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Excitatory Roles of Protein Kinase C in Striatal Cholinergic Interneurons

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Deng P, Pang Z-P, Lei Z, Xu ZC. Excitatory roles of protein kinase C in striatal cholinergic interneurons. J Neurophysiol 102: 2453–2461, 2009. First published August 5, 2009; doi:10.1152/jn.00325.2009. Protein kinase C (PKC) plays critical roles in neuronal activity and is widely expressed in striatal neurons. However, it is not clear how PKC activation regulates the excitability of striatal cholinergic interneurons. In the present study, we found that PKC activation significantly inhibited A-type potassium current ($I_A$), but had no effect on delayed rectifier potassium currents. Consistently, application of PKC activator caused an increase of firing in response to depolarizing currents in cholinergic interneurons, which was persistent in the presence of both excitatory and inhibitory neurotransmission blockers. These excitatory effects of PKC could be partially mimicked and occluded by blockade of $I_A$ with potassium channel blocker 4-aminopyridine. In addition, immunostaining demonstrated that PKCa, but not PKCy and PKCe, was expressed in cholinergic interneurons. Furthermore, activation of group I metabotropic glutamate receptors (mGluRs) led to an inhibition of $I_A$ through a PKC-dependent pathway. These data indicate that activation of PKC, most likely PKCa, increases the neuronal excitability of striatal cholinergic interneurons by down-regulating $I_A$. Group I mGluR-mediated $I_A$ inhibition might be important for the glutamatergic regulation of cholinergic tone in the neostriatum.

INTRODUCTION

Protein kinase C (PKC) has been implicated in many aspects of neuronal activities under both physiological and pathological conditions. At least 12 PKC isoforms are identified and grouped into three subfamilies, based on their sensitivity to the second messengers Ca$^{2+}$ and diacylglycerol (Nishizuka 1988; Poole et al. 2004). The conventional isoforms (PKCa, PKC$\beta$I, PKC$\beta$II, and PKC$\gamma$) are sensitive to both Ca$^{2+}$ and diacylglycerol, the novel isoforms (PKC$\delta$, PKCe, PKC$\theta$, and PKC$\eta$) are sensitive to diacylglycerol but not Ca$^{2+}$, and the atypical isoforms (PKC$\zeta$, PKM$\zeta$, and PKCa/\) are insensitive to either Ca$^{2+}$ or diacylglycerol (Poole et al. 2004). Most of these isoforms are expressed in neurons, with differential cellular distribution (Naik et al. 2000; Tanaka and Nishizuka 1994). In the neostriatum, medium-sized spiny neurons express PKC$\beta$I and PKC$\gamma$, whereas cholinergic interneurons express PKCa (Yoshihara et al. 1991). These observations suggest that activation of PKC may play isoform-specific roles in different striatal neurons. However, the roles of PKC in cholinergic interneurons remain to be elucidated.

PKC activation profoundly regulates the activities of ligand- and voltage-gated ion channels and thus the neuronal excitability. For instance, the neuronal N-methyl-d-aspartate receptor function is enhanced by PKC, through mechanisms of increasing channel opening rate and functional channel number on the cell membrane, which might contribute to potentiation of synaptic efficacy (Lan et al. 2001; Lin et al. 2006). Numerous studies have shown that PKC activation modulates the activities of voltage-gated potassium (Kv) channels (Jonas and Kaczmarek 1996). In CA1 hippocampal pyramidal cells, PKC activation inhibits dendritic A-type potassium current ($I_A$), accompanied by a depolarizing shift of the voltage dependence of activation (Hoffman and Johnston 1998). This modulation may be caused by the phosphorylation of Kv4.2 subunits that mediate somadendritic $I_A$ in neurons (Yuan et al. 2002). Although striatal cholinergic interneurons possess prominent $I_A$ (Song et al. 1998), their modulation by PKC is still unknown.

PKC may serve as an important intracellular pathway by which neurotransmitters regulate neuronal activity. It is well known that group I metabotropic glutamate receptors (mGluRs) positively couple to the phospholipase C, mobilize intracellular Ca$^{2+}$, and activate PKC (Ferraguti et al. 2008; Gerber et al. 2007). Striatal cholinergic interneurons express group I mGluRs, including both mGluR1 and mGluR5 (Bell et al. 2002; Pisani et al. 2001). Several studies have shown that activation of group I mGluRs induces an inhibition of background potassium currents in cholinergic interneurons (Berg et al. 2007; Bonsi et al. 2005; Takeshita et al. 1996). However, it remains to be determined whether activation of group I mGluRs modulates voltage-dependent potassium currents in these neurons.

