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Bidirectional Regulation of Ca^{2+}/Calmodulin-Dependent Protein Kinase II Activity by Dopamine D_4 Receptors in Prefrontal Cortex

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ABSTRACT

The dopamine D_4 receptor in prefrontal cortex (PFC) plays a key role in normal mental functions and neuropsychiatric disorders. However, the cellular mechanisms and physiological actions of D_4 receptors remain elusive. In this study, we found that activation of D_4 receptors in PFC exerts a complex regulation of Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII), a multifunctional enzyme critically involved in synaptic plasticity that is fundamental for cognitive and emotional processes. In PFC slices with high neuronal activity, application of the D_4 receptor agonist [4-phenylpiperazinyl)-methyl]benzamide (PD168077) produced a potent reduction of the CaMKII activity, whereas in PFC slices with low neuronal activity, PD168077 caused a marked increase of the CaMKII activity. The D_4 up-regulation of CaMKII activity was through the stimulation of phospholipase C pathway and elevation of intracellular Ca^{2+} via ionsitol-1,4,5-triphosphate receptors. These results reveal a bidirectional regulation of CaMKII activity by PFC D_4 receptors in response to changes in neuronal activity, and a nonclassic signaling pathway underlying the D_4 up-regulation of CaMKII activity. This modulation provides a unique and flexible mechanism for D_4 receptors to regulate CaMKII activity, which could lead to dynamic regulation of many targets of CaMKII by D_4 receptors.

Prefrontal cortex (PFC) is a brain region critically involved in the control of cognition, reasoning, perception, and emotion (Goldman-Rakic, 1995). Dysfunction of PFC has been implicated in a variety of neuropsychiatric disorders, including schizophrenia (Andreasen et al., 1997; Lewis and Lieberman, 2000). PFC functions are highly influenced by the dopaminergic input from the ventral tegmental area (Brozoski et al., 1979; Berger et al., 1988). Aberration of the dopaminergic system in PFC is considered a major factor in the pathophysiology of schizophrenia (Grace, 1991; Carlsson et al., 2001).

Dopamine D_4 receptors are highly enriched in PFC neurons (Mrzljak et al., 1996; Wedzony et al., 2000). The elevated D_4 receptors found in the PFC of patients with schizophrenia (Seeman et al., 1993) and the high affinities of D_4 receptors for antipsychotic drugs (Van Tol et al., 1991; Kapur and Remington, 2001) suggest that D_4 receptors may be critically involved in PFC functioning and neuropsychiatric disorders (Oak et al., 2000). In agreement with this, D_4 receptor antagonists ameliorate cognitive deficits caused by the psychotomimetic drug phencyclidine (Jentsch et al., 1997; Jentsch and Roth, 1999). Moreover, mice lacking D_4 receptors exhibit supersensitivity to psychomotor stimulants (Rubinstein et al., 1997) and reduced exploration of novel stimuli (Dulawa et al., 1999). To understand how D_4 receptors regulate PFC functions under normal and pathological conditions, we need to determine the potential targets of D_4 receptors that are critically involved in the regulation of cognitive and emotional processes subserved by PFC.

One potential target of such for D_4 receptors is the Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII). CaMKII

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ABBREVIATIONS: PFC, prefrontal cortex; CaMKII, Ca^{2+}/calmodulin-dependent protein kinase II; PLC, phospholipase C; CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione; APV, D(−)-2-amino-5-phophonopetanoic acid; 2APB, 2-aminoethoxydiphenylborane; DHBP, 1,1′-diheptyl-4-4′-bipyridinium; NEM, *N*-ethylmaleimide; ctl, control; IP_3R, ionsitol-1,4,5-triphosphate receptor; RyR, ryanodine receptor, ANOVA, analysis of variance; PD168077, [(4-phenylpiperazinyl)-methyl]benzamide; PKA, protein kinase A; BIC, bicuculline; TTX, tetrodotoxin; U73122, 1-[6-[[17β-methoxyestra-1,3,5(10)-trien-17-yl]amino]hexyl]-1*H*-pyrrole-2,5-dione; SCH23390, *R*-(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine; L-745870, (3-([4-(4-chlorophenyl)piperazin-1-yl]methyl)-1*H*-pyrollo[2,3-*b*]pyridine; BAPTA/AM, 1,2-bis(2-aminophenoxy)ethane-*N*,*N*,*N*′-tetraacetic acid/acetoxymethyl ester; cpt-cAMP, 8-(4-chlorophenylthio)adenosine 3′,5′-cyclic monophosphate.[C]

is highly expressed in the forebrain and concentrated at postsynaptic densities at glutamatergic synapses (Kennedy et al., 1983). This ideal position allows the multifunctional enzyme to play a central role in regulating several key postsynaptic targets required for synaptic plasticity that is integral for learning and memory (Malenka and Nicoll, 1999; Soderling et al., 2001). Mice with deficient CaMKII exhibit impairments in spatial learning (Silva et al., 1992) and permanent memory retention (Frankland et al., 2001). In addition to the cognitive deficit, these CaMKII mutant mice exhibit a spectrum of behavioral abnormalities associated with emotional disorders, including a decreased fear response and an increase in defensive aggression (Chen et al., 1994).

The function of CaMKII is shaped by its autoregulation and subcellular localization (Hudmon and Schulman, 2002). CaMKII is autophosphorylated at Thr^{286} when the enzyme is activated in the presence of Ca^{2+}/calmodulin, leading to the appearance of a sustained, Ca^{2+}-independent activity (Miller and Kennedy, 1986). This autoregulatory property enables CaMKII to act as a molecular memory device to detect synaptic activity and to coordinate and execute Ca^{2+} signal transduction. CaMKII also dynamically alters its subcellular distribution after *N*-methyl-D-aspartate receptor stimulation through a mechanism involving Ca^{2+}/calmodulin binding and autophosphorylation (Shen and Meyer, 1999). Given the convergent involvement in PFC functions for D_4 receptors and CaMKII, we sought to understand their interactions by examining the D_4 regulation of CaMKII activity in this study.

