) and KN-93 (5 μM), respectively, they were preincubated with the slices for ≥30 min before recording commenced. Preincubation of the slices in PLC inhibitor U-73122 (20 μM, Sigma/RBI, Natick, MA) required ≥1.5 h before experiments. In some neurons, recording pipettes were also filled with U-73122 (10 μM) for direct intracellular perfusion for ≥20 min before experiments. These enzyme inhibitors, along with nimodipine, forskolin, and KT-5720 (Sigma–Aldrich), were first dissolved in DMSO and stored frozen until use when they were diluted to final concentrations in the drug syringe. Control experiments using the same amount of DMSO vehicle (≤0.5%, vol/vol) in the pipette solution for recording did not change the excitability effects of SKF81297. All baseline-evoked spike counts were recorded in the presence of these inhibitors or antagonists.

Synaptic stimulations

Electrical stimulation was delivered by a concentric bipolar metal stimulating electrode (MCE-100X, David Korpf, Natick, MA) and positioned in layer V, roughly 200 μm from the adjacent recorded neuron to activate local afferents synaptically. Programmed two-train stimulations (square pulses of 0.2-ms pulse width, delivered at 20 Hz for 2 s, 30 μA) were delivered at 0.2 Hz by a Master-8 programmable pulse generator connected to an optically isolated stimulator (Isoflex, AMPI, Jerusalem, Israel).

Data analyses

The number of evoked spikes was measured and counted using an event-detection routine in the pClamp 9.0 software (Molecular Devices). Post-SKF81297 or posttetanic stimulation spike count data were expressed as percentage change from pre-SKF81927 or pretetani mean evoked spike counts. Individual cell response data used to compile posttreatment group histograms were taken from "mean area under the curve" (i.e., mean post-SKF81297 or posttetanic stimulation percentage change in evoked spikes during the entire posttreatment period). An ANOVA was used for multiple group data comparison, followed by post hoc Tukey's or Dunnett's multiple comparison test (GraphPad Prism, version 4.0.3). Student's *t*-test was also used to compare differences between two groups of data where appropriate. Differences between control and experimental responses with $P < 0.05$ were deemed significant. Mean values of the evoked spike firing responses from 10 μM SKF81297 application was reused to compare the effects of SKF81927 in the presence of various inhibitors and antagonists. All group data are expressed as means ± SE.

RESULTS

Stable somatic whole cell patch-clamp recordings in current-clamp mode were obtained from pyramidal neurons located in layers V/VI of the prelimbic and dorsal infralimbic regions of the PFC. The resting membrane potentials of these neurons were at least −65 mV or more negative. In response to intracellular suprathreshold depolarizing pulses, the evoked regenerative spikes had amplitude >70 mV. All experiments were performed in the continuous presence of a cocktail of amino acid receptor antagonists (CNQX, APV, Bic, SCH50911; see METHODS) to block all fast synaptic transmission.

Synaptically evoked release of endogenous DA augmented the neuronal excitability of layer V/VI PFC neurons by D1/D5 receptors

In a recent in vivo intracellular recording study in PFC pyramidal neurons, synaptically evoked DA release after high-frequency stimulation of VTA induced a prolonged increase in neuronal excitability in PFC pyramidal neurons (Lavin et al. 2005). Our previous study showed that our brain slice preparations also preserve some functional DA terminals that are capable of releasing endogenous DA when synaptically evoked the first 2–3 hours after slicing (Young and Yang 2005). Thus we determined whether synaptically evoked release of endogenous DA by focal PFC stimulation in the slice could also induce a change in neuronal excitability in layer V/VI pyramidal neurons in the continuous presence of a cocktail of ionotropic amino acid receptor antagonists that blocks all fast neurotransmission mediated by AMPA, NMDA, $GABA_A$, and $GABA_B$ receptors.

Single intracellular depolarizing pulses delivered at 0.05 Hz evoked three to four spikes. When a stable baseline (at least for 20 min) was achieved, focal synaptic train stimulation (two 20-Hz trains with 40 pulses per train, 2-s duration per train, 30 μA, delivered at 0.2 Hz), identical to that used in Lavin et al. (2005), was delivered by a locally placed bipolar stimulating electrode about 200 μM from the recorded layer V/VI PFC neuron. During tetanic stimulation, neither synaptic responses nor membrane potential changes were observed because all amino acid receptors mediating synaptic fast responses were blocked (Fig. 1*C*). After the focal synaptic tetani, switching back to single intracellular depolarizing pulses gradually evoked a greater number of spikes by the same depolarizing current pulses (e.g., reaching a maximum number of nine from an initial three to four spikes in some cells, $n = 7$) with little or no change in input resistance as monitored by the hyperpolarizing prepulse (Fig. 1, *A–C*). This increase in neuronal excitability after layer V synaptic tetani was long-lasting but some recovery was achieved 30 min posttetanus (Fig. 1*C*).

To determine whether endogenous DA release by the local synaptic tetanic stimulation activates D1/5 receptors to enhance the neuronal excitability, we first perfused the slices from a separate group of animals with (*R*)-(+)-8-chlor-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1H-3-benzazepin-7-ol(Z)-2-butenedioate (SCH23390, 1 μM) to block D1/5 receptors. Perfusion of SCH23390 alone (>20 min) did not alter the baseline-evoked firing in all five PFC neurons recorded, suggesting that before stimulation, there is little or no basal endogenous "DA tone" from spontaneously release in the PFC slice. After delivery of two tetanic train stimulations, there was

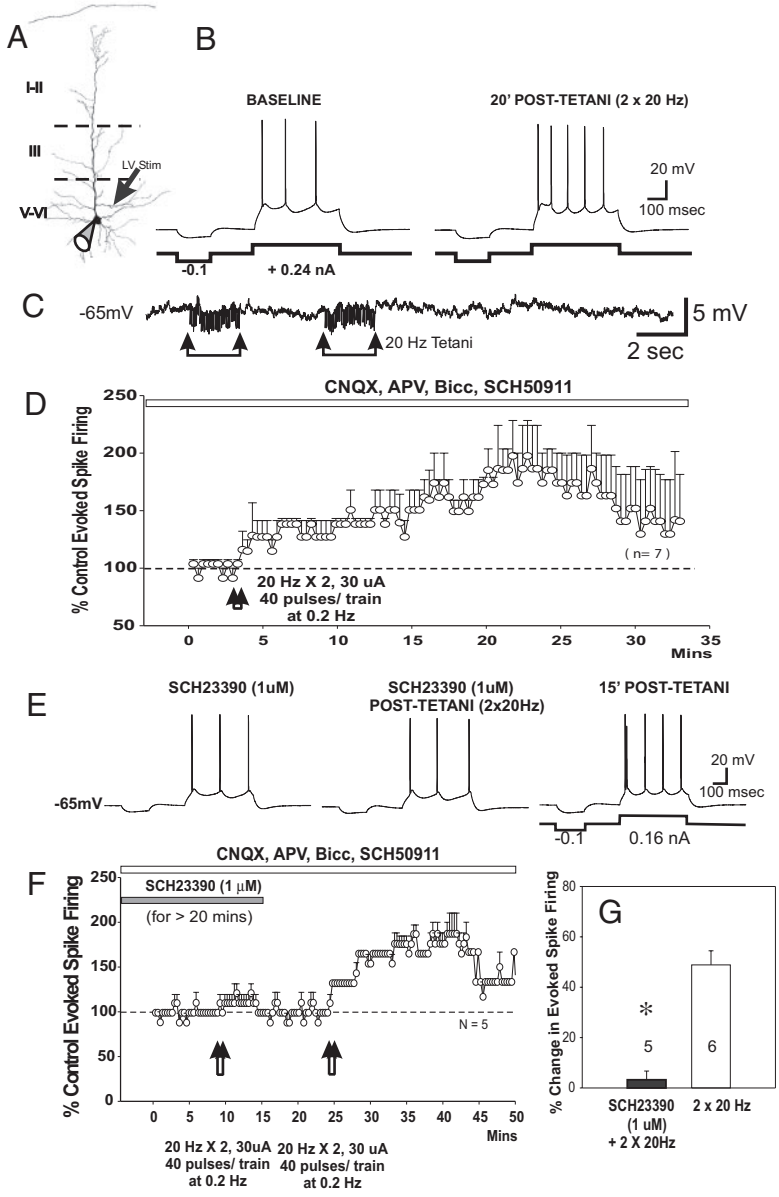

FIG. 1. In the continuous presence of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), *N*-methyl-D-aspartate (NMDA), γ-aminobutyric acid types A and B ($GABA_A$ and $GABA_B$, respectively) antagonists, tetanic stimulation of local synaptic inputs induces D1/5 receptor-mediated long-term potentiation of intrinsic excitability (LTP-IE) in layer V/VI prefrontal cortex (PFC) pyramidal neurons. *A*: schematic drawing showing a biocytin-stained pyramidal PFC neuron showing the recording pipette and the tetanic stimulation location (arrow). *B*: representative current-clamp voltage recordings of evoked spikes before and after a brief tetanic stimulation (20 Hz × 2 trains, 40 pulses per train, 30 μA, delivered at 0.2 Hz) of layer V. Holding potential was constantly clamped at −65 mV by appropriate DC injection. *Left*: note that the stimulation protocol consists of a hyperpolarizing prepulse (200 ms, −0.1 nA) to monitor any changes in input resistance. This is followed by a depolarizing pulse (500 ms, +0.24 nA) to evoke typically 3–4 spikes in the baseline every 20 s. *Right*: steady-state–evoked spike firing response showing an increase in the number of spikes evoked by the same depolarizing pulse at about 20 min after a brief tetanic stimulation of layer V. *C*: membrane voltage trace displaying a lack of instantaneous evoked responses or membrane potential change during and after tetanic stimulation in the presence of the cocktail of amino acid receptor antagonists. *D*: time course of the evoked spike response showing a slow gradual rise of the number of spikes evoked and they peak at about 20 min after the initial tetani. Double arrows indicate the time that the tetanic stimulations were delivered. *E*: representative voltage traces showing evoked spikes in the continuous presence of a D1/5 antagonist (SCH23390, 1 μM, for ≥20 min) (*left*), which did not change spike properties and input resistance. Tetanic stimulation of the adjacent layer V failed to induce any changes in evoked spike firing (*middle*). After washing off SCH23390 for 10 min, the same tetanic stimulation evoked greater number of spikes by the same intracellularly injected depolarizing pulse (*right*). Neurons were current clamped continuously with DC injection. *F*: time course of the evoked spike response that shows that in the continuous presence of SCH23390, brief tetani failed to induce any changes in neuronal excitability of PFC neurons. After washout of SCH23390, reapplication of tetani enhanced the number of evoked spikes by the same depolarizing current pulses. *G*: group histograms showing blockade of the D1/5-enhanced evoked spike by SCH23390. $*P < 0.05$.

no induced change in neuronal excitability in the continuous presence of SCH23390 (and amino acid receptor antagonists). After termination of SCH23390 application, the slice was washed for 10 min. After washout of SCH23390, redelivery of the two identical layer V train stimulations to the slice resulted in a gradual increase in the number of spikes evoked by the same depolarizing pulses ($n = 5$; Fig. 1, *E* and *F*). These results suggest that the D1/5 receptor antagonist SCH23390 (1 μM) significantly blocked [$t(11) = -4.26$; $P < 0.001$, the effects of endogenous DA released by layer V synaptic tetanic stimulation. These results in PFC brain slices replicated the data from in vivo intracellular recordings reported in Lavin et al. (2005).