In the present study, we investigated the PKC modulation of $I_A$ and its consequences on neuronal excitability in striatal cholinergic interneurons. In addition, we examined the effects of group I mGluRs on $I_A$ and the involvement of PKC in this modulation.

METHODS

Male adult (100–180 g) Wistar rats (Charles River Laboratories, Wilmington, MA) were used in the present study. Experimental protocols were approved by the Institutional Animal Care and Use Committee of Indiana University School of Medicine in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Brain slice and acute dissociation preparation

Brain slices were prepared using procedures similar to those previously described (Deng et al. 2005). Briefly, the animals were anesthetized with ketamine-HCl (80 mg/kg, intraperitoneally) and decapitated. The brains were quickly removed and immersed in ice-cold artificial cerebrospinal fluid (ACSF) containing (in mM): 130 NaCl, 3 KCl, 2 CaCl$_2$, 2 MgCl$_2$, 1.25 NaH$_2$PO$_4$, 26 NaHCO$_3$, and 10 glucose (pH 7.4, 295–305 mOsm/l). Transverse striatal slices of 300-μm thickness were cut using a vibratome (VT 1000; Leica, Nussloch, Germany) and incubated in ACSF (continuously equilibrated with 95% O$_2$-5% CO$_2$) for >1 h at room temperature (~24°C) before being transferred to the recording chamber.

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Acutely dissociated neurons, which have less dendrite and thus improved space clamp, were used in some experiments (Deng et al. 2004). Briefly, brain tissue containing the neostriatum was cut in 400-μm slices while being bathed in a low-Ca2+/Mg2+ buffer containing (in mM): 140 Na isethionate, 2 KCl, 0.1 CaCl2, 23 glucose, and 15 HEPES (pH 7.4, 300–305 mOsm/l). Slices were incubated at room temperature in oxygenated ACSF. The slices were then transferred into the low-Ca2+/Mg2+ buffer and regions of striatum were dissected and placed in an oxygenated HEPES-buffered Hank’s balanced salt solution containing 1–3 mg/ml protease. After 25–30 min of enzyme digestion at 35°C, tissue was rinsed three times in the low-Ca2+/Mg2+ buffer and mechanically dissociated with a graded series of fire-polished Pasteur pipettes. The cell suspension was plated onto a 12-mm coverslip, which was then placed in the recording chamber.

Electrophysiological recording

Recording electrodes were prepared from borosilicate glass (Warner Instruments, Hamden, CT) using a horizontal electrode puller (P-97; Sutter Instruments, Novato, CA), with resistances of 2–5 MΩ when filled with intracellular solutions. Oxygenated ACSF was used as extracellular solution and the flow rate was adjusted to 2–3 ml/min. Recordings were carried out at room temperature. Strialat cholinergic interneurons were visualized with an infrared– differential interference contrast microscope (BX50WI; Olympus Optical, Tokyo) and a CCD camera. Only those cells with large somata (>20 μm in diameter) were selected for recording. Recordings were performed with an Axopatch 200B amplifier (Molecular Devices, Foster City, CA). Signals were filtered at 2 kHz and digitized at a sampling rate of 5 kHz using a data-acquisition program (Axograph 4.6; Molecular Devices).

