

Potentiation of NMDA receptor currents by dopamine D₁ receptors in prefrontal cortex

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Interactions between dopamine and *N*-methyl-D-aspartate receptors (NMDARs) in prefrontal cortex (PFC) and other brain regions are believed to play an important role in normal mental function and neuropsychiatric disorders. In this study, we examined the regulation of NMDAR currents by the dopamine D₁ receptor in PFC pyramidal neurons. Application of the D₁ receptor agonist SKF81297 caused a prominent increase of the steady-state NMDA-evoked current in acutely isolated PFC pyramidal neurons. The D₁ effect on NMDARs was independent of protein kinase A or protein phosphatase 1, but was abolished by incubation of neurons in Ca²⁺-free medium. Intracellular application of the Ca²⁺ chelator, calmodulin, or calmodulin inhibitors largely prevented the D₁ modulation of NMDAR currents. Moreover, inhibiting PKC activity or disrupting PKC association with its anchoring protein also significantly reduced the D₁ effect on NMDAR currents. This up-regulation of NMDAR activity by dopamine D₁ receptors and the previous finding on up-regulation of dopamine D₁ receptors by NMDAR activation provide a cellular mechanism for the reciprocal interactions between D₁ and NMDARs. These interactions may play an important role in modulating synaptic plasticity and thus in cognitive and emotional processes.

Prefrontal cortex (PFC), a brain area highly associated with the control of cognition and emotion (1), is one of the most prominent regions affected by schizophrenia (2, 3). Schizophrenics often exhibit deficits in cognitive tasks requiring working memory subserved by PFC circuits (4). Despite the unclear etiology of schizophrenia, aberrations of several neurotransmitter systems have been implicated in this disorder. Among them, dysfunctions of the dopaminergic and glutamatergic systems in PFC are considered to be major contributing factors to the pathophysiology of schizophrenia (5).

It has long been recognized that dopamine receptors play a key role in the modulation of PFC working memory functions (1). Dopamine depletion in PFC results in impaired performances in PFC cognitive tasks, and these deficits can be ameliorated by the mixed dopamine receptor agonist apomorphine (6). The role of D₁ receptors in this process has been extensively explored. Iotophoresis of low concentrations of D₁ receptor antagonists enhances memory-related neuronal responses in monkeys performing challenging working memory tasks (7). On the other hand, administration of low, but not high, doses of D₁ receptor agonists improves spatial working memory performance (8). These results suggest that optimal levels of D₁ receptor stimulation are important for maintaining PFC functions.

Increasing evidence has suggested that, in addition to dopaminergic dysfunction, hypofunction of *N*-methyl-D-aspartate receptors (NMDARs) also plays a key role in schizophrenia (9). Administration of noncompetitive NMDAR antagonists in humans and animals produces behavioral symptoms that are remarkably similar to schizophrenia (10, 11). Mutant mice expressing 5% of the normal level of NR1 (12) exhibit a series of schizophrenia-like behaviors, which are ameliorated by administration of antipsychotic drugs that target dopamine receptors (12). To begin to understand how the “dopamine hypothesis” and “glutamate hypothesis” of schizophrenia may be mechanistically linked, we examined the

interactions between D₁ and NMDARs in PFC pyramidal neurons. A previous study has shown that dopamine D₁ signaling can be regulated by NMDAR activation (13). In this study, we provide evidence showing that NMDAR activity can be regulated by dopamine D₁ signaling.

Materials and Methods

Acute-Dissociation Procedure. PFC neurons from young adult (3–5 weeks postnatal) rats were acutely dissociated by using procedures similar to those described in ref. 14. All experiments were carried out with the approval of the State University of New York at Buffalo Animal Care Committee. After incubation of brain slices in a NaHCO₃-buffered saline, PFC was dissected and placed in an oxygenated Cell-Stir chamber containing papain (Sigma, 0.4 mg/ml) in HEPES-buffered Hanks' balanced salt solution at 35°C. After 20–40 min of enzyme digestion, tissue was rinsed three times in the low Ca²⁺, HEPES-buffered saline and mechanically dissociated with a graded series of fire-polished Pasteur pipettes. The cell suspension was then plated into a 35-mm Lux Petri dish, which was then placed on the stage of a Zeiss inverted microscope.