The D1/5 agonist SKF81297 dose-dependently enhanced neuronal excitability in PFC neurons

Although the tetani were brief (two trains) and of low intensity (30 μA), the tetani-induced increase in neuronal excitability does not rule out the possibility that the same stimulation could also release sufficient endogenous serotonin or glutamate that can activate 5HT2 and metabotropic glutamate receptors to increase neuronal excitability (Araneda and Andrade 1991; Sourdet et al. 2003). Moreover, SCH23390 was also previously shown to have affinity for 5HT2 receptors (Bischoff et al. 1986) and could also block tetani-induced excitability increase if it were mediated by 5HT2 receptors. To minimize all the potential non-dopamine-mediated effects evoked by tetanic stimulations, we opted to use bath application of the selective D1 agonist SKF81297 to test for the mechanisms of D1-mediated neuronal excitability. We thus used a full D1 agonist SKF81297 to establish a dose response of this D1/5 receptor-mediated effect. From here onward, all experiments were performed using SKF81297 as the D1/5 receptor agonist to determine the intracellular mechanisms that mediate the D1/5 receptor-mediated prolonged increase in neuronal excitability (i.e., LTP-IE).

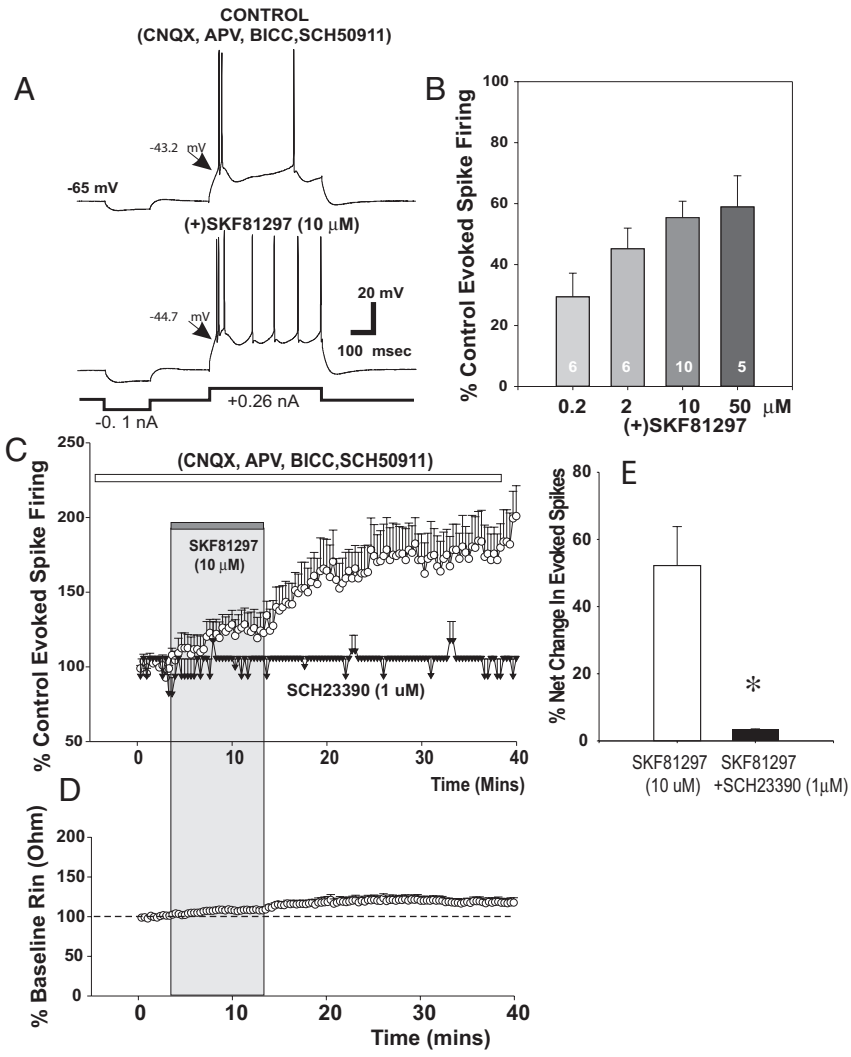

FIG. 2. D1/5 agonist (*R*)-(+)-SKF81297 dose-dependently increased the neuronal excitability of PFC neurons. Experiments were performed in the continuous presence of AMPA, NMDA, $GABA_A$, and $GABA_B$ antagonists. *A*: depolarizing pulse evoked firing is enhanced by SKF81297 with a small increase in input resistance. Note also a small negative shift in the threshold from −43.1 to −44.7 mV. All post-SKF–evoked spike representative traces in this and subsequent figures were taken at the steady-state response, roughly 20 min since the start of the 10-min SKF application. *B*: group histogram showing the dose-dependent increase of neuronal excitability. It appears that this D1/5 receptor-mediated effect peaked at 10 μM of the agonist used. *C*: time course of the evoked spike responses before, during, and after a 10-min application of the D1/5 agonist (*R*)-(+)-SKF81297 (10 μM) and their blockade by the D1/5 antagonist SCH23390 (1 μM). Note that the agonist also induced a slow gradual increase in neuronal excitability, similar to that induced by brief tetani of the adjacent putative afferents to the recorded cells in Fig. 1, *B* and *D*. Peak increase of neuronal excitability induced by the D1/5 agonist occurred at about 15 min from the start of the agonist exposure. Note that the potentiated evoked spike response is prolonged and outlasted the drug application period. *D*: hyperpolarizing prepulse monitored changes of membrane input resistance over the course of the response to the D1/5 agonist shows a small net increase of 16.2% in input resistance after SKF81297 application. This might also have contributed to the increase in neuronal excitability. *E*: group histograms showing that the D1/5 agonist effects on neuronal excitability are completely blocked by SCH23390 (1 μM). $*P < 0.001$.

In the continuous presence of the amino acid receptor antagonists, the D1/5 agonist dose-dependently enhanced the intracellular depolarizing pulse-evoked spike firing (Fig. 2) as we previously showed (Yang and Seamans 1996). We chose 10 μM SKF81297 as the test concentration for subsequent experiments because of the robust and replicable responses induced by this agonist (these data are also reused as control for a different series of experiments below using various enzyme inhibitors that block intracellular signaling).

Unlike in the case with the firing responses after tetanic stimulations of local DA afferents, a 10-min application of the D1/5 agonist (e.g., 10 μM) led to an enduring enhancement (56 ± 6.1%) of neuronal excitability that outlasted the agonist application period and persisted over the entire period of recording (≤50 min). In some but not all neurons, there was also a drop in the firing threshold. Thus after D1/5 receptor stimulation the increased number of evoked spikes could be elicited at a more negative potential by the same depolarizing pulse (Yang and Seamans 1996). However, we could not completely rule out that a small overall change (+16.2%) in mean input resistance (control R_{in} = 95.3 ± 5.82 MΩ; 10 μM SKF81297 = 109.5 ± 6.75 MΩ) might also have contributed to the increase in evoked firing by the depolarizing pulses.

In the continuous presence of bicuculline to block $GABA_A$ receptors (plus other amino acid antagonists), we did not observe any suppression of evoked Na^+ spikes by the D1/5 agonist in PFC pyramidal neurons during the entire period of recording as was reported previously (Geijo-Barrientos and Pastore 1995; Gulledge and Jaffe 1998). The peak excitability increase in response to the D1/5 agonist was not reached until about 5 min after the agonist application was terminated (about 15 min from the beginning of the drug application) and thereafter steady-state evoked firing was achieved (Fig. 2). Finally, preincubation of the D1/5 antagonist SCH23390 (1 μM) completely blocked the effects of SKF81297 on neuronal excitability (Fig. 2, *C–E*; $n = 4$). Histograms in Fig. 2*E* showed a significant [$t(11) = -4.05$; $P = 0.002$] blockade of the effects of SKF81297 on depolarizing pulse-evoked spike firing.