Whole cell voltage-clamp recordings were used to evoke Ik and delayed rectifier potassium current (IK). After tight-seal (>1 GΩ) formation, the electrode capacitance was compensated. Immediately after establishment of whole cell configuration, the membrane capacitance, series resistance, and input resistance of the recorded neurons were measured by applying a 5-mV (10-ms) hyperpolarizing voltage pulse from a holding potential of −60 mV. The series resistance was 8–15 MΩ. Cells with a series resistance >10% of the input resistance were discarded. During the experiment, the membrane capacitance and series resistance were periodically monitored. Cells with a series resistance change >20% during the experiment were excluded from the analysis. To record IK and Ik, electrodes were filled with an intracellular solution containing (in mM): 145 KCl, 1 MgCl2, 0 EGTA, 0.2 CaCl2, 10 HEPES, and 2 Na-ATP (pH 7.4, 280–295 mOsm/l). At a holding potential of −60 mV, the voltage-dependent outward potassium currents were evoked by voltage steps (from −80 to +70 mV in 10-mV increments, 400 ms) following a 300-ms hyperpolarizing pulse of −120 mV in the presence of tetrodotoxin (TTX, 1 μM) and CdCl2 (100–300 μM) to block voltage-activated Na+ and Ca2+ currents, as well as Ca2+-activated potassium currents. To isolate Ik, the IK was inactivated by a 100-ms prepulse of +10 mV following a 200-ms hyperpolarizing pulse of −120 mV. The current amplitude of Ik was measured as the average amplitude at 340–390 ms after the onset of the command voltage pulses (Deng et al. 2005). The Ik was isolated by subtracting the currents evoked after depolarized prepulses (+10 mV, 100 ms) from those evoked without depolarized prepulses. The current amplitude of Ik was measured at the peak of each current. The steady-state activation curves were established similarly to those previously reported (Deng et al. 2004). Briefly, the conductance (G) was calculated using the following equation: G = l/(V − V), where I is the current amplitude, V is the command potential, and V is the reversal potential of potassium (V = −98 mV). The conductance was then normalized with respect to the maximum value and plotted as a function of the membrane potential during the test pulse. The resulting activation curves were fitted with a normalized Boltzmann distribution: G/Gmax = 1/[1 + exp(Vm − V)/V], where Gmax is the maximum conductance at +70 mV, V1/2 is the membrane voltage at which the current amplitude was half-maximum, and k is the slope factor at V1/2. The inactivation kinetics of Ik was studied by fitting the decaying phase of currents with a biexponential function.

For whole cell current-clamp recordings, electrodes were filled with an intracellular solution containing (in mM): 125 KMeSO4, 20 KCl, 1 MgCl2, 1 EGTA, 0.2 CaCl2, 10 HEPES, 2 Mg-ATP, and 0.4 Na-GTP (pH 7.4, 280–295 mOsm/l). The fast I-clamp mode was used. Depolarizing current pulses (800 ms, 20–180 pA) were applied to evoke either a single firing or repetitive firings. The spike latency was measured from the onset of the current injection to the peak of the first action potential. The spike width was measured at the spike base.

Drug application

Phorbol-12,13-dibutyrate (PDBu), chelerythrine, 3-[1-(dimethylamino-propyl)indol-3-yl]-4-(indol-3-yl)maleimide (GF119203X), 4a-phorbol, 4-aminoypyridine (4-AP), (−)-2-amino-5-phosphonophenolphosphonatoic acid (APV), 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), and (−)-bicyculline methiodide (BIC) were purchased from Sigma–Aldrich (St. Louis, MO). Phorbol-12-myristate-13-acetate (PMA) and 12-(2-cyaanoethyl)-6,7,12,13-tetrahydro-13-methyl-5-oxo-5H-indolo(2,3-a)pyrrolo(3,4-c)-carbazole (Go6976) were purchased from Calbiochem (San Diego, CA). (S)-3,5-Dihydroxyphenylglycine (DHPG) and (S)-α-ethyl-4-carboxyphenylglycine (MCPG) were purchased from Tocris Bioscience (Ellisville, MO).

Drugs were prepared as concentrated stocks and stored at −20°C. Working solutions were prepared immediately before use and applied in bath solution. For studying the effects of PKC, an individual PKC activator or inhibitor was applied for 10–15 min after stable recording, at which the drugs reached stable effects. When coapplied, the PKC inhibitor was preapplied for >10 min prior to the activator application. Neurotransmitter antagonists (APV, CNQX, and BIC) and 4-AP were preapplied for >5 min prior to the application of PKC activator. For studying the effects of group I mGluRs, DHPG was applied for 3 min, after which the effects were detected. When coapplied, MCPG or Go6976 was preapplied for >10 min prior to DHG application. In all experiments, the effects of drugs were measured as the changes in each neuron before and after drug application.