Whole-Cell Recordings. Whole-cell recordings of ion channel currents used standard voltage clamp techniques (15, 16). The internal solution consisted of 180 mM *N*-methyl-D-glucamine, 40 mM HEPES, 4 mM MgCl₂, 0.1 mM 1,2-bis(2-aminophenoxy)ethane-*N,N,N',N'*-tetraacetate (BAPTA), 12 mM phosphocreatine, 2 mM Na₂ATP, 0.2 mM Na₃GTP, and 0.1 mM leupeptin, pH 7.2–7.3, osmolarity of 265–270. The external solution consisted of 127 mM NaCl, 20 mM CsCl, 10 mM HEPES, 1 mM CaCl₂, 5 mM BaCl₂, 12 mM glucose, 0.001 mM Tetrodotoxin, and 0.02 mM glycine, pH 7.3–7.4, osmolarity of 300–305. Recordings were obtained with an Axon Instruments (Foster City, CA) 200B patch-clamp amplifier that was controlled and monitored with an IBM PC running PCLAMP (version 8) with a DigiData 1320 series interface (Axon Instruments). Electrode resistances were typically 2–4 MΩ in the bath. After seal rupture, series resistance (4–10 MΩ) was compensated (70–90%) and periodically monitored. The cell membrane potential was held at –60 mV. NMDA (500 μM) was applied for 2 s every 30 s to minimize desensitization-induced decrease of current amplitude. The application of NMDA evoked a partially desensitizing inward current. Steady-state current amplitudes were measured at the end of a 2-s application of NMDA for generating the plot as a function of time and drug application. Drugs were applied with a gravity-fed “sewer pipe” system. The array of application capillaries (≈150 μm i.d.) was positioned a few hundred microns from the cell under study. Solution changes were effected

Abbreviations: AKAP, A-kinase anchoring protein; BAPTA, 1,2-bis(2-aminophenoxy)ethane-*N,N,N',N'*-tetraacetate; CaM, calmodulin; CDZ, calmidazolium; CsA, cyclosporin A; MLCK, myosin light chain kinase; NMDA, *N*-methyl-D-aspartate; NMDAR, NMDA receptor; OA, okadaic acid; PFC, prefrontal cortex; PKA, protein kinase A; PP1, protein phosphatase 1.

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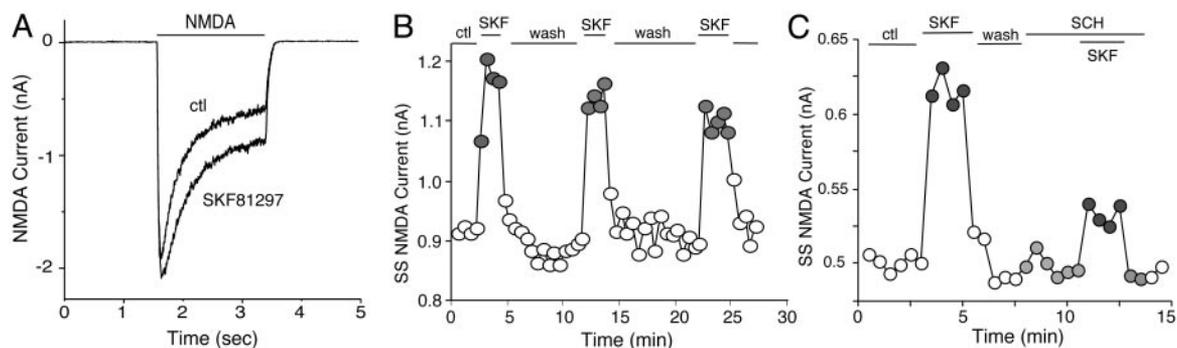


Fig. 1. Activation of D₁ receptors enhanced NMDAR currents in acutely dissociated PFC pyramidal neurons. (A) Current traces taken from a representative neuron showing the effect of SKF81297 (10 μ M) on NMDA (500 μ M)-evoked currents. (B) Plot of the steady-state NMDAR current (I_{ss}) as a function of time and agonist application. (C) Plot of I_{ss} showing that the selective D₁ antagonist SCH23390 (SCH) (10 μ M) largely blocked the effect of SKF81297 (SKF).

by the SF-77B fast-step solution stimulus delivery device (Warner Instruments, Hamden, CT).

Dopamine receptor ligands SKF81297 and SCH23390 (Sigma), as well as second messenger reagents PKI₆₋₂₂, myristoylated PKI₁₄₋₂₂, okadaic acid (OA), calmodulin (CaM), calmidazolium (CDZ), myosin light chain kinase (MLCK) peptide, KN-93, cyclosporin A (CsA), PKC₁₉₋₃₆, bisindolylmaleimide I, U73122, heparin, wortmannin, and genistein (Calbiochem) were made up as concentrated stocks and stored at -20°C . The final DMSO concentration in all applied solutions was $<0.1\%$. Stocks were thawed and diluted immediately before use. The amino acid sequence for the protein phosphatase 1 (PP1)-anchoring inhibitory peptide Gm[63-75] is GRRVSFADNFGFN.