D1/5 agonist enhancement of neuronal excitability is blocked by PLC inhibition, but not by adenylate cyclase inhibition

D1/5 receptors are classically coupled to Gs proteins that regulate the adenylate cyclase catalysis of cAMP formation. However, recent neurochemical studies also showed noncyclase, PLC/IP3 pathway activation by a novel receptor that possesses D1/5 receptor pharmacology in brain tissue (Bergson

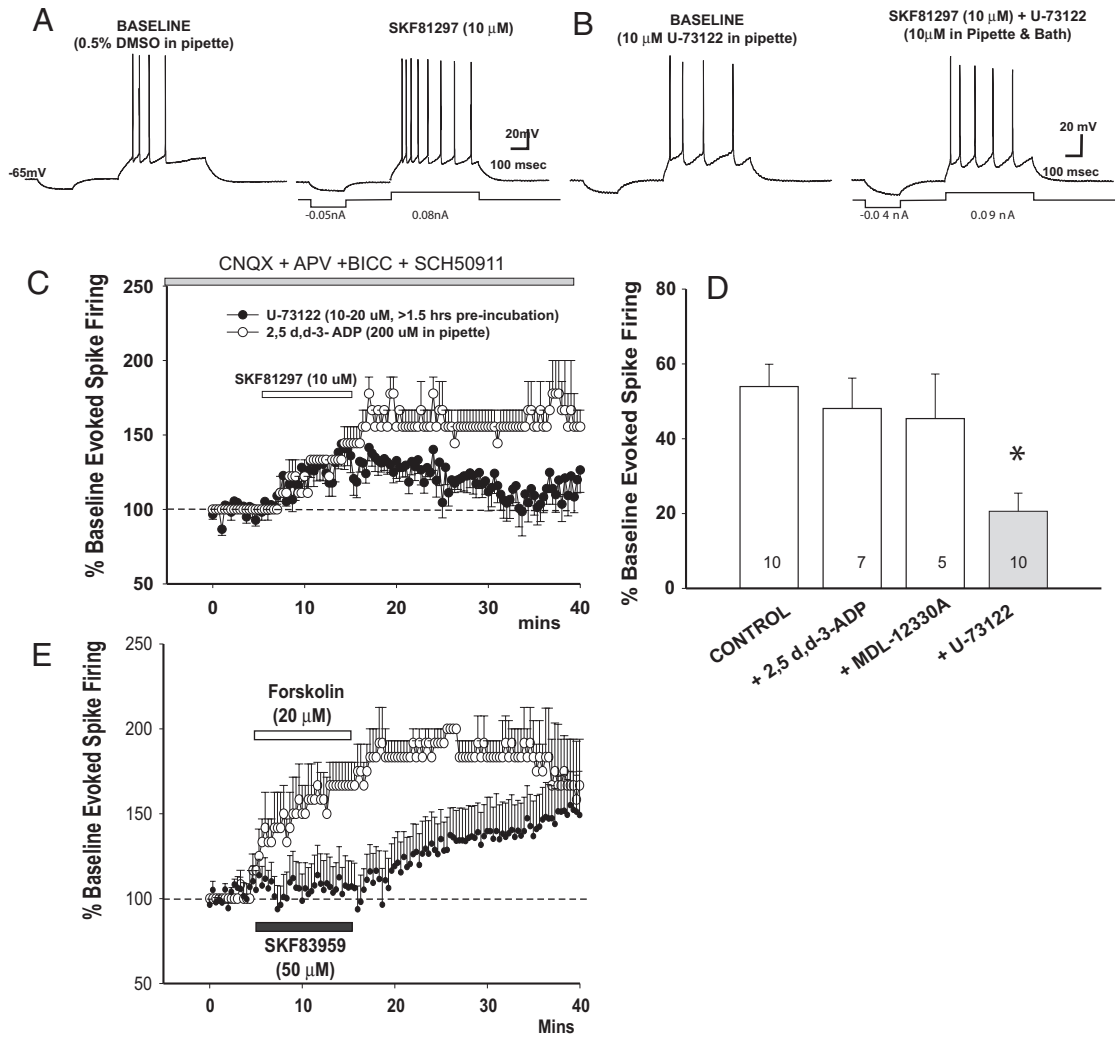

FIG. 3. D1/5 receptor-mediated increase in neuronal excitability is dependent on phospholipase C (PLC), but not adenylate cyclase activation. All experiments were performed in the continuous presence of AMPA, NMDA, $GABA_A$, and $GABA_B$ receptor blockade. *A*: representative control evoked spike traces showing that in PFC neurons recorded with patch pipette filled with patch solution that contained the PLC inhibitor vehicle DMSO (0.5% vol/vol). SKF81297 (10 μM) still augments evoked spike firing. Drug vehicle did not interfere with the effect of SKF81297 on enhancing neuronal excitability. Post-SKF traces taken at about 20 min from the start of the 10-min D1/5 agonist application. *B*: evoked spike traces recorded from a PFC neuron using a patch pipette that contained U-73122 (10 μM) in a slice that was preincubated with U-73122 (10 μM for >1.5 h) and then perfused continuously with the same PLC inhibitor during the recording. Note that the SKF81297 (10 μM) induced increase in neuronal excitability was greatly attenuated. *C*: time course of the D1/5 agonist-induced increase in neuronal excitability in the presence of adenylate cyclase inhibitor 2,5,d,d-3-ADP (intrapipette, 200 μM, open circles) or the PLC inhibitor U-73122 (10 μM, bath applied, and/or 10 μM in the pipette, filled circles). Note that the PLC inhibitor appreciably attenuated the D1/5 agonist-induced LTP-IE, especially the late phase, whereas the adenylate cyclase inhibitor was ineffective in blocking this effect. *D*: group histograms showing insignificant change of the D1/5 agonist-induced increase in neuronal excitability (Control, SKF81297) did not change significantly in the presence of the 2 adenylate cyclase inhibitors (2,5,d,d-3-ADP, in pipette, and MDL-12230, bath-applied) but the PLC inhibitor U-73122 significantly reduced the SKF81297-induced increase in neuronal excitability. $*P < 0.01$. *E*: direct adenylate cyclase stimulation by forskolin induced a rapid strong increase in neuronal excitability ($n = 4$), whereas application of SKF83959, a D1-like agonist that specifically stimulates the PLC/IP3 pathway, induced only a delayed increase in neuronal excitability ($n = 10$). Note that SKF83959 appears to activate the late phase of the LTP-IE.

et al. 2003; Friedman et al. 1997; Jin et al. 2001; Undie et al. 1994; Yu et al. 1996; Zhen et al. 2004). We thus first tested the effects of SKF81297 in the presence of two different adenylate cyclase inhibitors. In one group of PFC neurons, we included 2′,5′,d,d-3′-ADP (200 μM) in the patch pipette and the neurons ($n = 7$) were dialyzed for ≥15 min after whole cell "break-in" before baseline recordings were acquired. In another group of PFC neurons ($n = 7$), another adenylate cyclase inhibitor, MDL-12330A (30 μM), was preincubated for ≥30 min before recordings were acquired. Under both inhibitor treatments, the effects of SKF81297 in enhancing neuronal excitability did not significantly differ from SKF81297 alone [Fig. 3, *C* and *D*, $F_{(3,27)} = 5.9$, $P > 0.5$]. On the other hand, in a separate group of four PFC neurons, direct stimulation of adenylate cyclase by forskolin (20 μM) rapidly and markedly enhanced the evoked spike firing by 69.7 ± 6.8% (Fig. 3*E*). These data suggest that the D1/5 receptor-coupled adenylate cyclase was not contributing significantly to the D1/5 agonist-induced increase in neuronal excitability, but a direct stimulation of adenylate cyclase by forskolin is still capable of increasing neuronal excitability as reported previously (Cudmore and Turrigiano 2004).

Besides adenylate cyclase, D1/5 receptor activation is also known to activate the PLC/IP3/DAG pathway and thereby

increase intracellular Ca^{2+} (Felder et al. 1989; Friedman et al. 1997; Undie and Friedman 1990; Undie et al. 1994; Yu et al. 1996). To determine whether the D1/5 receptor-mediated increase in neuronal excitability is mediated by PLC activation, we preincubated the slices for ≥1.5 h (incubation <1 h was ineffective) with the PLC inhibitor U-73122 (10–20 μM) before experiments. During the experiment, the slices were continuously perfused with U-73122 (10–20 μM). Neurons recorded from these slices showed a significantly [$F_{(3,27)} = 5.9$, $P < 0.05$] attenuated (to 21 ± 4.8%) excitability increase by SKF81297 (10 μM, 56 ± 6.1% increase in excitability) ($n = 10$; Fig. 3, *A–D*). Furthermore, in three of the 10 PFC neurons tested, U-73122 (10 μM) was included in the pipette solution for intracellular application (for >20 min before baseline recording starts), along with continuous bath perfusion of the same PLC inhibitor (10 μM). All three neurons showed an attenuated SKF81297-induced excitability increase (Fig. 3, *A–B*). In control neurons recorded (with DMSO, 0.5% in the pipette solution), SKF81297 (10 μM) still robustly enhanced the neuronal excitability (Fig. 3*A*). These data suggest that the D1/5 receptor activation activated the D1/PLC pathway to induce an increase in neuronal excitability in PFC layer V neurons.

Another D1/5 agonist, SKF83959, was shown to exhibit affinity for a novel D1-like receptor that couples to Gq, and by PLC/IP3/DAG activation to increase intracellular Ca^{2+} (Jin et al. 2001, 2003; Lezcano and Bergson 2002; Lin et al. 1995; Mahan et al. 1990; Panchalingam and Undie 2005; Tang and Bezprozvanny 2004; Wang et al. 1995; Yasumoto et al. 2004; Zhen et al. 2005). We used SKF83959 as a tool to test whether a D1 receptor-linked PLC/IP3 pathway may participate in the LTP-IE. Unlike the rapid and robust effect of forskolin on neuronal excitability illustrated above (see Fig. 3*E*), bath-application of SKF83959 (50 μM) caused a weak, delayed onset (with increase in neuronal excitability typically beginning at about 10 min after termination of the application of the D1/5 agonist), but prolonged enhancement of LTP-IE in PFC neurons ($n = 10$; Fig. 3*E*). The delayed onset of the LTP-IE after SKF83959 suggested that the PLC/IP3 and its downstream mechanisms may contribute to the late effects of the LTP-IE. However, because of the lower potency of SKF83959, the mean post-SKF83959 effects achieve only a 27 ± 15.4% increase from baseline-evoked firing (vs. 56 ± 6% with SKF81297, $P < 0.05$).

D1/5 agonist-induced increase in neuronal excitability is predominantly PKC dependent

Our previous voltage-clamp studies showed that PKC mediated D1/5 receptor induced shift in the activation of a slowly inactivating (persistent) Na^+ current to a more hyperpolarizing potential. This may contribute to the mechanism(s) that mediate a spike threshold lowering and D1/5 receptor-mediated enhancement of evoked spike firing (Astman et al. 1998; Franceschetti et al. 2000; Gorelova and Yang 2000). We tested the effects of inhibiting PKA and PKC intracellularly on the D1/5 agonist-induced increase in neuronal excitability.