Immunohistochemistry

The following primary antibodies were used: mouse anti-PKCα (1:50, Santa Cruz Biotechnology, Santa Cruz, CA), mouse anti-PKCγ (1:500, Sigma), mouse anti-PKCε (1:50, Santa Cruz Biotechnology), and rabbit anticholine acetyltransferase (ChAT, 1:250, Chemicon, Temecula, CA). Animals were anesthetized and perfused through the ascending aorta with a solution of phosphate-buffered saline (PBS, 0.01 M, pH 7.4) for about 5 min, followed by 4% paraformaldehyde in PBS for 20–30 min. Brains were removed and postfixed in 4% paraformaldehyde at 4°C overnight. Coronal sections containing striatum were cut (50 μm) with a vibratome and collected in PBS. Sections were blocked with normal serum (10% in PBS containing 0.5% Triton X-100) for 2 h at room temperature and incubated in a solution containing primary antibody against PKC isoform for 18 h at 4°C. Then, sections were incubated in a solution containing fluorescein-conjugated anti-mouse secondary antibody (1:100; Vector Laboratories, Burlingame, CA) for 2 h at room temperature. After repeated washes in PBS, the stained sections were incubated with rabbit anti-ChAT at 4°C overnight, followed by rhodamine-conjugated anti-rabbit secondary antibody (1:100; Vector Laboratories) for 4 h at room temperature. Finally, the sections were washed in PBS and mounted with an antifading medium for fluorescent microscopic observation.
Data analysis

The data are presented as means ± SE. Statistical difference was detected using paired or unpaired Student’s t-test (StatView 5.0; Abacus Concepts, Berkeley, CA). Changes were considered significant if \( P < 0.05 \).

RESULTS

All the data were obtained from striatal cholinergic interneurons, which were easily identified based on their morphological and electrophysiological features (Bennett and Wilson 1999; Deng et al. 2007, 2008; Kawaguchi 1993).

PKC inhibits \( I_A \) in cholinergic interneurons

Numerous studies have demonstrated that PKC activation modulates \( I_A \) in other neurons (Hoffman and Johnston 1998; Yuan et al. 2002), which may potentially alter the firing patterns (Bean 2007; Storm 1990). We examined the PKC modulation of \( I_A \) in striatal cholinergic interneurons. Consistent with previous studies (Deng et al. 2004; Song et al. 1998), these neurons expressed prominent \( I_A \) with current amplitude of 1.3–4.8 nA when evoked at +30 mV (2.8 ± 0.7 nA, \( n \) = 12; Fig. 1, A and C). We found that application of PKC activator PDBu (1 μM) led to a decrease in the amplitude of \( I_A \) by 39.1 ± 7.5% (\( n \) = 10; evoked at +30 mV; \( P < 0.01 \); Figs. 1, A and B and 2A), whereas the inactive phorbol ester 4α-phorbol (1 μM) caused no change in \( I_A \) amplitude (102.8 ± 9.6% of control, \( n \) = 5; evoked at +30 mV; \( P > 0.1 \)). The inhibitory effects could be completely eliminated in the presence of GF109203X (1 μM), a selective PKC inhibitor (98.2 ± 5.1% of control, \( n \) = 5; evoked at +30 mV; \( P > 0.1 \); Fig. 2A). Furthermore, no voltage dependence was observed in the effect of PDBu on \( I_A \) amplitude (\( n \) = 10; Figs. 1, A and B and 2B). Additionally, application of selective PKC inhibitor, either chelerythrine (10 μM, \( n \) = 6) or GF109203X (1 μM, \( n \) = 5), had no effect on \( I_A \) amplitude (\( P > 0.1 \); Fig. 2A), indicating that there is no tonic PKC modulation of \( I_A \) in cholinergic interneurons.

PKC has been shown to affect the voltage dependence of \( I_A \) activation (Hoffman and Johnston 1998), which might account for the decrease of \( I_A \) amplitude at a certain membrane potential. However, no obvious shift in the activation curve was detected after PDBu (1 μM) application (\( V_{1/2} \) in control: −21.2 ± 1.6 mV; \( V_{1/2} \) after PDBu: −23.9 ± 2.8 mV; \( n \) = 6 in each group; \( P > 0.1 \); Fig. 2B). In addition, both fast and slow time constants of \( I_A \) inactivation remained unchanged in the presence of PDBu (1 μM), suggesting that PKC activation had no effect on the inactivation kinetics of \( I_A \) (Fig. 2C). For example, the fast time constants were 24.0 ± 1.4 and 20.1 ± 7.7 ms in control and PDBu application, respectively.