Data analyses were performed with AXOGRAPH (Axon Instruments), KALEIDAGRAPH (Albeck, Reading, PA), ORIGIN 6 (OriginLab, Northampton, MA), and STATVIEW (Abacus Concepts, Berkeley, CA). For analysis of statistical significance, Mann-Whitney *U* tests were performed to compare the current amplitudes in the presence or absence of agonists. ANOVA tests were performed to compare the differential degrees of current modulation between groups subjected to different treatment.

Results

Activation of D₁ Receptors Enhances NMDA-Evoked Currents in Dissociated PFC Pyramidal Neurons. To test the potential impact of D₁ receptors on NMDA signaling, we examined the effect of SKF81297, a selective D₁ receptor agonist, on NMDAR-mediated currents in acutely isolated PFC pyramidal neurons. As shown in Fig. 1A and B, application of NMDA (500 μ M) evoked a partially desensitizing inward current, and SKF81297 (10 μ M) caused a potent and reversible increase in the amplitude of NMDA-evoked currents in the PFC pyramidal neuron. Moreover, the enhancing effect of SKF81297 was most prominent in the steady-state component of NMDA-evoked currents, suggesting a suppression of desensitization or inactivation of NMDARs. In a sample of PFC pyramidal neurons we tested, SKF81297 produced a $19.8 \pm 5.4\%$ ($n = 98$, $P < 0.01$, Mann-Whitney) enhancement of steady-state NMDAR currents (I_{ss}).

To verify that D₁ receptors were responsible for the modulation seen with SKF81297, we examined the ability of SCH23390, a selective D₁ receptor antagonist, to prevent the action of SKF81297. As shown in Fig. 1C, SKF81297 (10 μ M) produced a reversible enhancement of the steady-state NMDAR current in the PFC neuron, and this effect was greatly reduced by SCH23390 (10 μ M). In a sample of cells we examined, SKF81297 had a significantly ($P < 0.005$, ANOVA) smaller effect on steady-state NMDAR currents in the presence of SCH23390 ($9.8 \pm 3.8\%$, $n = 4$), compared to the effect of SKF81297 in the absence of SCH23390 ($22.3 \pm 2.0\%$, $n = 4$). These results suggest

D₁ as the receptor underlying the SKF81297-induced potentiation of NMDAR currents.

The D₁ Enhancement of NMDAR Currents in PFC Pyramidal Neurons Is Independent of Protein Kinase A (PKA)/PP1 but Involves Ca²⁺/CaM. We next examined the signal transduction pathway mediating the D₁ potentiation of NMDAR currents in PFC pyramidal neurons.

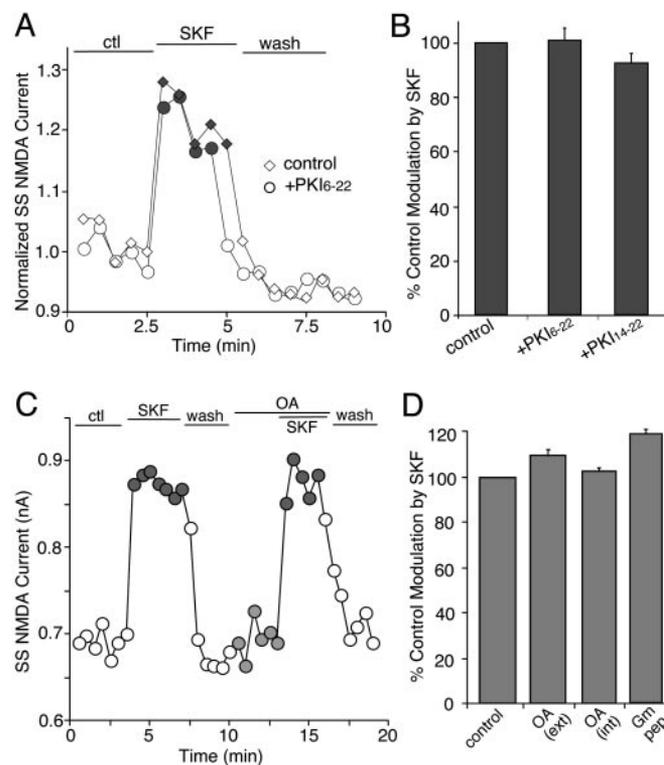


Fig. 2. The effect of SKF81297 (SKF) on NMDAR currents was independent of PKA/PP1. (A) Plot of I_{ss} showing that dialysis with the PKA inhibitory peptide PKI₆₋₂₂ (20 μ M) did not prevent the SKF81297-induced potentiation of NMDAR currents. (B) Cumulative data (mean \pm SE) showing the percentage control modulation of I_{ss} by SKF81297 in the absence ($n = 16$) or presence of PKI₆₋₂₂ ($n = 15$), or the membrane-permeable myristoylated PKA inhibitor PKI₁₄₋₂₂ (0.2 μ M, $n = 10$). (C) Plot of I_{ss} showing that inhibiting PP1 activity with OA (0.1 μ M) failed to affect the SKF81297-induced potentiation of NMDAR currents. (D) Cumulative data (mean \pm SE) showing the percentage control modulation of I_{ss} by SKF81297 in the absence ($n = 34$) or presence of OA (external application: 0.1 μ M, $n = 8$; internal application: 1 μ M, $n = 23$) or the PP1-anchoring inhibitory peptide Gm (20 μ M, $n = 20$).