Materials and Methods

Western Blot Analysis. PFC slices were prepared as described previously (Gu et al., 2003). After treatment with different agents as indicated in the text, equal amounts of protein from slice homogenates were separated on 7.5% acrylamide gels and transferred to nitrocellulose membranes. The blots were blocked with 5% nonfat dry milk for 1 h at room temperature. Then the blots were incubated with the anti–Thr^{286}-phosphorylated α-CaMKII antibody (Santa Cruz, 1:2000) for 1 h at room temperature. After being rinsed, the blots were incubated with horseradish peroxidase-conjugated anti-rabbit antibodies (Amersham, 1:2000) for 1 h at room temperature. After 3 washes, the blots were exposed to the enhanced chemiluminescence substrate. Then the blots were stripped for 1 h at 50°C followed by saturation in 5% nonfat dry milk and incubated with an anti–α-CaMKII antibody (Upstate Biotechnology, 1:5000) for the detection of the total α-CaMKII. Quantification was obtained from densitometric measurements of immunoreactive bands on films.

Dopamine receptor ligands PD168077 maleate, L-745870 trihydrochloride (Tocris Cookson Inc., Ellisville, MO), quinpirole, sulpiride, and SCH23390 (Sigma-Aldrich, St. Louis, MO), as well as second-messenger reagents U73122, genistein, BAPTA/AM, 2-aminoethoxydiphenylborane (2APB), 1,1′-diheptyl-4–4′-bipyridinium (DHBP), thapsigargin (Calbiochem, San Diego, CA), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid/kainate receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), and *N*-methyl-D-aspartate receptor antagonist D(−)-2-amino-5-phosphonopetanoic acid (APV) (Sigma-Aldrich) were made up as concentrated stocks and stored at −20°C. The final dimethyl sulfoxide concentration in all applied solutions was less than 0.1%. Stocks were thawed and diluted immediately before use.

Results

Bidirectional Regulation of CaMKII Activity by D_4 Receptors in PFC Neurons. CaMKII is activated by the binding of Ca^{2+}/calmodulin, and then it undergoes autophosphorylation at Thr^{286}, which renders the enzyme to obtain Ca^{2+}-independent autonomous activity (Miller and Kennedy, 1986). Thus, the activated CaMKII (Thr^{286}-phosphorylated) should be sensitive to stimuli that can change cellular Ca^{2+}, such as neuronal activity. So we first examined whether the activation levels of CaMKII might be dynamically regulated by D_4 receptors in response to different patterns of neuronal activity. PFC slices were incubated for 1 h with either bicuculline (BIC, 10 μM) to increase activity through block of inhibitory transmission, or with CNQX (10 μM) and APV (20 μM) to decrease activity through block of excitatory transmission, followed by a 10-min treatment with the specific D_4 receptor agonist PD168077 (Glase et al., 1997; Wang et al., 2002). As shown in Fig. 1A, the basal level of activated CaMKII in PFC slices was higher after BIC treatment compared with after CNQX/APV treatment. PD168077 (20 μM) caused a significant decrease of the activated CaMKII in PFC with high neuronal activity (BIC-treated), but it caused a potent increase of the activated CaMKII in PFC with low neuronal activity (CNQX/APV-treated). In contrast to the bidirectional effect of PD168077, the D_2 receptor agonist quinpirole (20 μM) only produced a reduction of the activated CaMKII irrespective of the neuronal activity. Total CaMKII levels exhibited no change with any of the treatment. Quantitative data from a series of experiments are summarized in Fig. 1B. PD168077 decreased Thr^{286}-phosphorylated CaMKII by 65 ± 11% (n = 8) in BIC-treated PFC slices, whereas it increased Thr^{286}-phosphorylated CaMKII by 270 ± 52% (n = 8) in CNQX/APV-treated PFC slices. Quinpirole reduced Thr^{286}-phosphorylated CaMKII by 72 ± 12% (n = 6) or 36 ± 6% (n = 6) in PFC slices treated with BIC or CNQX/APV, respectively.

Similar experiments were performed in PFC slices pretreated with TTX (0.5 μM, 1 h) to suppress spike activity. As shown in Fig. 1, C and D, PD168077 caused a significant decrease (59 ± 10%, n = 8) of the Thr^{286}-phosphorylated CaMKII in PFC with high neuronal activity (no TTX pretreatment), but it caused a marked increase (240 ± 48%, n = 8) of the Thr^{286}-phosphorylated CaMKII in PFC with low neuronal activity (TTX-pretreated). Quinpirole reduced the level of Thr^{286}-phosphorylated CaMKII by 64 ± 12% (n = 6) or 31 ± 5% (n = 6) in PFC slices pretreated without or with TTX, respectively. These results indicate that D_4 receptors exert a dynamic bidirectional regulation of CaMKII activity depending on the neuronal activity.