FIG. 1. Protein kinase C (PKC) inhibits A-type potassium current (\( I_A \)) in cholinergic interneurons. A: representative recordings showing the isolation of \( I_A \). The \( I_A \) (\( A_i \)) was isolated by subtracting the currents evoked with depolarizing steps following a hyperpolarizing step (\( A_h \)) by the currents evoked by the same voltage steps with a prepulse of +10 mV (\( A_p \)). B: representative traces of \( I_A \) (\( B_i \)) recorded during application of phorbol-12,13-dibutyrate (PDBu, 1 μM). PDBu application suppressed \( I_A \) at each voltage step (\( A_i \), \( B_i \)). C: protocols used to evoke voltage-gated potassium (Kv) currents and isolate \( I_A \).

FIG. 2. PKC inhibits \( I_A \) without affecting the voltage dependent of activation and the inactivation kinetics. A: group data showing that PDBu application reduced the \( I_A \) amplitude, which was blocked by 3-[1-(dimethylamino)propyl]indol-3-yl]-4-(indol-3-ylmaleimide (GF109203X, 1 μM). Application of either chelerythrine (1 μM) or GF109203X (1 μM) had no obvious effect on \( I_A \). The \( I_A \) was evoked at +30 mV. B: the activation curve of \( I_A \) was unchanged by application of PDBu (1 μM). C: both fast and slow time constants of \( I_A \) inactivation were not affected by PDBu application. The \( I_A \) was evoked at +30 mV (\( * P < 0.01 \)).
FIG. 4. Conventional PKC isoforms may be involved in the PKC inhibition of \( I_A \) in cholinergic interneurons. Space-clamp error occurred during recordings on brain slices might affect the voltage dependence of \( I_A \) activation. We thus used acutely dissociated neurons to verify the effects of PDBu on \( I_A \) activation curve. It has been shown that addition of \( \text{Cd}^{2+} \) to extracellular solution positively shifts the voltage dependence of \( I_A \) activation (Song et al. 1998), which may interact with the effects of PKC. Therefore \( \text{Ca}^{2+} \)-free extracellular solution was used to avoid addition of \( \text{Cd}^{2+} \) during recordings.

Similar to those recorded on brain slices, application of PDBu (1 \( \mu \text{M} \)) induced an inhibition of \( I_A \), without influencing the activation curve (\( V_{1/2} \), control: -35.2 \( \pm \) 3.0 mV; \( V_{1/2} \) after PDBu: -30.4 \( \pm \) 2.3 mV; \( n = 6 \) in each group; \( P > 0.1 \); Fig. 3, A and B). We also found that application of PDBu caused a decrease of the saturated conductance of \( I_A \) (control: 24.1 \( \pm \) 3.8 pA/mV; PDBu: 17.3 \( \pm \) 2.7 pA/mV; \( n = 6 \) in each group; evoked at +70 mV; \( P < 0.05 \); Fig. 3C).

The effects of PKC activation on \( I_A \) were further examined with a more specific activator PMA (1 \( \mu \text{M} \)) and an inhibitor Go6976 (1 \( \mu \text{M} \)) that is specific for conventional isoforms (PKC\( \alpha \) and PKC\( \beta \)). Similar to the above-cited data, application of PMA caused a voltage-independent decrease of \( I_A \) amplitude (\( n = 6 \); \( P < 0.01 \)), which could be eliminated in the presence of Go6976 (\( n = 8 \); \( P > 0.1 \); Fig. 4). In addition, Go6976 application had no effect on \( I_A \) amplitude (\( n = 5 \); \( P > 0.1 \); Fig. 4). These data indicate that the inhibition of \( I_A \) might be mainly mediated by conventional PKC isoforms.

**Group I mGluRs inhibits \( I_A \) in a subgroup of cholinergic interneurons**

Considering that striatal cholinergic interneurons express group I mGluRs that may activate PKC (Bell et al. 2002; Ferraguti et al. 2008; Pisani et al. 2001), we tested whether activation of group I mGluRs inhibits \( I_A \). Interestingly, application of DHPG (50 \( \mu \text{M} \)), a selective agonist of group I mGluRs, significantly suppressed \( I_A \) in six of nine cells, in a voltage-independent manner (by 38.9 \( \pm \) 4.4\%); evoked at +30 mV; \( P < 0.01 \); Fig. 5). The inhibition of \( I_A \) by DHPG could be completely blocked by preapplication of MCPG (100 \( \mu \text{M} \)), a nonselective group I mGluR antagonist (104.2 \( \pm \) 9.1\% of control, \( n = 6 \); evoked at +30 mV; \( P > 0.1 \); Fig. 5). Moreover, preapplication of Go6976 (1 \( \mu \text{M} \)) also blocked the inhibitory effects of DHPG on \( I_A \) (100.5 \( \pm \) 5.6\% of control, \( n = 8 \); evoked at +30 mV; \( P > 0.1 \); Fig. 5). Thus group I mGluRs inhibit \( I_A \) in a subgroup of cholinergic interneurons through a PKC-dependent pathway.