The PKA inhibitory peptide [5–24] (10 μM) was added to the internal pipette solution and neurons were dialyzed for ≥15 min after "break-in" before baseline recordings. Intracellular dialysis of the PKA inhibitory peptide [5–24] failed to significantly suppress ($P > 0.5$) the D1 agonist effects on neuronal excitability (70 ± 15%; $n = 5$) (Fig. 4, *A*, *C*, and *D*). In a separate experiment, we ensure that this concentration of PKA-inhibitory peptide [5–24] was effective in blocking PKA by dialyzing this inhibitory peptide for ≥20 min before bath application of forskolin (20 μM). The forskolin-induced increase in neuronal excitability (to 69.7 ± 7%) was appreciably attenuated (to 0.79 ± 5.3%) in these neurons ($n = 5$; Fig. 4, *E* and *F*), suggesting that the concentration of PKA [5–24] was effective in blocking a known agent (i.e., forskolin) that activates PKA. Additionally, we used a different PKA inhibitor, KT-5720 (Otani et al. 2002), to determine whether the D1/5 agonist-induced increase in excitability was indeed PKA independent. Continuous bath application of KT-5720 (2 μM, for 25 min before the D1/5 agonist application) also failed to block the SKF81297 effects on increase excitability ($n = 4$; Fig. 4*G*). This concentration of KT-5720 was also sufficient to block a forskolin-induced increase in neuronal excitability (Fig. 4*H*). These findings suggest that the D1/5 agonist-induced increase in neuronal excitability was not affected by two inhibitors that effectively block PKA.

We observed that after PKA inhibition, SKF81297 had in fact a stronger tendency to evoke firing (to 93.2 ± 4.2%, $P < 0.05$) (Fig. 4*G*). This may be explained by a constitutive PKA inhibitory action on PLC (Dodge and Sanborn 1998; Yue et al. 1998) and when PKA was inhibited (e.g., by KT-5720 and PKA-I [5–24] peptide) D1/5 receptor activation of the PLC pathway fully engaged the downstream intracellular signaling cascades to mediate the enhanced neuronal excitability. Conclusive evidence for this interpretation awaits future investigations.

In a separate group of PFC neurons, intracellular PKC inhibition by PKC inhibitory peptide 19–36 (PKC-I) significantly [$F_{(2,23)} = 9.3$, $P < 0.05$] suppressed the ability of SKF81297 to enhance evoked spike firing with little (<10%) or no change in input resistance ($n = 9$). The histograms summarize the mean post-SKF81297–evoked spike counts (Fig. 4*C*) and show that the PKC-I–treated group (24 ± 7.4%) is significantly lower than the control SKF81297 alone group (56 ± 6.1%). Collectively, these data strongly suggest that the D1/5 receptor-mediated enhanced excitability in PFC neurons is primarily PKC mediated. Nonetheless, the PKC inhibition did not totally abolish the SKF81297-induced increase in excitability, suggesting that under PKC inhibition, a D1/PKA-mediated mechanism could still operate to modulate K^+ (I_D) current to induce a small change in neuronal excitability (Dong and White 2003; Yang and Seamans 1996).

D1/5 agonist-induced increase in neuronal excitability was not affected by blockade of L-type Ca^{2+} channels, but was attenuated by chelation of intracellular Ca^{2+}

The above experiments showed that the D1/5 agonist-induced increase in neuronal excitability was PLC and PKC mediated. IP3 elevation from PLC activation leads to increases in $[Ca^{2+}]_i$ release and PKC is known to be activated by intracellular Ca^{2+}. Ca^{2+} can come from intracellular store release and extracellular influx. Thus we next addressed whether the D1/5 agonist effects on neuronal excitability required influx of extracellular Ca^{2+} by L-type Ca^{2+} channels. In the continuous presence of the L-type Ca^{2+} channel blocker nimodipine (10 μM) with a cocktail of amino acid receptor

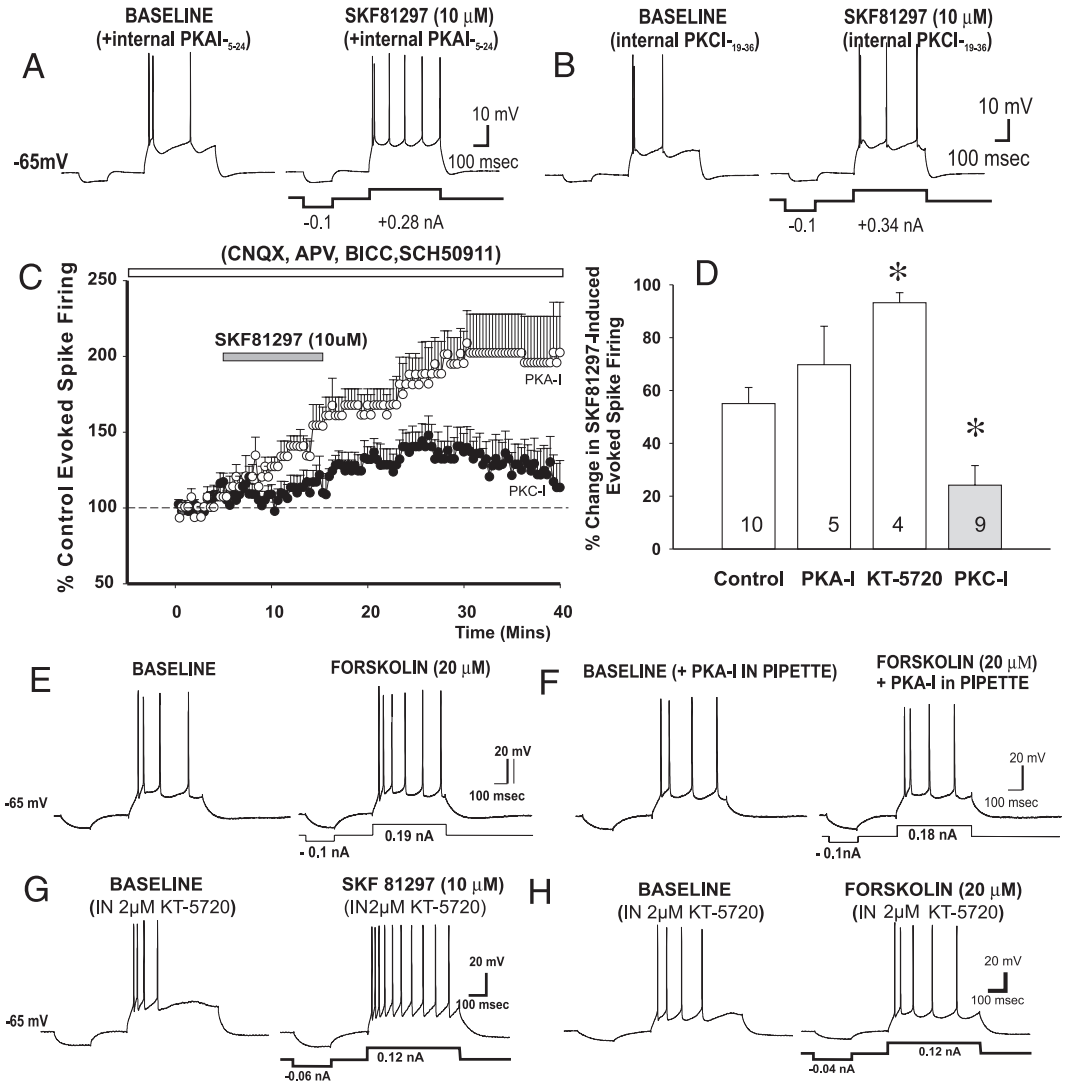

FIG. 4. SKF81297-induced increase in evoked spike firing is protein kinase C (PKC), but not protein kinase A (PKA) dependent. *A*: in the continuous presence of AMPA, NMDA, $GABA_A$, and $GABA_B$ antagonists, intracellular perfusion of PKA inhibitory peptide [5–24] (PKA-I, present in the patch pipette) failed to block spike firing evoked by the same depolarizing pulse before (*left*) and after (*right*) SKF81297 (10 μM) application. Baseline-evoked spikes were collected in the presence of PKA-I in the pipette. *B*: intracellular perfusion of PKC inhibitory peptide [19–36] (PKC-I) suppressed the SKF81297-induced increase in evoked spike firing. *C*: time course of the excitability response to SKF81297 recorded either in PKA-I–filled pipettes (open circles) or in PKC-I–filled pipettes (filled circles). Note that PKC inhibition dramatically attenuated the effects of SKF81297 on LTP-IE. *D*: group histograms showing that PKC, but not PKA, inhibition suppressed the SKF81297 (Control) induced increase in neuronal excitability (evoked spike firing) in PFC neurons. $*P < 0.05$. *E*: as a control experiment to show that the PKA-I used (at 10 μM) was still effective in blocking PKA activation, forskolin (20 μM) was used to demonstrate that forskolin-induced PKA activation was capable of enhancing neuronal excitability similar to that shown in Fig. 3*E*. Representative spike traces show that forskolin (20 μM) did enhance neuronal excitability with normal patch solution–filled micropipettes. *F*: however, when PKA-I–filled electrodes were used, forskolin (20 μM) failed to enhance neuronal excitability, as shown by the lack of increase in postforskolin steady-state evoked spike discharge. This suggests that intracellular perfusion of PKA-I (10 μM) was effective in blocking forskolin-induced PKA activation. Findings that the D1/5 agonist-induced increase neuronal excitability is not blocked by internal PKA-I perfusion (*A–D*) suggests that the D1/5 receptor-induced effect is not dependent on PKA activation. *G*: bath application of another PKA inhibitor (KT-5720, 2 μM) failed to block SKF81297-induced increase in evoked spike firing. *H*: evoked spike traces show that the concentration of this PKA inhibitor (at 2 μM) is sufficient to block forskolin (20 μM) induced increase in neuronal excitability in another cell.

antagonists, SKF81297 (10 μM) still enhanced the neuronal excitability ($n = 4$; Fig. 5, *A–D*). This suggested that influx of extracellular Ca^{2+} by L-type Ca^{2+} channel was not required for the effect, although we cannot rule out the participation of other high-voltage–activated (HVA) Ca^{2+} channels.