To confirm that neuronal activity is manipulated by drugs that affect synaptic transmission, glutamatergic excitation, or GABAergic inhibition, we compared the level of activated (Thr^{286}-phosphorylated) CaMKII in PFC slices treated with saline or various drugs. As shown in Fig. 1, E and F, compared with saline-treated slices (ctl), slices treated with TTX, CNQX/APV, or the nonselective glutamate receptor antagonist kynurenic acid (1 mM) showed a substantial decrease of the activated CaMKII (TTX: 67 ± 12%, n = 10; CNQX/APV: 72 ± 13%, n = 10; and kynurenic acid: 70 ± 13%, n = 8). Moreover, the reduction of CaMKII activity by TTX or CNQX/APV was not blocked by the PKA activator cpt-cAMP

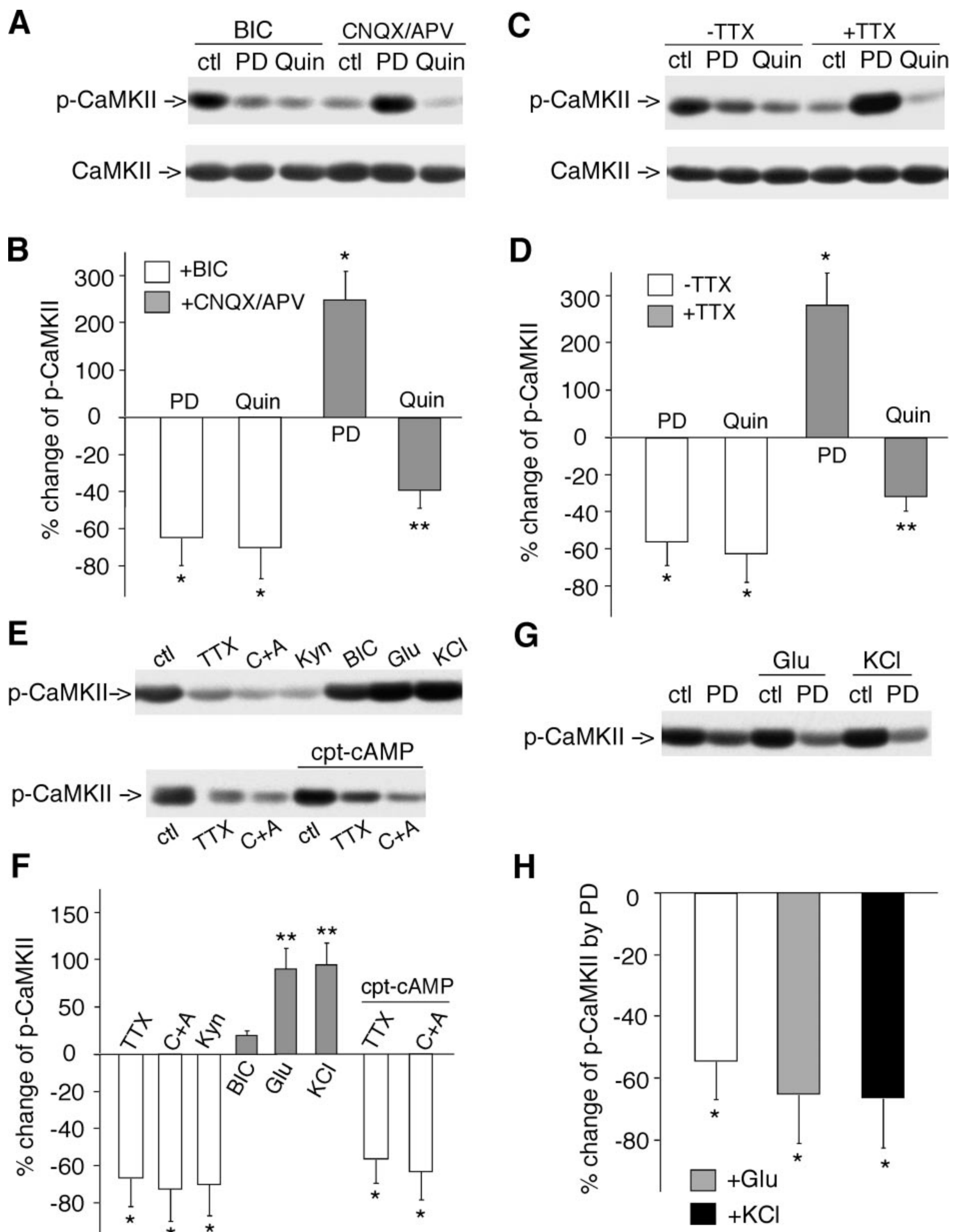

Fig. 1. The D_4 receptor agonist PD168077 induced an up- and down-regulation of CaMKII activity depending on the neuronal activity in PFC slices. A and C, immunoblots of phospho-CaMKII and CaMKII. PFC slices were incubated in BIC (10 μM) or CNQX (10 μM)/APV (20 μM) for 1 h (A), or were pretreated without or with TTX (0.5 μM for 1 h, C), followed by a 10-min treatment with PD168077 (PD, 20 μM) or quinpirole (Quin, 20 μM). Extracts of slices were immunoblotted with an anti–phospho-α-CaMKII antibody. After stripping out signals, membranes were reblotted with an antibody recognizing the total α-CaMKII. B and D, percentage changes of *p*-CaMKII induced by PD168077 or quinpirole in PFC slices incubated with bicuculline- or CNQX/APV (B), or pretreated without or with TTX (D). E, immunoblots of phospho-CaMKII. Top, PFC slices were treated with TTX, CNQX/APV (C+A), kynurenic acid (kyn, 1 mM), bicuculline for 30 min, or glutamate/glycine (Glu, 100/10 μM) or KCl (30 μM) for 20 min. Bottom, cpt-cAMP (100 μM) was added 10 min before TTX or CNQX/APV treatment. G, slices were pretreated with saline (ctl), glutamate/glycine, or KCl for 10 min, followed by a 10-min treatment with PD168077. F and H, percentage changes of *p*-CaMKII induced by treatments corresponding to E and G, respectively. ⋆⋆, $p < 0.01$; ⋆, $p < 0.001$, ANOVA.