**PKC has no effect on \( I_k \) in cholinergic interneurons**

We also examined whether PKC modulates \( I_k \) in striatal cholinergic interneurons. However, the \( I_k \) amplitude showed no change in the presence of either PMA (1 \( \mu \text{M} \), 101.9 \( \pm \) 4.2\% of control, \( n = 6 \); evoked at +30 mV; \( P > 0.1 \)) or Go6976 (1 \( \mu \text{M} \), 106.4 \( \pm \) 6.8\% of control, \( n = 5 \); evoked at +30 mV; \( P > 0.1 \); Fig. 6). These data suggest that \( I_k \) might not be modulated by PKC in cholinergic interneurons.

**Cholinergic interneurons express PKC\( \alpha \)**

To reveal the PKC isoforms involved in the modulation of \( I_A \) in cholinergic interneurons, the expression of some conventional and novel PKC isoforms was examined. Cholinergic interneurons were identified by immunostaining with antibodies against ChAT. In agreement with previous studies (Yoshihara et al. 1991), among three isoforms tested (PKC\( \alpha \), PKC\( \gamma \), and PKC\( \epsilon \)), only PKC\( \alpha \) was detected in cholinergic interneurons, with positive immunoreactivity located mainly in the perikarya. Both PKC\( \gamma \) and PKC\( \epsilon \) were detected in the majority of medium-sized striatal neurons (Fig. 7). These results suggest that, although other isoforms (e.g., PKC\( \beta \)) cannot be excluded, PKC\( \alpha \) is the major isoform responsible for the inhibitory effects of PKC on \( I_A \) in cholinergic interneurons.
PKC increases excitability of cholinergic interneurons

Modulation of IA may alter neuronal excitability (Hoffman and Johnston 1998; Storm 1990). Therefore the voltage responses to current injection were compared before and after application of selective PKC activator PDBu (1 μM). Because most of cholinergic interneurons in vitro exhibit spontaneous firings (Bennett and Wilson 1999; Deng et al. 2007), a small negative current (10–60 pA) was applied continuously in these neurons to suppress the spontaneous activities. We found that the number of action potentials evoked with positive currents (20–80 pA) was significantly increased in the presence of PDBu (Fig. 8, A and C). For example, the firing number increased by 38.2 ± 8.1% (n = 7; P < 0.01) when evoked with 60 pA (800 ms). Moreover, PDBu application caused a decrease of latency to the first spike (by 36.7 ± 10.3%, n = 6; P < 0.01; Fig. 8, A and C) and an increase of spike width (by 17.4 ± 2.2%, n = 5, P < 0.05; Fig. 8, B and C). These data indicate that, by enhancing the voltage response to positive currents, PKC activation may play excitatory roles in cholinergic interneurons.

If the enhanced voltage responses in cholinergic interneurons were caused by PKC modulation of IA, partial blockade of IA with channel blocker should produce comparable changes. Indeed, application of 4-AP at a concentration of 1 mM, which blocks nearly 50% of IA in cholinergic interneurons (Song et al. 1998), induced an increase of number of action potential during a depolarizing current of 40–60 pA (by 45.4 ± 14.1%, n = 5; P < 0.05; Fig. 9, A and B). Similar to PDBu, application of 4-AP (1 mM) led to a decrease of latency to the first spike (by 40.1 ± 7.3%, n = 5; P < 0.01; Fig. 9C) and an increase of the spike width (by 60.2 ± 11.3%, n = 5, P < 0.01; Fig. 9D). Importantly, in the presence of 4-AP (1 mM), application of PDBu (1 μM) showed no further effects on the voltage responses to current pulses (n = 7; P > 0.1; Fig. 9). These data strongly suggest that IA inhibition, at least partially, contributes to the excitatory effects of PKC in cholinergic interneurons.