In five neurons under whole cell recording, BAPTA (5–10 mM) was dialyzed in the recording pipette for ≥20 min before baseline recording commenced. The postspike burst afterhyperpolarization (AHP) evoked by a strong depolarizing pulse (200 ms, +0.2 nA) was used to determine the effectiveness of intracellular Ca^{2+} chelation. The postburst AHP typically disappeared after a 10-min intracellular perfusion of BAPTA (not shown). In the presence of BAPTA in the recording pipette and continuous bath presence of nimodipine (10 μM) in the perfusate, there was a significant ($P < 0.05$) reduction of the effect of SKF81297 (10 μM) on evoked spike firing [$F_{(2,15)} = 4.3$; $P < 0.05$]. Figure 5, *C* and *D* shows the time course and group data of the D1/5 agonist effect in the presence of nimodipine (10 μM) and nimodipine (10 μM) plus BAPTA (in pipette) when compared with the effects with SKF81297 (10 μM) alone. It appears that chelation of intracellular Ca^{2+} delayed the

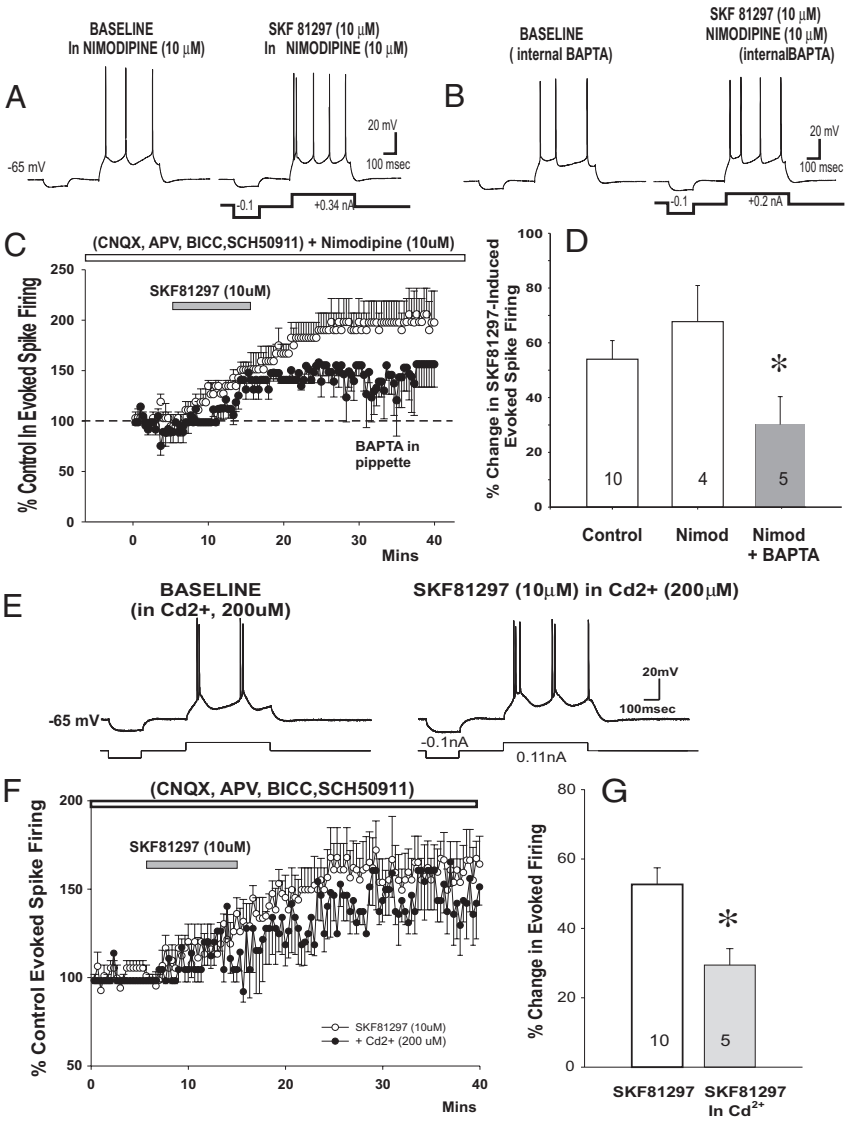

FIG. 5. D1 agonist-induced increase in neuronal excitability is intracellular Ca^{2+} and CaMKII dependent. *A*: increase in neuronal excitability after bath application of SKF81297 (10 μM) in the continuous presence of the L-type Ca^{2+} channel antagonist nimodipine (10 μM). This suggests that activity of the L-type Ca^{2+} channels did not contribute to the mechanisms that mediate D1 receptor induction of increase in neuronal excitability. *B*: intracellular perfusion of BAPTA to chelate intracellular Ca^{2+} significantly attenuated the D1 agonist-induced increase in neuronal excitability, suggesting that intracellular Ca^{2+} contributes to the D1 mechanisms. *C* and *D*: time course (*C*) and group histogram (*D*) summarizing the effects of nimodipine and BAPTA on the D1-induced increase in neuronal excitability (Control). Intracellular Ca^{2+} chelation by BAPTA but not blockade of L-type Ca^{2+} channels by nimodipine, attenuated the SKF81297-induced neuronal excitability (Control). *E*: evoked spike burst after perfusion of Cd^{2+} arising from blockade of afterhyperpolarization (AHP). Application of SKF81297 in the presence of Cd^{2+} induced a much smaller enhancement of evoked spike burst 20 min from the beginning of the 10-min D1/5 agonist application. *F* and *G*: time course (*F*) and histograms (*G*) showing the attenuated SKF81297-induced LTP-IE in the presence of Cd^{2+}, suggesting that Ca^{2+} influx by non-L-type Ca^{2+} channel contributes to the D1/5 agonist-induced LTP-IE.

onset of the D1-induced increase in neuronal excitability. These data suggest that an increase of intracellular Ca^{2+} and an influx of extracellular Ca^{2+}, possibly through non-L-type Ca^{2+} channels (below), contribute to the D1/5 receptor-induced increase in neuronal excitability in pyramidal PFC neurons.

To determine whether non-L-type Ca^{2+} channels contribute to the D1/5 receptor-mediated neuronal excitability increase, we also blocked all the HVA Ca^{2+} channels by continuous bath-application of Cd^{2+} (200 μM). Minutes after Cd^{2+} application PFC neurons started to fire spike bursts when evoked by depolarizing pulses. This was likely a result of the Cd^{2+} blockade of Ca^{2+}-activated K^+ channels (Schwindt et al. 1988) (Fig. 5*E*). On achieving steady-state–evoked burst firing in the continuous presence of Cd^{2+}, application of SKF81297 (10 μM) still enhanced neuronal excitability and LTP-IE (Fig. 5, *E–G*), but the magnitude of evoked spike firing was significantly attenuated (to 29 ± 5.5%; $P < 0.05$; Fig. 5*G*), thus suggesting that Ca^{2+} influx by non-L-type HVA Ca^{2+} channels indeed contributed to the D1/5 receptor-mediated increase in neuronal excitability.

D1/5 receptor-mediated increase in neuronal excitability is MAPK independent, but CAMKII dependent

A downstream kinase that is activated by D1 receptor or action potentials is the MAPK family (Rosen et al. 1994; Valjent et al. 2005), including the extracellular signal regulated kinase (ERK) that is known to mediate neural plasticity (Sweatt 2004; Thomas and Huganir 2004). We incubated the slices with the MAPK inhibitor U-0126 (20 μM) for ≥30 min before patch recording. We found that MAPK inhibition failed to change the D1/5 agonist-induced increase in neuronal excitability ($n = 5$; Fig. 6, *E* and *F*).

Evoked spikes can themselves induce dendritic Ca^{2+} influx by non-L-type Ca^{2+} channels and the increased Ca^{2+} influx can bind to calmodulin to activate Ca^{2+}/CaMKII, which may cause multiple long-term changes in neuronal plasticity (Lisman et al. 2002; Park et al. 2002; Roeper et al. 1997; Varga et al. 2004). Slices were preincubated in the CaMKII inhibitor KN-93 (5 μM) together with the amino acid antagonist cocktail for ≥20 min. The presence of KN-93 attenuated (to 4.2 ± 14%, $n = 7$) the SKF81927-induced increase in evoked spike firing (Fig. 6). Group data comparisons show that the presence