(100 μM, cpt-cAMP + TTX: 58 ± 13%, n = 8; cpt-cAMP + CNQX/APV: 63 ± 10%, n = 8), suggesting that it is not mediated by PKA inhibition. On the other hand, compared with saline-treated slices (ctl), slices treated with bicuculline caused little change on the activated CaMKII (20 ± 4%, n = 8), and slices treated with glutamate/glycine (100/10 μM) or high KCl (30 mM) further increased the level of activated CaMKII (glutamate: 89 ± 17%, n = 8; KCl: 92 ± 17%, n = 8). These data suggest that PFC neurons are switched to the "low activity" state by the treatment with TTX, CNQX/APV, or kynurenic acid, whereas they are at the "high activity" state in saline- (ctl), bicuculline-, glutamate/glycine-, or high KCl-treated slices.

We further compared the effect of PD168077 on CaMKII activity in PFC slices at the high-activity state. As shown in Fig. 1, G and H, PD168077 caused a potent reduction of the activated CaMKII in saline-, glutamate/glycine-, or high KCl-treated slices (saline: 58 ± 12%, n = 8; glutamate: 69 ± 15%, n = 8; KCl: 68 ± 15%, n = 8), similar to the effect of PD168077 in bicuculline-treated slices (Fig. 1, A and B). These data further indicate that D_4 receptors decrease the level of CaMKII activation in PFC with high neuronal activity.

Mediation by D_4 Receptors of the Up-Regulation of CaMKII Activity in PFC Slices. BecauseD_4 receptors couple to the "classic" inhibition of PKA pathway in transfected cell lines (Chio et al., 1994), it is surprising that D_4 receptors increased the activation level of CaMKII in PFC neurons with low neuronal activity. Thus, in subsequent experiments, we further examined the mechanisms underlying D_4 up-regulation of CaMKII activity in TTX-pretreated PFC slices.

The dose-dependence of PD168077-induced CaMKII activation is shown in Fig. 2, A and B. A small effect could be detected after a 10-min exposure to 5 μM PD168077, and a saturating effect was seen at 20 μM PD168077. Quantification data exhibited a 3.4 ± 0.8-fold increase of CaMKII activity (n = 8, $p < 0.001$, ANOVA) by PD168077 (20 μM, 10 min). The kinetics of PD168077-induced activation of CaMKII was also tested. As demonstrated in Fig. 2, C and D, the CaMKII activation induced by PD168077 (40 μM) showed rapid and transient kinetics, reaching a peak at 10 min and declining to basal levels within 30 to 60 min.

To verify that D_4 receptors were mediating the PD168077 activation of CaMKII, we examined the ability of selective D_4 receptor antagonists to prevent the action of PD168077. As shown in Fig. 3, A and B, PD168077 (40 μM) produced a potent increase (3.7 ± 0.9-fold, n = 12) of activated CaMKII in PFC slices, and this effect was significantly ($p < 0.001$, ANOVA) blocked by L-745870 (20 μM, 0.98 ± 0.2-fold, n = 8), a highly selective D_4 antagonist (Patel et al., 1997). In contrast to the strong effect of PD168077 on CaMKII activation in PFC slices, PD168077 failed to regulate CaMKII activity in striatal slices (1.1 ± 0.2-fold, n = 6) (Fig. 3, A and B), consistent with the highly enriched expression of D_4 receptors in PFC but not in striatum.