It is possible that PKC activation may enhance neuronal excitability by influencing the synaptic inputs of cholinergic interneurons. We therefore examined the effects of PKC activator under conditions with neurotransmission blockade. Consistent with previous studies (Bennett and Wilson 1999), the firing pattern of cholinergic interneurons showed no obvious change after application of APV (50 μM), CNQX (10 μM), and BIC (30 μM) to block both excitatory and inhibitory neurotransmissions (n = 7; data not shown). In the presence of APV, CNQX, and BIC, application of PMA (1 μM) caused a decrease of latency to the first spike (by 31.5 ± 8.9%, n = 5; P < 0.01; Fig. 10, A and C) and an increase of firing number (by 33.9 ± 10.1%, n = 5; evoked with 20–80 pA, 800 ms; P < 0.05; Fig. 10, A and C) and spike width (by 24.8 ± 4.5%, n = 5, P < 0.01; Fig. 10, B and C). These data support the conclusion that synaptic inputs are not involved in the excitatory roles of PKC in cholinergic interneurons.

DISCUSSION

Our findings demonstrate that activation of PKC, most likely PKCα, increases the excitability of striatal cholinergic interneurons. These effects may be, at least in part, attributable to the PKC inhibition of IA. Because cholinergic interneurons are the major source of cholinergic inputs in the neostriatum, PKC modulation of neuronal excitability may be critical to control the cholinergic tone in this brain region.

PKC modulation of Kv channels

It has been shown that PKC activation modulates the activities of ion channels in neurons, including Kv channels. For example, activation of PKCα, but not PKCβ and PKCγ, inhibited Kv currents (such as IA and IL) in neurons cultured from the hypothalamus and brain stem (Pan et al. 2001). The dendritic IA in CA1 pyramidal neurons is also decreased by PKC activation (Hoffman and Johnston 1998; Yuan et al. 2002). In agreement with those studies, our data demonstrated that application of PKC activator, PDBu, or PMA dramatically inhibited IA in striatal cholinergic interneurons and indicated that conventional and novel PKC isoforms might be involved in the IA inhibition. Immunostaining revealed that cholinergic interneurons expressed PKCα, but not PKCγ and PKCε. Since...
cholinergic interneurons do not express PKCβ1 and PKCβII (Yoshihara et al. 1991), it is strongly suggested that PKCα might be the major contributor to $I_A$ inhibition.

Previous studies have demonstrated that activation of group I mGluRs leads to a suppression of several types of potassium currents, including slowly inactivating D-type outward currents ($I_D$) and apamin-insensitive currents responsible for the slow afterhyperpolarization in hippocampal neurons (Krause et al. 2002; Wu and Barish 1999), M-type potassium currents in superior cervical ganglion neurons (Ikeda et al. 1995), and 4-AP–sensitive transient currents and 4-AP–insensitive potassium currents in visceral sensory neurons (Hay and Lindsey 1995). In particular, group I mGluRs have been shown to inhibit background potassium currents in cholinergic interneurons, which induces membrane depolarization (Berg et al. 2007; Bonsi et al. 2005; Takeshita et al. 1996). In the present study, we found that application of selective agonists for group I mGluRs significantly reduced $I_A$ in most of cholinergic interneurons, which could be blocked by preapplication of PKC inhibitors. Thus PKC-induced $I_A$ inhibition might be one of the mechanisms underlying the excitatory roles of group I mGluRs in striatal cholinergic interneurons.

The downstream signaling pathways of PKC modulation of $I_A$ in striatal cholinergic interneurons are still unclear. The Kv currents in cholinergic interneurons are characterized by the predominant $I_A$, which is mainly mediated by Kv4.2 (in all interneurons) and Kv4.1 (in 60% of interneurons) subunits, but not Kv1.4 or Kv1.1 and β1 subunits (Song et al. 1998). Previous studies have shown that activation of PKC suppresses heterologous Kv4.2-mediated currents expressed in Xenopus oocytes (Nakamura et al. 1997). Thus PKC-induced $I_A$ inhibition in cholinergic interneurons might be attributable to the modulation of Kv4.2 subunits. Direct phosphorylation of Kv subunits by PKC might be one of the pathways. Accumulating evidence demonstrates that some Kv subunits, such as Kv2.1 (Misonou et al. 2004; Murakoshi et al. 1997) and Kv4.2 (Varga et al. 2000), are extensively phosphorylated in native neurons. Furthermore, several phosphorylation sites for protein kinase A (Anderson et al. 2000), calcium-calmodulin-dependent kinase II (Varga et al. 2004), and extracellular-regulated kinase
(Schrader et al. 2006) in Kv4.2 subunits have been identified. Based on the studies on CA1 pyramidal neurons, PKC-mediated $I_A$ inhibition may be caused by the direct phosphorylation of Kv4.2 subunits by extracellular-regulated kinase (Schrader et al. 2006; Yuan et al. 2002). It is possible that similar pathways might be involved in the PKC modulation of Kv4.2-mediated $I_A$ in striatal cholinergic interneurons.