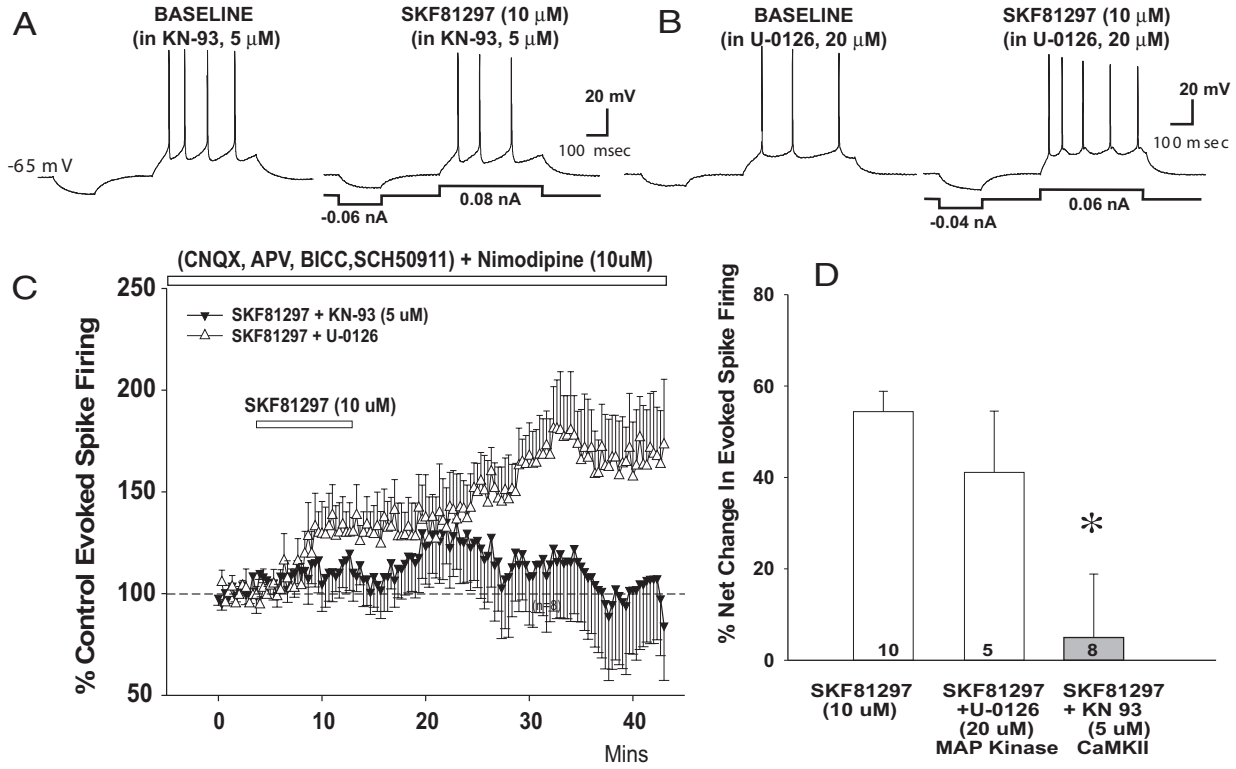

FIG. 6. D1/5 agonist-induced increase in neuronal excitability and LTP-IE is dependent on CaMKII and not mitogen-activated protein kinase (MAPK) activation. *A*: representative evoked spike traces showing in a PFC neuron perfused with the CaMKII inhibitor KN-93 (5 μM), SKF81927 (10 μM) no longer enhanced the neuronal excitability. In some cases, there was a reduction of evoked firing resulting from a reduction of input resistance in an otherwise healthy neuron (judging from the capability of firing regenerative spikes that overshot 0 mV, and smooth time constant of the membrane capacitance charging of the membrane voltage in the beginning the hyperpolarizing response to the prepulse). *B*: in another PFC neuron, the presence of MAPK inhibitor U-0126 (20 μM) failed to significantly block the effects of SKF81297 (10 μM) on excitability. *C*: time course of evoked spike firing response to SKF81297 in the presence of a MAPK inhibitor U-0126 (20 μM) or a CaMKII inhibitor KN-93 (5 μM). *D*: group histograms showing that only CaMKII inhibitor induced a significant (*$P < 0.05$) suppression of the SKF81297 (Control) enhancement of neuronal excitability in PFC neurons.

of KN-93 significantly [$F_{(3,28)} = 4$; $P < 0.05$] reduced the D1/5 agonist-induced increase in neuronal excitability despite some variability in the later time points in some of these responses. The blockade of LTP-IE by continuous KN-93 perfusion was observable even during the 10-min application period of the D1/5 agonist SKF81297, suggesting an early temporal involvement of CaMKII in the LTP-IE induction. Collectively, it appears that the D1/5 receptor-induced increase in neuronal excitability is dependent on Ca^{2+} influx by non-L-type Ca^{2+} channels, elevation of intracellular Ca^{2+}, and activation of PLC, PKC, and CaMKII (see Fig. 7 for a summary).

DISCUSSION

The principal findings of this study were that a D1-class receptor mediates tetanic stimulation- and D1/5 agonist-induced increase in LTP-IE in layer V/VI pyramidal PFC neurons in the absence of fast synaptic transmission. This LTP-IE was blocked by a D1 class antagonist SCH23390. The long-term changes in D1/5 receptor-mediated increase in neuronal excitability was dependent on Ca^{2+} influx, PLC, intracellular Ca^{2+}, and activation of PKC and CaMKII, but not adenylate cyclase, PKA, MAPK, or Ca^{2+} influx by L-type Ca^{2+} channels. This novel mechanism mediates D1/5 receptor modulation of ion channels that regulate threshold and repetitive generation of spikes in PFC neurons.

In the absence of fast synaptic transmission arising from the continuous presence of amino acid receptor antagonists, brief and moderate-frequency (20 Hz) local tetanic stimulation of layer V/VI that mimics phasic DA cell firing (Marinelli et al. 2006) led to a prolonged increase in neuronal excitability in layer V/VI pyramidal neurons, similar to what was observed in vivo (Lavin et al. 2005). Similarly, application of the full D1/5 agonist SKF81297 also induced a slow onset, but robust and prolonged enhancement of evoked spike firing in pyramidal PFC neurons. The finding that both the tetanic stimulation and D1/5 agonist-induced LTP-IE were completely blocked by a D1/5 antagonist SCH23390 suggests that a D1-like receptor is mediating the prolonged increase in neuronal excitability, as previously shown by multiple laboratories (Ceci et al. 1999; Gulledge and Jaffe 2001; Henze et al. 2000; Lavin and Grace 2001; Lavin et al. 2005; Penit-Soria et al. 1987; Shi et al. 1997; Tseng and O'Donnell 2005; Yang and Seamans 1996).

D1/5 receptor activation of PLC- and PKC-dependent intracellular mechanism mediates LTP-IE in PFC neurons

In the present study, we found that activation of a D1 class receptor-mediated increase in neuronal excitability was independent of adenylate cyclase because SKF81297 still induced LTP-IE in the presence of two different cyclase inhibitors: 2′,5′d,d-3′-ADP or MDL-12330A. Nonetheless, direct stimulation of adenylate cyclase by forskolin alone still induced a

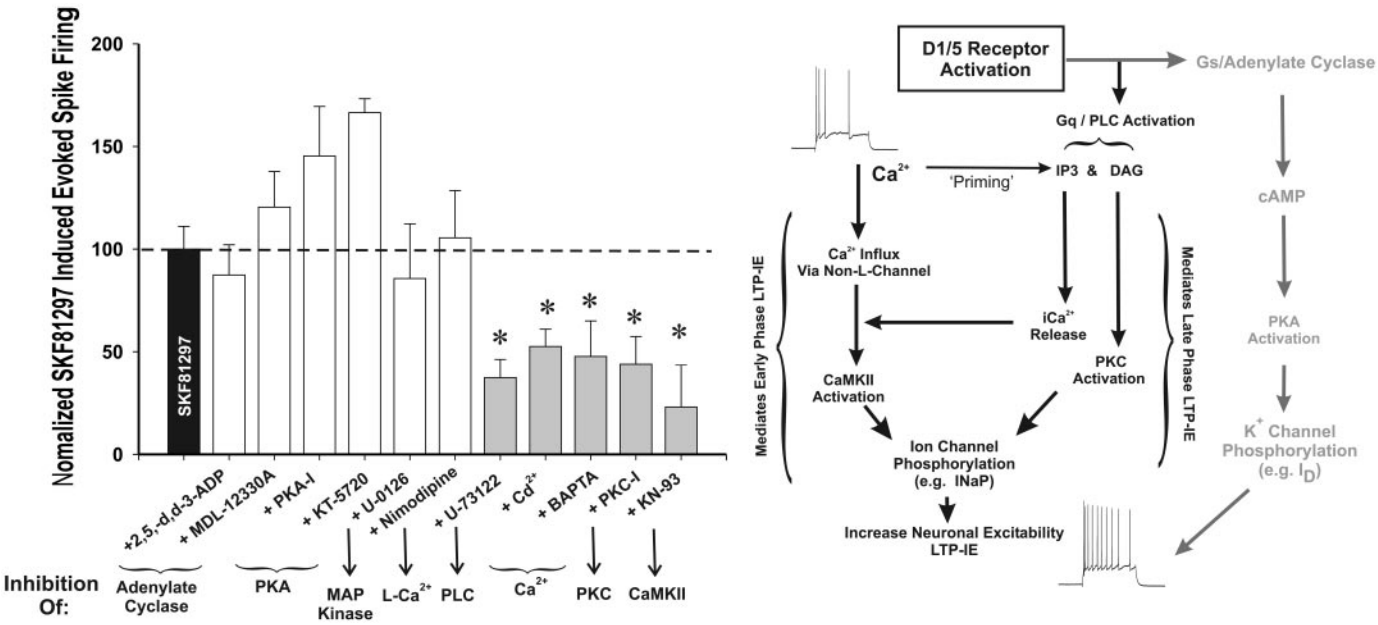

FIG. 7. Model of D1/5 receptor-mediated LTP-IE based on pharmacological data from this study. *A*: group data summary using normalized SKF81297 responses (100%) to compare with the effects of various inhibitors and antagonists on the D1/5 agonist-induced LTP-IE. Group histograms showed clearly that suppressing the activity of PLC, Ca^{2+}-dependent kinases (CaMKII, PKC), non-L-type Ca^{2+} channels, and chelation of intracellular Ca^{2+} all significantly reduce LTP-IE. Interestingly, PKA inhibition (e.g., by KT-5720) could enhance the SKF81297-induced LTP-IE, perhaps attributable to a removal of the normal constitutive PKA suppression of PKC activity (Dodge and Sanborn 1998; Yue et al. 1998), thus allowing the PKC-dependent LTP-IE to occur. *B*: model of intracellular signaling cascades that are mediating the D1/5 receptor-induced LTP-IE in PFC neurons. We propose that before and during D1 receptor activation, evoked spike firing elicited by depolarizing inputs can trigger influx of Ca^{2+} through non-L-type HVA Ca^{2+} channels. This Ca^{2+} influx can activate a fast and a slow process. Ca^{2+} influx quickly activates CaMKII, which then phosphorylates ion channel(s) to mediate LTP-IE. In addition to SKF81297 directly activating a novel D1-like receptor that coupled to PLC/IP3/DAG pathway, the Ca^{2+} influx may also provide a "priming" mechanism for D1 receptor to couple with Gq/11 protein to trigger the PLC/IP3/DAG pathway. Subsequent release of intracellular Ca^{2+} (by IP3 actions) then activates CaMKII and the DAG activates PKC to phosphorylate ion channel(s) that regulate spike firing and an increase in neuronal excitability. This latter process is slower and contributes to the late, prolonged phase of LTP-IE. Classic D1/5 receptor-activated Gs/adenylate cyclase/cAMP pathway that activates PKA to phosphorylate K^+ channels (e.g., that conduct I_D) also contributes, in part, to the intracellular mechanisms that mediates LTP-IE in PFC pyramidal neurons.

rapid and robust increase in neuronal excitability, suggesting that activation of adenylate cyclase not linked to D1/5 receptors in PFC neurons could still enhance neuronal excitability. Furthermore, the adenylate cyclase independence of the D1/5 receptor-mediated LTP-IE is consistent with our finding that inhibition of PKA was ineffective in blocking SKF81297-induced LTP-IE. It should also be emphasized that our present experimental protocols are biased against detecting an effect of PKA-dependent modulation of K^+ currents that would also be activated by D1 receptors to enhance excitability (Dong and White 2003; Yang and Seamans 1996). In all experiments PKA inhibitors were included in the patch pipette (PKA-I) or bath applied (KT-5720) and therefore subsequent application of SKF81297 could not influence targets downstream of PKA, such as K^+ currents. Thus the present series of experiments emphasized the critical role of the novel PKC and CaMKII regulation of neuronal excitability increase after D1/5 receptor activation.