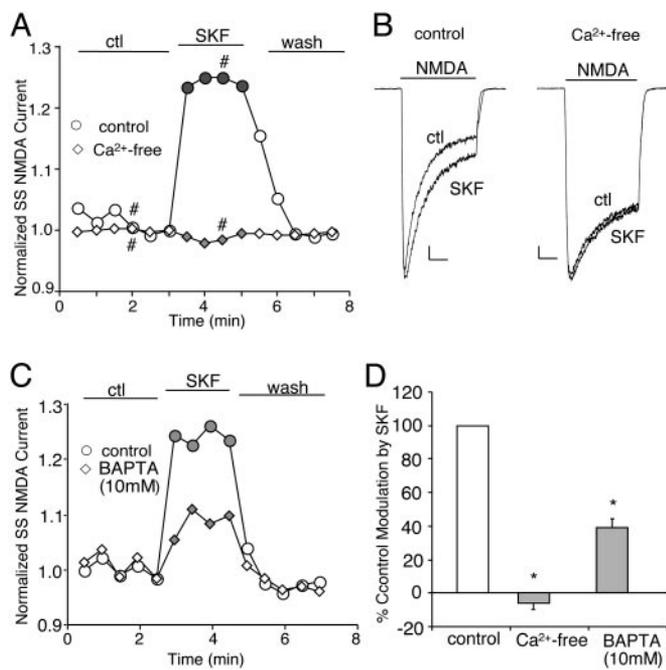


Fig. 3. The effect of SKF81297 (SKF) on NMDAR currents dependent on Ca^{2+} . (A) Plot of I_{ss} as a function of time and agonist application in neurons perfused in a Ca^{2+} -free solution (10 μM EGTA added) or in the normal external solution (containing 1 mM Ca^{2+}). (B) Representative current traces taken from the records used to construct A (at time points denoted by #). (Scale bars: 0.2 nA, 0.5 s.) (C) Plot of I_{ss} as a function of time and agonist application in neurons dialyzed with the high BAPTA internal solution (10 mM) or the normal internal solution (containing 0.5 mM BAPTA). (D) Cumulative data (mean \pm SE) showing the percentage control modulation of I_{ss} by SKF81297 in normal conditions ($n = 20$), in the Ca^{2+} -free solution ($n = 7$), or in neurons dialyzed with high BAPTA ($n = 15$). *, $P < 0.001$, ANOVA.

The “classical” signaling cascade of D_1 receptors is to stimulate adenylate cyclase and cAMP formation. The D_1 -induced activation of PKA could directly modulate NMDAR currents through increased phosphorylation of NR1 subunits on the PKA sites (17). Alternatively, the activation of PKA could cause the inhibition of PP1 via increased phosphorylation of regulatory proteins, such as dopamine and cAMP-regulated phosphoprotein DARPP-32 (18) and the PP1 inhibitory protein I-1, leading to the decreased dephosphorylation of NMDAR subunits by PP1 (19) and up-regulation of NMDAR currents. To evaluate these potential signaling mechanisms, we examined the effect of SKF81297 on NMDARs in the presence of PKA or PP1 inhibitors.

As shown in Fig. 2A and B, dialysis with the PKA inhibitory peptide PKI₆₋₂₂ (20 μM) failed to block the SKF81297-induced increase of NMDAR currents ($101.3 \pm 9.7\%$ of control modulation, $n = 15$). The effect of SKF81297 was also intact in the presence of the membrane-permeable PKA inhibitory peptide, myristoylated PKI₁₄₋₂₂ (0.2 μM , $91.9 \pm 4.3\%$ of control modulation, $n = 10$, Fig. 2B). Moreover, bath application of the PP1 inhibitor OA (0.1 μM) did not affect the ability of SKF81297 to enhance NMDAR currents (Fig. 2C). As summarized in Fig. 2D, inhibiting PP1 catalytic activity with external or internal application of OA failed to block the SKF81297 enhancement of NMDAR currents (external OA: $109.2 \pm 2.3\%$ of control modulation, $n = 8$; internal OA: $102.2 \pm 1.9\%$ of control modulation, $n = 23$). The effect of SKF81297 on NMDAR currents was also unaffected when PP1 targeting was disrupted by 20 μM of peptide Gm[63–75] (20) ($118.9 \pm 2.5\%$ of control modulation, $n = 20$). These results suggest that the classical