PKC modulation of Kv currents may result from the changes in the biophysics of Kv channels, such as the voltage dependence of steady-state activation and the inactivation kinetics. For example, PKC activation leads to a depolarizing shift in the activation curve of $I_A$ in CA1 pyramidal neurons, causing a decrease in $I_A$ amplitude when evoked at a certain membrane potential (Hoffman and Johnston 1998). In some neurons, PKC activation dramatically alters the inactivation kinetics of $I_A$ (Hoffman and Johnston 1998) and Kv3.3- and Kv3.4-mediated potassium currents (Covarrubias et al. 1994; Desai et al. 2008). However, we found that, in cholinergic interneurons, neither the voltage dependence of the activation curve nor the time constant of inactivation of $I_A$ was changed in the presence of PKC activator. Instead, the inhibitory effects of PKC in $I_A$ were observed at all command voltages (from −40 to +50 mV). Moreover, the saturated conductance of $I_A$ was significantly reduced by PKC activation. Our data are consistent with previous findings that PKC has little effect on voltage dependence and inactivation kinetics of heterologous Kv4.2-mediated currents (Nakamura et al. 1997). These data suggest that the decrease of Kv channel conductance, rather than the changes of the steady-state activation and the inactivation kinetics, might contribute to the PKC inhibition of $I_A$ in cholinergic interneurons. It is also possible that internalization of Kv channels might be involved in the PKC inhibition of $I_A$ because phosphorylation of Kv subunits influences the channel trafficking (Hammond et al. 2008; Yang et al. 2007).

Possible roles of PKC in striatal function

The roles of PKC in neurons have been extensively studied, because PKC is believed to be essential for specific signal transduction involved in neuronal development, synaptic plasticity, and cell death (Bright and Mochly-Rosen 2005; Malenka and Nicoll 1999; Tanaka and Nishizuka 1994). It has been shown that, in striatal cholinergic interneurons, PKC activation reduces γ-aminobutyric acid type A receptor–mediated currents (Yan and Surmeier 1997). The present study revealed that PKC activation inhibited $I_A$, but not $I_K$. The latency to the first spike was signifi-
spike, the repolarization of action potential, and the firing frequency are largely determined by $I_A$ (Rudy 1988; Storm 1990). Indeed, partial blockade of $I_A$ (50%) with 4-AP induced a shortening of the latency to the first spike and an increase of firing during positive current pulse, which mimicked the effects of PKC on neuronal activities. Importantly, PKC had no further effect on neuronal excitability in the presence of 4-AP. Application of 1 mM 4-AP may block $I_D$ in other neurons, which may influence membrane properties, including the latency to the first spike and the spike repolarization (Storm 1990). However, $I_D$ is not obvious in striatal cholinergic interneurons (Song et al. 1998). Furthermore, highly negative resting membrane potentials may be necessary for effective $I_D$ (Storm 1990). Since cholinergic interneurons have less-negative resting potentials (Bennett et al. 2000; Deng et al. 2007), $I_D$ in these interneurons, if any, may have little impact on the membrane properties. Therefore our findings indicate that PKC activation inhibits $I_A$ and thus increases the neuronal excitability in cholinergic interneurons.

Striatal cholinergic interneurons are critical to the functions of basal ganglia, including movement, cognition, and associative learning (Graybiel et al. 1994; Pisani et al. 2007). Although comprising only 1–2% of the entire striatal neuronal population, cholinergic interneurons are the major source of striatal acetylcholine (Kawaguchi et al. 1995). Thus by modulating the neuronal activity of cholinergic interneurons, PKC may be important for controlling the acetylcholine release that influences the basal ganglia circuit. Additionally, mGluRs have been implicated in the pathophysiology of Parkinson’s disease (Bonsi et al. 2007). Inhibition of $I_A$ might be a novel mechanism by which group I mGluRs contribute to the excitation of cholinergic interneurons in movement disorders.

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PKC IN STRIATAL INTERNEURONS