To further confirm the involvement of D_4 receptors in the

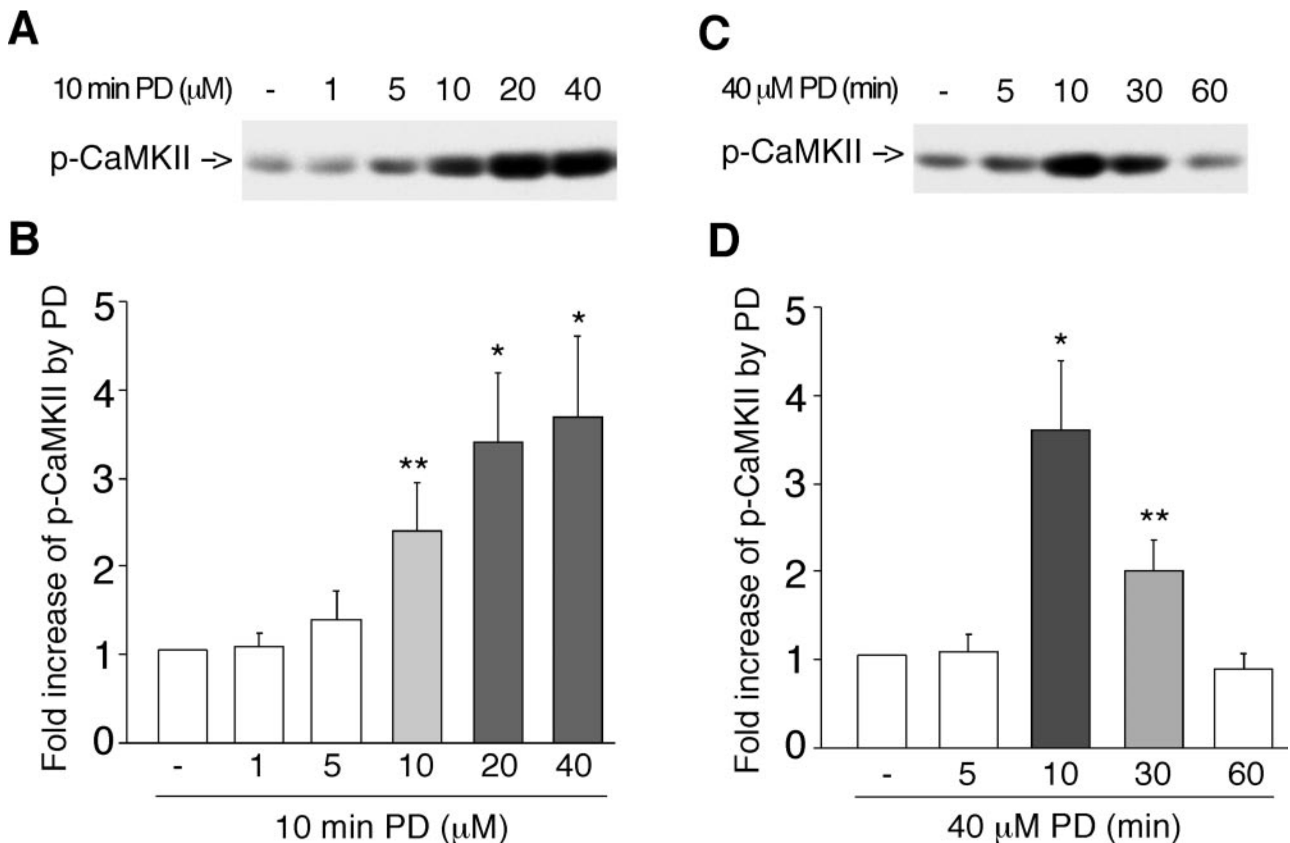

Fig. 2. PD168077 increased CaMKII activity in a dose- and time-dependent manner. A, dose-dependence of the PD168077-induced activation of CaMKII. PFC slices (TTX-pretreated) were treated with PD168077 for 10 min at the indicated concentrations. B, quantification of p-CaMKII induced by different concentrations of PD168077. C, time course of the PD168077-induced activation of CaMKII. PFC slices (TTX-pretreated) were treated with PD168077 (40 μM) for the indicated durations. D, quantification of p-CaMKII induced by PD168077 treatment for different lengths of time. ⋆⋆, $p < 0.01$; ⋆, $p < 0.001$, ANOVA, compared with control (−).

up-regulation of CaMKII activity, we tested the effect of dopamine (50 μM) on CaMKII in the presence of D_1/D_5 antagonist SCH23390 (10 μM) and D_2/D_3 antagonist sulpiride (10 μM). As shown in Fig. 3, C and D, when D_1/D_5 and D_2/D_3 receptors were blocked, dopamine produced an enhancement of CaMKII activity (3.4 ± 0.8-fold, n = 8), mimicking the PD168077 effect. Moreover, this effect of dopamine on CaMKII was blocked by the D_4 antagonist L-745870 (1.15 ± 0.2-fold, n = 6). These results suggest that dopamine released on PFC neurons could indeed elevate CaMKII activity via D_4 receptors.

Signaling Mechanisms Underlying the D_4 Enhancement of CaMKII Activity in PFC Slices. Our previous study has shown that D_4 receptors decrease CaMKII activity in PFC slices (no TTX pretreatment) through a cascade involving the inhibition of PKA and ensuing disinhibition of protein phosphatase 1 (Wang et al., 2003). We next examined the signal transduction pathways mediating the increase of CaMKII activity by D_4 receptors in PFC slices when the neuronal activity was suppressed by TTX pretreatment. As shown in Fig. 4A, application of the phospholipase C (PLC) inhibitor U73122 (1 μM) but not the broad-spectrum tyrosine kinase inhibitor genistein (100 μM) blocked the PD168077-induced increase of CaMKII activity. Moreover, application of the $G_{i/o}$ protein alkylating agent *N*-ethylmaleimide (NEM, 30 μM) failed to prevent PD168077 from elevating CaMKII activity. As summarized in Fig. 4B, the PD168077-induced activation of CaMKII (3.6 ± 0.8-fold, n = 14) was abolished in the presence of U73122 (0.9 ± 0.2-fold, n = 8) but was intact in the presence of genistein (3.5 ± 1.0-fold, n = 8) or NEM (3.9 ± 1.3-fold, n = 8). These results suggest that the D_4 enhancement of CaMKII activity is through a mechanism dependent on the stimulation of PLC pathway but not the activation of tyrosine kinases or the coupling to $G_{i/o}$ proteins.

Given the dependence of CaMKII activation on Ca^{2+}, we then examined whether the D_4 enhancement of CaMKII activity required the Ca^{2+} entry from extracellular regions or the Ca^{2+} elevation from intracellular stores. To test this, PFC slices were incubated in Ca^{2+}-free solutions or with the membrane-permeable Ca^{2+} chelator BAPTA/AM. As shown in Fig. 5, A and B, PD168077 induced a potent increase (4.1 ± 1.2-fold, n = 8) in CaMKII activity under Ca^{2+}-free conditions, similar to the PD168077 effect in normal Ca^{2+}-containing solutions (3.8 ± 0.7-fold, n = 15). However, when intra-