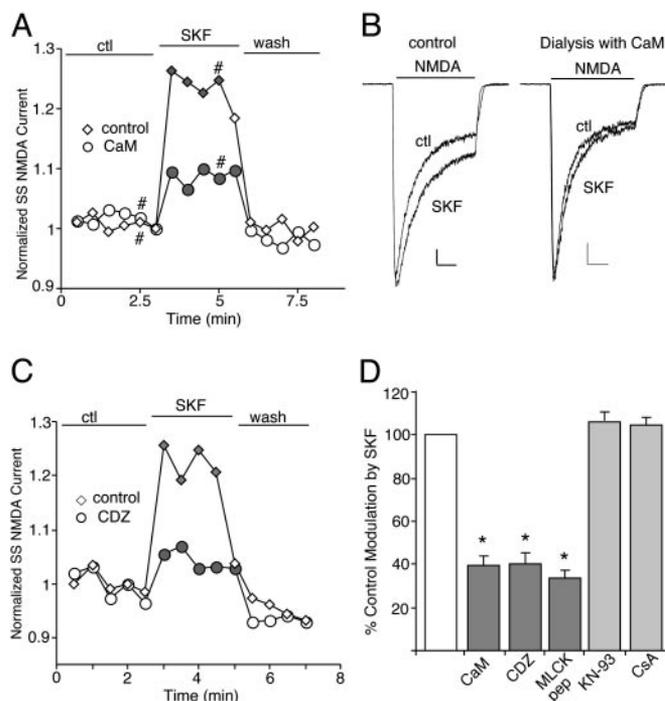


Fig. 4. The effect of SKF81297 (SKF) on NMDAR currents dependent on CaM. (A) Plot of I_{ss} as a function of time and agonist application in neurons loaded with or without CaM (10 μM). (B) Representative current traces taken from the records used to construct A (at time points denoted by #). (Scale bars: 0.2 nA, 0.5 s.) (C) Plot of I_{ss} as a function of time and agonist application in neurons dialyzed with or without the CaM antagonist CDZ (20 μM). (D) Cumulative data (mean \pm SE) showing the percentage control modulation of I_{ss} by SKF81297 in normal conditions ($n = 10$) or in neurons dialyzed with CaM ($n = 7$), CDZ ($n = 14$) or the CaM inhibitory peptide MLCK peptide (MLCKP) ($n = 12$), or in the presence of the CaM-dependent protein kinase II inhibitor KN-93 (10 μM , $n = 14$) or the calcineurin inhibitor CsA (50 μM , $n = 5$). *, $P < 0.001$, ANOVA.

PKA/PP1 cascade does not link D_1 receptors to the potentiation of NMDAR currents in PFC pyramidal neurons, at least under the experimental conditions of the present study.

The SKF81297-induced potentiation of peak NMDAR current (I_p) is relatively small in saturating concentrations of NMDA and glycine, whereas the enhancement of steady-state NMDAR current (I_{ss}) is prominent, suggesting that the major effect of D_1 receptors on NMDA-evoked response in PFC pyramidal neurons is the inhibition of Ca^{2+} -dependent inactivation of NMDAR channels (21–23). We therefore examined the role of Ca^{2+} in D_1 modulation of NMDAR currents in PFC. At first we incubated neurons in a Ca^{2+} -free solution (with 10 μM EGTA), and examined NMDAR currents in the absence and presence of SKF81297 under this condition. NMDA (500 μM)-evoked currents in neurons under the Ca^{2+} -free condition had much smaller inactivation, as calculated by the ratio of I_{ss} to I_{peak} (I_{ss}/I_{peak} ; Ca^{2+} -free: 0.59 ± 0.06 , $n = 7$; Ca^{2+} -containing: 0.32 ± 0.07 , $n = 32$). As shown in Fig. 3A and B, SKF81297 completely lost the ability to enhance NMDAR currents in the neuron treated with the Ca^{2+} -free solution. We then dialyzed neurons with a high concentration (10 mM) of BAPTA, a potent and rapid Ca^{2+} chelator, and examined D_1 modulation of NMDAR currents under this condition. Dialysis with high BAPTA reduced the inactivation of NMDAR currents (I_{ss}/I_{peak} ; high BAPTA: 0.42 ± 0.06 , $n = 13$; low BAPTA: 0.32 ± 0.07 , $n = 32$), and substantially attenuated the effect of SKF81297 on NMDAR currents (Fig. 3C). In a set of neurons we tested (Fig. 3D), the enhancing effect of D_1 on NMDAR currents was eliminated under the Ca^{2+} -free condition ($-6.0 \pm 2.0\%$ of control modulation, $n = 7$) and was signif-