Besides the classical D1/adenylate cyclase/cAMP pathway, D1/5 receptor activation was also previously shown to activate a Gq/PLC/IP3/intracellular Ca^{2+} pathway (Jin et al. 2001, 2003; Lezcano and Bergson 2002; Lin et al. 1995; Mahan et al. 1990; Panchalingam and Undie 2005; Tang and Bezprozvanny 2004; Wang et al. 1995; Yasumoto et al. 2004; Zhen et al. 2005). Indeed, in the present study and others, the SKF81297 increased neuronal excitability (or intracellular Ca^{2+} elevation; Tang and Bezprozvanny 2004) was significantly attenuated by an adequate inhibition of PLC by U-73122 (after >1.5 h preincubation, as well as intracellular perfusion by patch pipette). This finding suggests that the D1/5 receptor activation of the PLC pathway is involved in LTP-IE. Using SKF83959, a D1/5 agonist that specifically activates a D1-like receptor that couples to Gq/PLC/IP3 pathway resulted in a delayed though moderate enhancement of LTP-IE in PFC neurons. This delayed LTP-IE response may indicate that the D1/Gq/PLC/IP3 pathway may be mediating the later phase of the LTP-IE (see Fig. 3*C*).

Like the PFC pyramidal neurons shown in the present study, DA also induces a remarkably similar LTP-IE in medium spiny neurons of the nucleus accumbens. The main difference in the two cell types is that the accumbens effect is mediated by a coactivation of both D1 and D2 receptors (Hopf et al. 2003, 2005). The accumbens mechanism requires a D2 receptor-dependent release of a subunit of the D2-coupled $G_{i/o}$ protein, G-$\beta\gamma$, which acts together with the D1-coupled G-αs protein to activate adenylate cyclase and, subsequently, PKA-dependent phosphorylation inactivation of a K^+ channel to increase neuronal excitability (Hopf et al. 2003, 2005). It is not known whether D1/5 receptor activation alone in PFC neurons also leads to a dissociated release of G-$\beta\gamma$ subunit to trigger downstream intracellular signaling cascades to mediate LTP-IE. Based on the present findings, it appears that DA can use multiple intracellular mechanisms to achieve LTP-IE in different brain regions.

In addition to being blocked by a PLC inhibitor, the SKF81297-induced LTP-IE was also blocked by intracellular

dialysis of PKC inhibitory peptide [19–36]. Evoked spikes may induce sufficient Ca^{2+} influx by non-L-type Ca^{2+} channels, when combined with D1/5 receptor-mediated change in intracellular Ca^{2+}, to activate diacyglycerol (DAG)-dependent and -independent PKC (Lee et al. 2004; Mogami et al. 2003; Oancea and Meyer 1998; Yasumoto et al. 2004). Similar to findings observed in our study in PFC neurons, Hopf et al. (2005) also showed in accumbens neurons that DA, or combined D1 and D2 agonists, induced increases in neuronal excitability that is blocked by intracellular PKC inhibition. Their data suggest that the DA action may be dependent on an atypical form of membrane-bound PKC (PKCζ) that does not require PLC and diacyglycerol for its activation (Gschwendt 1999; Hopf et al. 2005). Although our data show that the PLC/IP3-activating SKF83959 can elicit a late, moderate increase in neuronal excitability in PFC neurons, we cannot rule out that D1/5 receptor activation by SKF81297 also activates a putative atypical PKC (PKCζ or PKMζ) (Hopf et al. 2005; Huang and Huang 1993; Ling et al. 2002; Muslimov et al. 2004; Pastalkova et al. 2006; Serrano et al. 2005).

Roles of intracellular Ca^{2+} and CaMKII that mediate LTP-IE

The importance of $[Ca^{2+}]_i$ was exemplified by the finding that an intracellular chelation of Ca^{2+} by BAPTA or blocking of Ca^{2+} influx by Cd^{2+}-sensitive channels attenuated the D1/5 agonist-induced LTP-IE. In our baseline recordings before D1/5 agonist application, each depolarizing pulse triggered multiple Na^+ spikes that could activate soma-dendritic nimodipine-insensitive voltage-gated Ca^{2+} channels to enable Ca^{2+} influx by these non-L-type HVA Ca^{2+} channels (Jacobs and Meyer 1997; Markram et al. 1995). We propose here that the spike-induced increase in intracellular Ca^{2+}, like the large membrane depolarization induced by elevated extracellular K^+ (Lezcano and Bergson 2002), can provide a rapid "priming" mechanism to enable D1 receptors to couple with Gq/11 and subsequent D1/5 receptor stimulation (by SKF81297) activates the PLC/IP3 pathway to increase in intracellular Ca^{2+} release.

Our present data also show that CaMKII is also critically involved in LTP-IE. CaMKII is long associated with long-term synaptic plasticity through its unique autophosphorylation properties (Lisman et al. 2002; Merrill et al. 2005; Xu et al. 2005). Thus Ca^{2+} influx from evoked somatic spikes may initially provide a very localized rapid "priming" mechanism to promote coupling of the D1 receptor to Gq/11 protein so that D1/5 receptor activation could activate the PLC/IP3 pathway to increase $[Ca^{2+}]_i$, and lead to subsequent activation of Ca^{2+}-dependent CaMKII and PKC to mediate LTP-IE (Jacobs and Meyer 1997; Llano et al. 1994). Furthermore, the blockade of the early phase of LTP-IE by CaMKII inhibitor KN-93 and by Ca^{2+} chelator BAPTA suggests that this early phase is strongly Ca^{2+} dependent, whereas the late phase is likely to involve downstream steps that require activation of PKC.

Ionic currents that may mediate D1/5 receptor modulation of LTP-IE

Both D1/5 receptor-activated PKC and CaMKII may serve to phosphorylate a number of ion channels that are likely to be the ultimate downstream targets that mediate changes in neuronal excitability. These targets include Na^+ and K^+ channels (Astman et al. 1998; Dong and White 2003; Dong et al. 2004; Franceschetti et al. 2000; Gorelova and Yang 2000; Pedarzani and Storm 1995; Schrader et al. 2002; Varga et al. 2004; Yang and Seamans 1996). Based on the early findings that a D1 agonist suppresses outward rectification in the subthreshold voltage range and prolongs a pharmacologically isolated Na^+ plateau potential, it was hypothesized (Yang and Seamans 1996) that D1/5 receptor activation facilitates a slowly inactivating persistent Na^+ current (I_{NaP}) and suppresses a slowly inactivating persistent K^+ current (I_D) to reduce spike threshold and promote regenerative spike firing in response to depolarizing inputs.

In a follow-up voltage-clamp study in PFC slices, D1/5 receptor stimulation (after $GABA_A$ receptor blockade) was shown to shift the activation voltage of a persistent Na^+ current (I_{NaP}) to more negative potentials in a PKC- but not PKA-dependent manner (Gorelova and Yang 2000). Other voltage-clamp studies of neocortical pyramidal neurons also show that direct PKC activation by OAG shifts the activation of the I_{NaP} to more hyperpolarized potential despite causing a suppression of the peak amplitude of the transient fast inactivating Na^+ current that is responsible for Na^+ spikes (Astman et al. 1998; Franceschetti et al. 2000; Numann et al. 1991; West et al. 1991). Therefore the D1-dependent reduction in Na^+ spike peak (Maurice et al. 2001) coupled with a shift in I_{NaP} (Gorelova and Yang 2002) is consistent with these mechanisms and the effects of D1 receptors on the Na^+ current is PKC mediated.

PKC phosphorylates the α-subunit of Na^+ channels to slow the inactivation of the $Na_v1.2$ isoform Na^+ channel (Chen et al. 2006). However, it is the $Na_v1.6$ isoform Na^+ channels (insensitive to PKA phosphorylation) that contribute largely to I_{NaP} (Maurice et al. 2001; Vega-Saenz de Miera et al. 1997), even though earlier studies suggested that modal gating switched the same Na^+ channel between both I_{NaP} and fast inactivating I_{Na} (Alzheimer et al. 1993; Brown et al. 1994; Crill 1996; Stafstrom et al. 1984). It is still not known whether PKC phosphorylates the $Na_v1.6$ channels to specifically regulate I_{NaP} and to play a role in the D1 enhancement of evoked spike firing in pyramidal neurons (Astman et al. 1998; Franceschetti et al. 2000; Gorelova and Yang 2000; Li et al. 1993; Numann et al. 1991; West et al. 1991). Furthermore, G-$\beta\gamma$ subunits of an activated G protein, when dissociated from the Gα-subunit, can directly enhance I_{NaP} (Ma et al. 1997; Mantegazza et al. 2005). Although the DA-mediated increase in neuronal excitability in accumbens neurons requires a coactivation of D1 and D2 receptors to liberate the G-$\beta\gamma$ subunit (Hopf et al. 2003), whether D1/5 receptor activation alone in PFC neurons leads to dissociation of active $\beta\gamma$ subunits to modulate I_{NaP} to induce an increase in neuronal excitability remains to be tested.