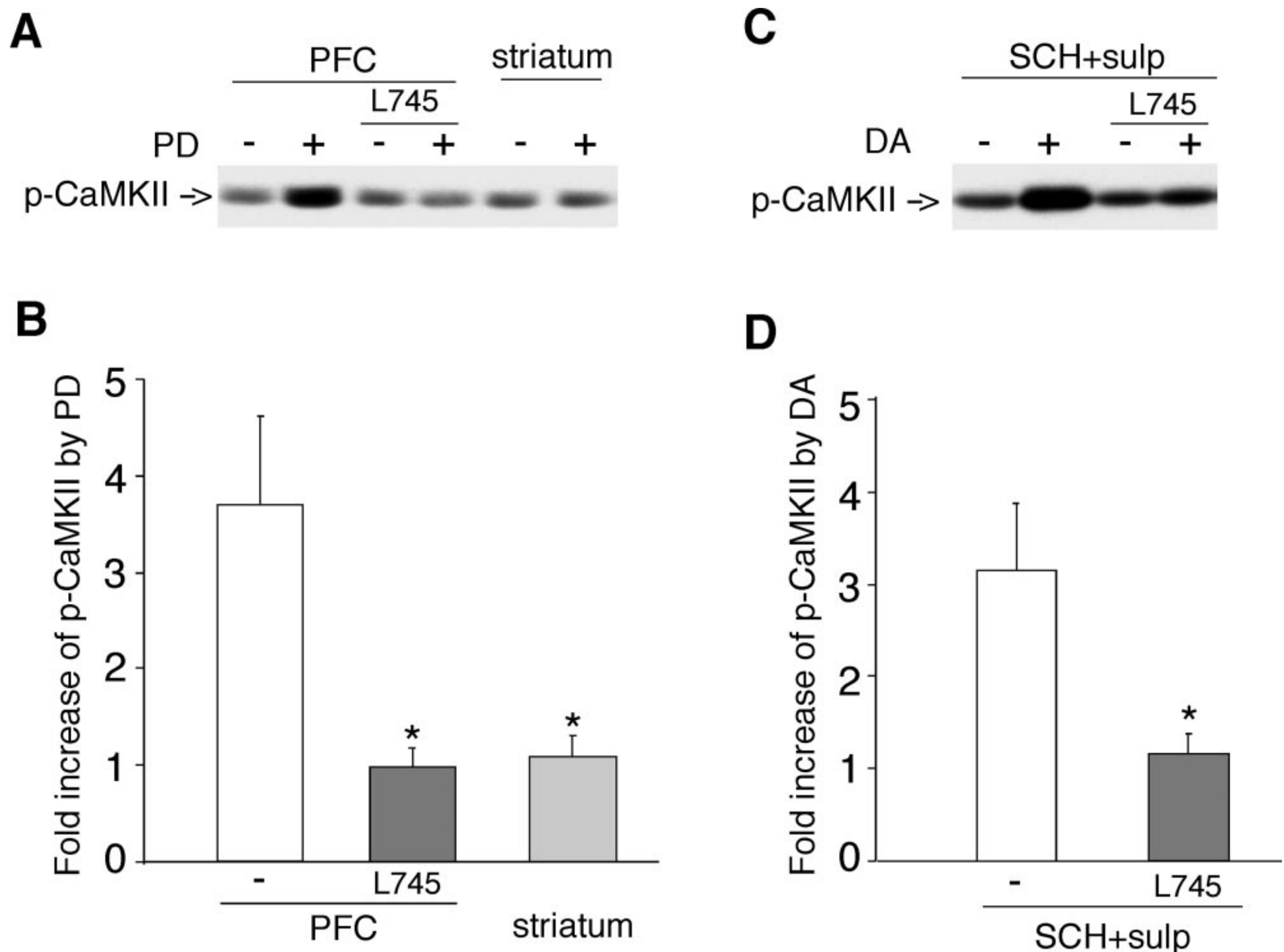

Fig. 3. The PD168077-induced up-regulation of CaMKII activity was mediated by D_4 receptors. A, immunoblots of phospho-CaMKII. PFC or striatal slices (TTX-pretreated) were incubated in the absence or presence of the selective D_4 antagonist L-745870 (20 μM, 15 min) followed by a 10-min treatment with PD168077. B, quantification of *p*-CaMKII induced by PD168077 (PD) in PFC or striatal slices. C, immunoblots of phospho-CaMKII. PFC slices (TTX-pretreated) were incubated with SCH23390 (10 μM) and sulpiride (10 μM) for 30 min to block D_1/D_5 and D_2/D_3 receptors. Then they were incubated in the absence or presence of L-745870 (20 μM, 15 min), followed by a 10-min treatment with dopamine (50 μM). D, quantification of *p*-CaMKII induced by dopamine (coapplied with SCH23390 plus sulpiride) in the absence or presence of L-745870. ⋆, $p < 0.001$, ANOVA, compared with the effect under control conditions (−).

cellular Ca^{2+} increase was blocked by BAPTA/AM (50 μM), PD168077 failed to enhance CaMKII activity (1.12 ± 0.25-fold, $n = 8$). These results suggest that an increase of intracellular Ca^{2+} from internal stores is required for the D_4 enhancement of CaMKII activity.

In neurons, a major source of internal calcium is the stores present in the endoplasmic reticulum network. Both ionsitol-1,4,5-triphosphate receptors (IP_3Rs) and ryanodine receptors (RyRs) on endoplasmic reticulum are responsible for releasing calcium from this internal source (Kostyuk and Verkhratsky, 1994; Simpson et al., 1995). To determine which one was involved in the D_4 activation of CaMKII, PFC slices were pretreated with pharmacological agents to block these receptors. As shown in Fig. 5, A and B, application of 2APB (30 μM), a membrane-permeable IP_3R antagonist (Hamada et al., 1999), abolished the D_4 effect on CaMKII activation (0.9 ± 0.2-fold, $n = 10$). In contrast, DHBP (30 μg/ml), a potent RyR antagonist (Kang et al., 1994), failed to alter the D_4 enhancement of CaMKII activity (3.6 ± 0.9-fold, $n = 6$). Pretreatment of PFC slices with the intracellular calcium pump inhibitor thapsigargin (5 μM, 30 min) to deplete internal stores of Ca^{2+} also eliminated the D_4 effect on CaMKII activation (1.0 ± 0.2-fold, $n = 8$). These results suggest that D_4 receptors elevate intracellular calcium via IP_3Rs to increase CaMKII activity.