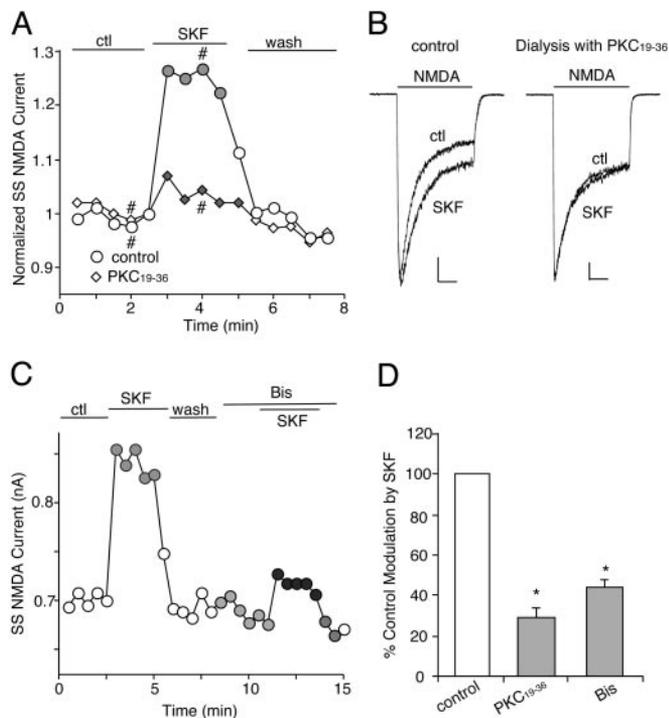


Fig. 5. The effect of SKF81297 (SKF) on NMDAR currents was attenuated by inhibiting PKC. (A) Plot of I_{ss} as a function of time and agonist application in neurons loaded with or without the PKC inhibitory peptide PKC₁₉₋₃₆ (4 μ M). (B) Representative current traces taken from the records used to construct A (at time points denoted by #). (Scale bars: 0.2 nA, 0.5 s.) (C) Plot of I_{ss} showing that the enhancing effect of SKF81297 was largely diminished in the presence of the PKC inhibitor bisindolylmaleimide (Bis, 1 μ M). (D) Cumulative data (mean \pm SE) showing the percentage control modulation of I_{ss} by SKF81297 in normal conditions ($n = 11$) or in neurons dialyzed with PKC₁₉₋₃₆ ($n = 17$), or in the presence of bisindolylmaleimide (Bis, $n = 21$). *, $P < 0.001$, ANOVA.

icantly ($P < 0.001$, ANOVA) diminished by buffering intracellular Ca^{2+} with BAPTA in the patch pipette ($39.0 \pm 5.8\%$ of control modulation, $n = 15$).

The binding of Ca^{2+} /CaM to the NR1 subunit is thought to underlie Ca^{2+} inactivation of NMDARs (23–25). Thus, we examined the role of CaM in D₁ modulation of NMDAR currents in PFC pyramidal neurons. Intracellular infusion of active CaM (10 μ M) caused more inactivation of NMDA-evoked currents (I_{ss}/I_{peak} ; with CaM, 0.22 ± 0.06 , $n = 9$; without CaM: 0.32 ± 0.07 , $n = 32$), and substantially blocked the SKF81297-induced potentiation of NMDAR currents (Fig. 4A and B). On the other hand, inclusion of the CaM antagonist CDZ (20 μ M) or the CaM inhibitory peptide MLCK peptide (26) in patch electrodes reduced the inactivation of NMDAR currents (I_{ss}/I_{peak} ; with CaM inhibitors: 0.41 ± 0.04 , $n = 19$; without CaM inhibitors: 0.32 ± 0.07 , $n = 32$), and markedly attenuated the enhancing effect of SKF81297 (Fig. 4C). In a sample of neurons we tested (Fig. 4D), the SKF81297 enhancement of steady-state NMDAR currents was significantly ($P < 0.001$, ANOVA) smaller in neurons dialyzed with CaM ($39.4 \pm 4.5\%$ of control modulation, $n = 7$), CDZ ($40.6 \pm 4.2\%$ of control modulation, $n = 14$), or the CaM inhibitory peptide MLCK peptide ($33.8 \pm 3.6\%$ of control modulation, $n = 12$).