K^+ current consideration

With regard to the D1/5 modulation of relevant K^+ channels, an α-dendrotoxin–sensitive slowly inactivating K^+ current (I_D) that activates at subthreshold potentials and normally delays fast Na^+ spike initiation and reduces repetitive spike firing is modulated by D1 class receptor (Bekkers 2000a,b; Bekkers and Delaney 2001; Dong and White 2003; Hammond and Crepel 1992; Korngreen and Sakmann 2000; Storm 1988; Yang and Seamans 1996). However, the suppressive D1 effects

on I_D in PFC pyramidal neurons was shown to be mediated by a cAMP-dependent PKA-mediated (Dong and White 2003), but not a PKC-mediated mechanism as was found in the present study. Our studies also show that PLC, PKC inhibition, or $[Ca^{2+}]_i$ chelation never completely prevent an increase in neuronal excitability increase by the D1/5 agonist and the known D1/PKA/I_D mechanism (Dong and White 2003) may still contribute to a smaller extent in enhancing neuronal excitability in PFC neurons under these circumstances. As pointed out earlier, PKA inhibitors were included in the patch pipette (PKA-I) or bath applied (KT-5720) and therefore subsequent application of SKF81297 could not influence targets downstream of PKA, such as K^+ currents. Thus our study was biased against detecting an effect of PKA-dependent modulation of K^+ currents that would normally be activated by D1 receptors to enhance excitability.

The other K^+ current often associated with other non-ligand-induced LTP-IE is the reduction of Ca^{2+}-activated K^+ current that normally serves to generate multiple forms of postspike or postburst afterhyperpolarization (I_{AHP}) (Debanne et al. 2003; Schrader et al. 2002). Our intracellular BAPTA chelation of $[Ca^{2+}]_i$ data indicated that elevation of intracellular Ca^{2+} was required for D1/LTP-IE to occur in PFC neurons. In hippocampal neurons, a reduction of AHP by DA and D1 agonist by a PKA-mediated mechanism was thought to cause an increase in neuronal excitability (Malenka and Nicoll 1986; Pedarzani and Storm 1995), although the inferred D1/5 receptor-mediated increase in $[Ca^{2+}]_i$ did not increase the I_{AHP} in PFC neurons. It is likely that the D1/5 receptor-mediated elevation of intracellular Ca^{2+} could not interact with discretly distributed Ca^{2+}-activated K^+ channels (Abel et al. 2004; Pineda et al. 1998, 1999). The Ca^{2+} influx via non-L-type Ca^{2+} channels triggered by baseline-evoked spike discharge and/or from Ca^{2+} release from intracellular stores are compartmentalized locally and are regulated tightly by spatial buffering (Abel et al. 2004; Allbritton and Meyer 1993; Augustine and Neher 1992; Berkefeld et al. 2006; Berridge et al. 2003; Jacobs and Meyer 1997; Pineda et al. 1998, 1999; Stewart and Foehring 2001). It is thus less likely that the above K^+ channels have a major role in D1/5 receptor-mediated LTP-IE. At this point, we cannot rule out other K^+ channels that could contribute to the LTP-IE induced by D1/5 receptor activation.

Ca^{2+} current consideration

The D1/5 agonist-induced LTP-IE in PFC does not involve L-type Ca^{2+} channels because the LTP-IE is unaffected by the L-type channel antagonist nimodipine. In addition, the nimodipine-sensitive HVA Ca^{2+} spike potential is strongly suppressed by D1/5 receptor activation by a PKC-mediated mechanism (Young and Yang 2004). Furthermore, a subthreshold voltage-activated, nimodipine-sensitive, Ca^{2+} "hump" potential can be enhanced by a D1/PKA-dependent mechanism (Young and Yang 2004). Again, like the D1/PKA-dependent modulation of K^+ currents, this D1/PKA modulation of L-type Ca^{2+} currents was not assessed here because PKA inhibitors were preapplied before SKF81297 stimulation of D1/5 receptor. It is unlikely that the D1/PKC enhancement of neuronal excitability in PFC neurons found in this study involved a nimodipine-sensitive L-type Ca^{2+} channel because: *1*) the presence of nimodipine did not interfere with D1/LTP-IE and that *2*) the D1/PKC activation normally leads to suppression of a nimodipine-sensitive L-type Ca^{2+} channel (Young and Yang 2004). Nonetheless, non-L-type Ca^{2+} channels contribute significantly to the Ca^{2+} influx during the evoked spike firing (e.g., by back-propagated Na^+ spikes to trigger dendritic Ca^{2+} signaling by non-L-type Ca^{2+} channels) and play a role in the D1/5 receptor-mediated enhancement of neuronal excitability and LTP-IE.

Taken together, it appears that baseline-evoked firing already augments intracellular Ca^{2+} elevation (Jacobs and Meyer 1997) and may lead to a "priming" action for the D1/5 receptor to couple to Gq/11 protein, along with activation of PLC/IP3/DAG pathways, increase in intracellular Ca^{2+} release and leading to PKC, and CaMKII, activation downstream. Subsequently, these kinases catalyze phosphorylation of Na^+ channel ($Na_v^+1.6$?) or K^+ channels to enable LTP-IE (see Fig. 7 for a schematic summary).

Functional consideration for D1/5 receptor modulation of LTP-IE

LTP-IE is an integral mechanism in synaptic plasticity. Although induction of LTP-IE of evoked spike firing response is based on D1/5 receptor activation alone, a D1 modulation of the LTP of use-dependent, glutamate-receptor–mediated, synaptic efficacy also occurs in the PFC in vitro and in vivo (Gurden et al. 2000; Herry and Garcia 2002; Hirsch and Crepel 1990, 1992; Jay et al. 1998; Matsuda et al. 2006; Morris et al. 1999; Otani 2003; Sun et al. 2005; Vickery et al. 1997). The finding in the present study of a D1/PKC-dependent LTP-IE may reflect specific direct modulation of ion channels that regulates subthreshold voltage of EPSPs, thereby boosting the EPSP to reach spike firing. This is also consistent with the increase in neuronal excitability of intracellularly recorded PFC neurons after activation of the DA input from the VTA in vivo using identical induction protocols (Lavin et al. 2005).

The LTP of EPSP → spike generation is an integral mechanism operating at the subthreshold voltage range by depolarizing EPSPs (mostly glutamatergic) and may serve as a part of the long-term, use-dependent augmentation of synaptic efficacy. Increased spike firing and the associated increase in $[Ca^{2+}]_i$ can also lead to diverse changes in gene expression and dendritic gene translation, processes that form proteins that are essential for long-term changes in neural plasticity (Goldin and Segal 2003; Havik et al. 2003; Smith et al. 2005; Steward and Schuman 2001). The D1/5 receptor-dependent increase in intrinsic excitability most likely affects these latter processes.

PFC has a well-established role in processing short-term working memory (WM) (Goldman-Rakic 1998; Seamans and Yang 2004) and the persistent firing within a reverberatively active neural network could subserve WM maintenance (Lau and Bi 2005; Sakurai and Takahashi 2006; Wang 2001). Computational models predict that D1/5 receptor activation stabilizes PFC networks so they become more "robust" to a myriad of distractions and noise by enhancing signal-to-ratios to focus processing on the strongest inputs (Cohen et al. 2002; Compte et al. 2000; Durstewitz and Seamans 2002; Durstewitz et al. 2000; Lapish et al. 2006). This process involves LTP-IE as well as similar long-lasting enhancements of NMDA and GABA synaptic currents (Chen G et al. 2004; Lavin and Grace 2001; Lewis and O'Donnell

2000; Seamans and Yang 2004; Seamans et al. 2001; Trantham-Davidson et al. 2004; Tseng and O'Donnell 2004). However, PKC and CaMKII may mediate novel aspects of PFC activity during WM processing because PKC and CaMKII inhibition actually *improves* memory performance in animals (Birnbaum et al. 2004; Runyan et al. 2005).

Furthermore, the timing of the DA-dependent increase spike firing, when repeatedly paired with incoming synaptic inputs, may also play a crucial role in spike-timing–dependent LTP/D (Dan and Poo 2006; Nelson et al. 2002; Sjostrom and Nelson 2002). A sustained increase in neuronal excitability during or beyond the short period of WM maintenance (e.g., extending into tens and hundreds of minutes) could be used to strengthen the association of items or representations, allowing reordering of these items in an organized manner for long-term memory formation in PFC (Blumenfeld and Ranganath 2006; Ranganath and Blumenfeld 2005; Ranganath et al. 2005). The slow onset of the D1/5 receptor-mediated excitability increase and the longer timescale that corresponds to synaptic LTP are both PKC and CaMKII dependent (Gurden et al. 1999, 2000; Huang et al. 2004; Lisman et al. 2002; Matsuda et al. 2006; Muller et al. 1991; this study). Dopamine D1/5 receptor may have a crucial role in this switch and D1/LTP-IE is an integral part in this process.

LTP at synapses and LTP-IE may regulate the way PFC networks deal with short-term WM information. These long-term forms of plasticity are excellent candidate mechanisms for storing rule-based information in PFC. Rules in turn affect the manner in which trial-unique information is processed. Perhaps the best example of how these processes relate can be found in Wallis and Miller (2003), Wallis et al. (2001), and White and Wise (1999), who investigated the activation of PFC neurons in situations where two different abstract rules could be applied. PFC neurons showed different degrees of activation during a delay period depending on the preference of the neuron for a specific task rule. Therefore stable long-standing rules regulate how strongly a cell in PFC exhibits short-term memory–related activity. As shown here and by others (Gurden et al. 1999, 2000; Lisman and Grace 2005; Matsuda et al. 2006), long-term plasticity mechanisms are subjected to modulation by dopamine. In this formulation, dopamine may regulate the establishment of rules in the PFC networks that ultimately maintain transient trial unique information.

Finally, given the critical roles of mesocortical DA modulation of Ca^{2+}-dependent intracellular signaling cascades (e.g., PKC, CaMKII) that may mediate neuronal plasticity and cognitive processes (Birnbaum et al. 2004; Lidow et al. 2001; Runyan et al. 2005; Seamans and Yang 2004), a disruption of the D1/5 receptor-mediated intracellular signaling will have a profound impact in PFC functions and may also contribute to the cognitive deficits in schizophrenia (Abi-Dargham and Moore 2003; Bai et al. 2004; Baracskay et al. 2006; Clinton et al. 2005; Koh et al. 2003; Yang and Chen 2005).

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