As a control, we also examined the involvement of PLC/IP_3R signaling in D_4 reduction of CaMKII activity in high neuronal activity conditions. As shown in Fig. 5, C and D, application of the PLC inhibitor U73122 (1 μM) or IP_3R antagonist 2APB (30 μM) failed to block the PD168077-induced decrease of activated CaMKII (62 ± 13%, $n = 8$; U73122: 74 ± 12%, $n = 8$; 2APB: 71 ± 11%, $n = 8$), suggesting that the PLC/IP_3R signaling is not involved in D_4 reduction of CaMKII activity.

Discussion

CaMKII has been regarded as a cognitive kinase because of its involvement in regulating learning and memory, and its autoregulatory properties that can be viewed as a type of molecular memory (Hudmon and Schulman, 2002). A variety of extracellular signals triggers the activation of CaMKII by elevating the intracellular Ca^{2+} level through Ca^{2+} influx or Ca^{2+} release from internal stores. We show here that stimulation of D_4 receptors can lead to either up- or down-regulation of CaMKII activity depending on basal neuronal activity. In PFC slices with suppressed neuronal activity, the level of activated CaMKII was increased by D_4 receptors, whereas in PFC slices with elevated neuronal activity, the level of activated CaMKII was decreased by D_4 receptors. This dual regulation of CaMKII activity was unique for D_4 receptors, because it was not observed with D_2 receptor activation. By regulating CaMKII activity in such a dynamic activity-dependent fashion, D_4 receptors could fine-tune the functions of CaMKII flexibly and precisely.

How can D_4 receptors either decrease or increase CaMKII activity? Emerging evidence has suggested that G protein-mediated signal transduction is a complex signaling network with divergent and convergent pathways intimately intertwined (Gudermann et al., 1997). The classic signaling cascade for D_4 receptors is to couple to $G_{i/o}$-type G proteins to

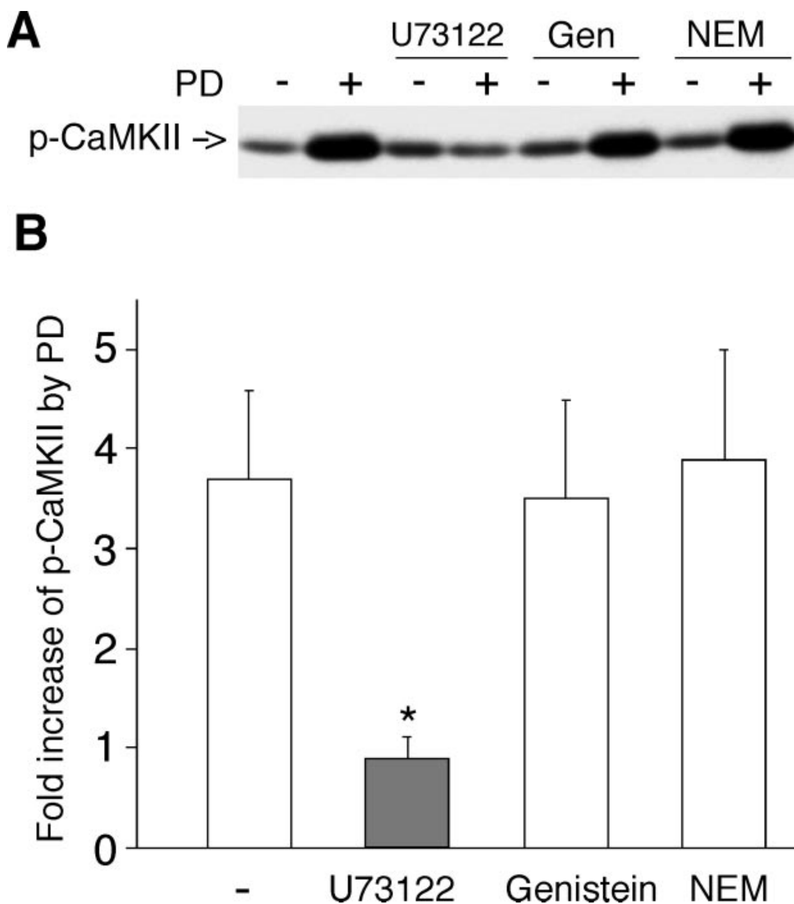

Fig. 4. The D_4 potentiation of CaMKII activity was dependent on the stimulation of PLC pathway. A, immunoblots of phospho-CaMKII. PFC slices (TTX-pretreated) were incubated in the absence or presence of various agents for 30 min, followed by a 10-min treatment with PD168077. Agents included the PLC inhibitor U73122 (1 μM), the tyrosine kinase inhibitor genistein (100 μM), and the $G_{i/o}$ protein alkylating agent NEM (30 μM). B, Quantification of *p*-CaMKII induced by PD168077 under different treatments. ⋆, $p < 0.001$, ANOVA, compared with the effect under control conditions (−).