We also examined the effect of SKF81297 on NMDAR currents in the presence of the Ca^{2+} /CaM-dependent protein kinase II inhibitor KN-93 or the calcineurin inhibitor CsA, to determine whether these Ca^{2+} -dependent enzymes were involved. The presence of KN-93 (10 μ M) or CsA (50 μ M) failed to reduce the SKF81297-induced potentiation of NMDAR currents (Fig. 4D, KN-93: $105.8 \pm 3.6\%$ of control modulation, $n =$

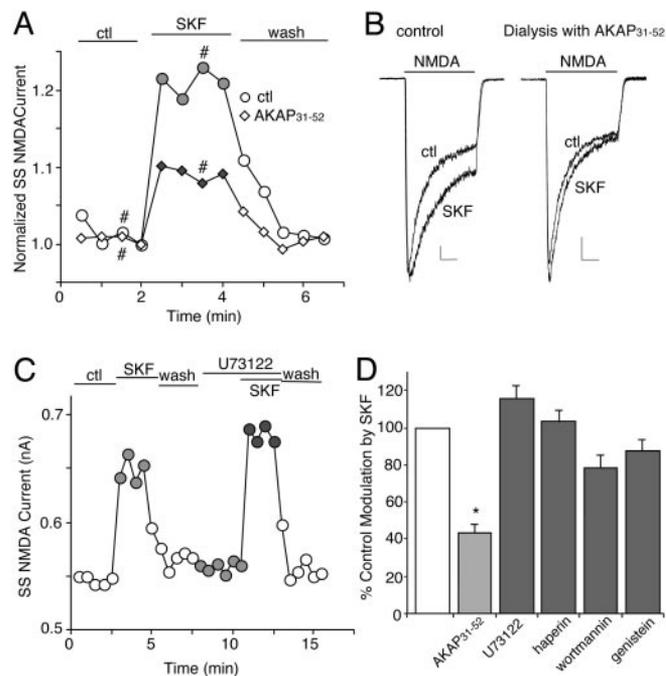


Fig. 6. The effect of SKF81297 (SKF) on NMDAR currents was reduced by disrupting the PKC/AKAP interaction but was not affected by inhibiting phospholipase C (PLC) or other signaling molecules. (A) Plot of I_{ss} as a function of time and agonist application in neurons loaded with or without the peptide AKAP₃₁₋₅₂ (40 μ M). (B) Representative current traces taken from the records used to construct A (at time points denoted by #). (Scale bars: 0.2 nA, 0.5 s.) (C) Plot of I_{ss} showing that the enhancing effect of SKF81297 was intact in the presence of the PLC inhibitor U73122 (1 μ M). (D) Cumulative data (mean \pm SE) showing the percentage control modulation of I_{ss} by SKF81297 in normal conditions ($n = 10$) or in neurons dialyzed with AKAP₃₁₋₅₂ ($n = 15$), or in the presence of U73122 ($n = 4$), the inositol-1-4-5-triphosphate receptor antagonist heparin (2 mg/ml, $n = 4$), the phosphoinositide 3-kinase inhibitor wortmannin (3 μ M, $n = 12$), or the protein tyrosine kinase inhibitor genistein (100 μ M, $n = 16$). *, $P < 0.001$, ANOVA.

14; CsA: $104.4 \pm 2.7\%$ of control modulation, $n = 5$). Collectively, these results suggest that the D₁ potentiation of NMDAR currents in PFC pyramidal neurons is caused by suppression of Ca^{2+} /CaM-dependent inactivation of NMDARs.

The D₁ Enhancement of NMDAR Currents in PFC Pyramidal Neurons Is Through a Mechanism Involving PKC. Previous studies in hippocampal neurons have shown that PKC activation enhances Ca^{2+} /CaM-dependent inactivation of NMDAR channels (27), presumably because of a phosphorylation-dependent regulation of the interactions between NMDAR subunits, CaM, or other postsynaptic density proteins (27). We therefore examined the role of PKC in D₁ modulation of NMDAR currents in PFC pyramidal neurons. As shown in Fig. 5A and B, intracellular infusion of the PKC inhibitory peptide PKC₁₉₋₃₆ (4 μ M) substantially diminished the SKF81297-induced potentiation of NMDAR currents. In the presence of bisindolylmaleimide (1 μ M), a membrane-permeable selective PKC inhibitor, SKF81297 also had a much smaller effect on NMDAR currents (Fig. 5C). As summarized in Fig. 5D, the SKF81297 enhancement of steady-state NMDAR currents was significantly ($P < 0.001$, ANOVA) reduced in neurons dialyzed with PKC₁₉₋₃₆ ($29.3 \pm 5.3\%$ of control modulation, $n = 17$) or treated with bisindolylmaleimide ($43.9 \pm 4.2\%$ of control modulation, $n = 21$).

Given the involvement of both Ca^{2+} /CaM and PKC in the D₁ modulation of NMDAR currents in PFC pyramidal neurons, we

examined their possible interconnections. It has been shown that Ca^{2+}/CaM competes with PKC for binding to A-kinase anchoring protein (AKAP) 79, releasing the inhibited PKC from its association with the anchoring protein (28). Thus, we dialyzed neurons with a peptide AKAP₃₁₋₅₂ (29) to disrupt the interaction of PKC with AKAP79, followed by the examination of SKF81297 effects on NMDAR currents. As shown in Fig. 6A and B, the SKF81297-induced potentiation of NMDAR currents was markedly reduced in neurons loaded with the AKAP₃₁₋₅₂ peptide (40 μ M, $43.6 \pm 4.1\%$ of control modulation, $n = 15$, $P < 0.001$, ANOVA, Fig. 6D). On the other hand, application of U73122 (1 μ M), a phospholipase C (PLC) inhibitor, failed to affect SKF81297 enhancement of NMDAR currents (Fig. 6C, $115.8 \pm 6.1\%$ of control modulation, $n = 4$, Fig. 6D), indicating that the D_1 regulation of NMDARs was independent of the PLC pathway.

The potential role of several other signaling molecules in the D_1 regulation of NMDARs in PFC pyramidal neurons was also examined. As summarized in Fig. 6D, the enhancing effect of SKF81297 was intact in the presence of the inositol-1-4-5-triphosphate receptor antagonist heparin (2 mg/ml, $103.5 \pm 5.5\%$ of control modulation, $n = 4$), the phosphoinositide 3-kinase inhibitor wortmannin (3 μ M, $78.3 \pm 6.7\%$ of control modulation, $n = 12$), or the broad-spectrum protein tyrosine kinase inhibitor genistein (100 μ M, $87.1 \pm 4.0\%$ of control modulation, $n = 16$), indicating the lack of involvement of these molecules. Taken together, these results suggest that the D_1 potentiation of NMDAR currents in PFC pyramidal neurons is through a mechanism involving PKC that is regulated by CaM.

Discussion

Because the schizophrenia-like behaviors induced by administration of NMDAR antagonists (10, 11) or NMDAR knockdown (12) have implicated NMDARs in mental disorders associated with PFC dysfunction, it is conceivable that D_1 receptors may influence PFC functions through the regulation of NMDAR channels. In agreement with this, D_1 and NMDARs are enriched in dendritic spines (30–33). This synaptic organization enables interactions between dopamine and glutamate signaling in the same neuron. In this study, we demonstrated that activation of D_1 receptors significantly enhanced NMDAR currents in acutely dissociated PFC pyramidal neurons, consistent with previous findings on D_1 potentiation of NMDAR-mediated excitatory

postsynaptic currents in PFC slices (34, 35), and D_1 increase of NMDAR currents in medium spiny neostriatal neurons (36). Our data suggest that the postsynaptic NMDAR is a potential key target of D_1 receptors. On the other hand, application of NMDA in cultured striatal neurons increases the recruitment of D_1 receptors to dendritic spines in a Ca^{2+} -dependent manner (13), suggesting that NMDARs can also regulate D_1 signaling. The reciprocal positive interactions between D_1 and NMDAR channels could play a significant role in regulating the cognitive and emotional status.

NMDAR channel activity can be regulated by a wide array of extracellular agents, including Mg^{2+} , glycine, and Zn^{2+} (37), intracellular agents like Ca^{2+} and CaM (21, 24), and protein kinases/phosphatases, such as PKA, PKC, and PP1 (19, 38, 39). Our results show that the D_1 enhancement of steady-state NMDAR currents in PFC pyramidal neurons is through the suppression of Ca^{2+}/CaM -dependent inactivation of NMDAR channels, but not via the classical PKA/PP1 cascade as found in striatal neurons (36, 40). Unlike the D_4 -mediated down-regulation of NMDAR channels in PFC pyramidal neurons (16), Ca^{2+}/CaM -dependent protein kinase II is not involved in this D_1 action. Inhibiting PKC activity or disrupting PKC association with its anchoring protein AKAP79 attenuated the D_1 effect on NMDAR channels, suggesting the involvement of PKC in this modulation. Ca^{2+} entry from NMDAR channels may regulate NMDAR inactivation by two mechanisms. One is through CaM binding to NR1 subunits directly (23, 24). The other is through CaM competing with PKC for binding to AKAP79, leading to the release and disinhibition of PKC (28) and PKC-dependent potentiation of NMDAR inactivation (27). By inhibiting Ca^{2+}/CaM through an unknown mechanism, activation of D_1 receptors suppresses the NMDAR inactivation, resulting in an enhanced steady-state NMDAR current. Given the key role of D_1 and NMDARs in schizophrenia and other neuropsychiatric disorders, the present results provide a possible cellular mechanism that could underlie the D_1 regulation of cognitive functions associated with PFC.

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