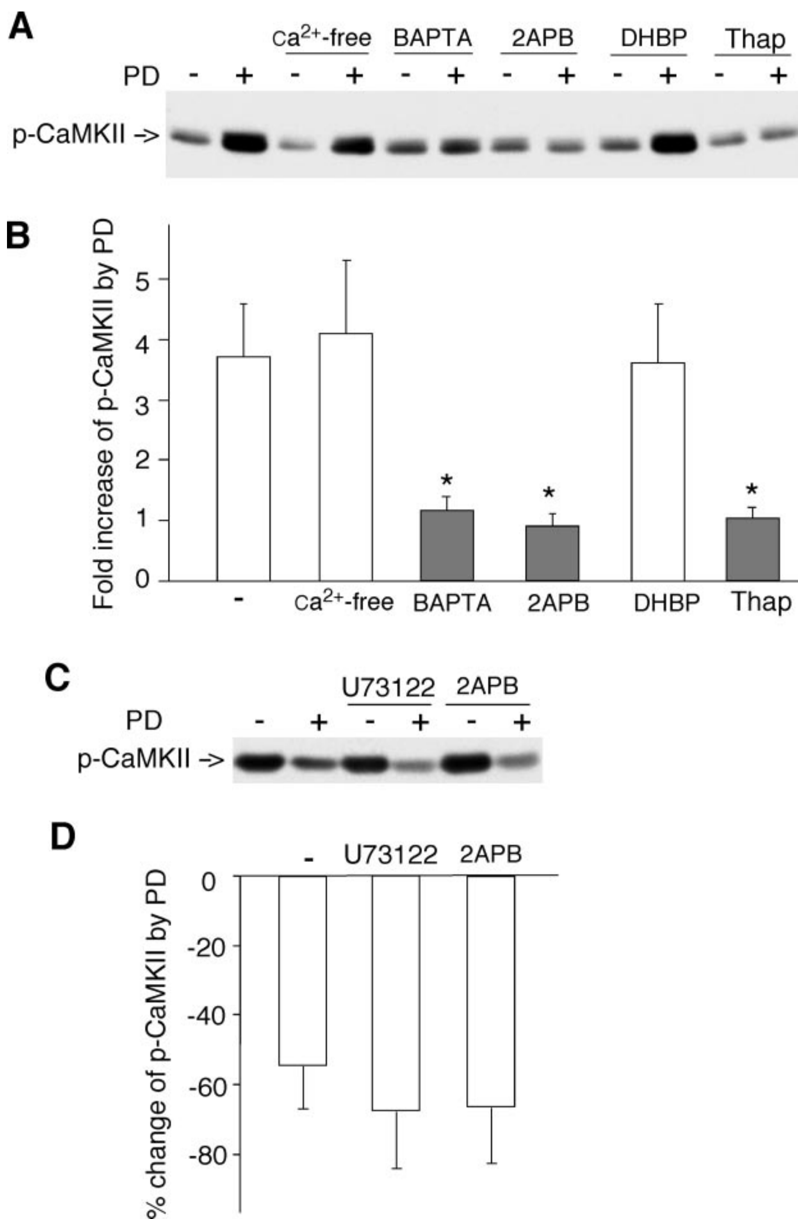

Fig. 5. D_4 receptors augmented CaMKII activity through elevation of intracellular Ca^{2+} via IP_3 receptors. A, immunoblots of phospho-CaMKII. PFC slices (TTX-pretreated) were incubated in the absence or presence of various agents or in a Ca^{2+}-free solution for 30 min, followed by a 10-min treatment with PD168077. Agents included the membrane-permeable Ca^{2+} chelator BAPTA/AM (50 μM), the IP_3R antagonist 2APB (30 μM), the RyR antagonist DHBP (30 μg/ml), and the intracellular calcium-pump inhibitor thapsigargin (5 μM). C. PFC slices (bicuculline-pretreated) were pretreated with saline, U73122 (1 μM), or 2APB for 30 min, followed by a 10-min treatment with PD168077. B and D, quantification of *p*-CaMKII induced by PD168077 under different treatments. ⋆, $p < 0.001$, ANOVA, compared with the effect under control conditions (−).

inhibit adenylate cyclase and cAMP formation (Chio et al., 1994). The inhibition of PKA could cause the activation of protein phosphatase 1 via decreased phosphorylation of the inhibitory protein I-1 (Ingebritsen and Cohen, 1983), leading to the dephosphorylation of CaMKII and a decrease of CaMKII activity. Our previous study confirmed this mechanism for the D_4 down-regulation of CaMKII activity (Wang et al., 2003). In this study, we show that the D_4 up-regulation of CaMKII activity is through the stimulation of PLC pathway and elevation of intracellular Ca^{2+} via IP_3Rs. How D_4 receptors activate the PLC pathway is not clear. One potential mechanism is that activation of D_4 receptors in PFC neurons leads to the release of G protein $\beta\gamma$ subunits and thus potentiates the stimulation of PLC by $\beta\gamma$ subunits (Camps et al., 1992).

This study mechanistically links together D_4 receptors and CaMKII, both of which have been implicated in cognitive and emotional processes associated with PFC. The D_4 regulation of CaMKII activity enables D_4 receptors to affect many aspects of cellular function via changing numerous CaMKII substrates, such as K^+ channels, glutamate receptors, synapsin, cAMP response element-binding protein, tau, and so on (Hudmon and Schulman, 2002). A novel feature of this D_4 modulation of CaMKII is that it is bidirectional depending on neuronal activity, and a "dual signaling" (i.e., inhibition of adenylate cyclase and stimulation of PLC) underlies the D_4-induced suppression or potentiation of CaMKII activity. This supports the notion that many neuromodulators, such as dopamine and serotonin, can have dual roles not only because they can act on a variety of different targets, but also because they can act differently on the same target under different physiological conditions (Cai et al., 2002). This mechanism ensures that the modulation provides a feedback system to effectively maintain normal neuronal activity.

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