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β-Amyloid Peptides Impair PKC-Dependent Functions of Metabotropic Glutamate Receptors in Prefrontal Cortical Neurons

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Tyszkiewicz, Joanna P. and Zhen Yan. β-amyloid peptides impair PKC-dependent functions of metabotropic glutamate receptors in prefrontal cortical neurons. *J Neurophysiol* 93: 3102–3111, 2005. First published January 19, 2005; doi:10.1152/jn.00939.2004. The metabotropic glutamate receptors (mGluRs) have been implicated in cognition, memory, and some neurodegenerative disorders, including the Alzheimer's disease (AD). To understand how the dysfunction of mGluRs contributes to the pathophysiology of AD, we examined the β-amyloid peptide (Aβ)-induced alterations in the physiological functions of mGluRs in prefrontal cortical pyramidal neurons. Two potential targets of mGluR signaling involved in cognition, the GABAergic system and the *N*-methyl-D-aspartate (NMDA) receptor, were examined. Activation of group I mGluRs with (S)-3,5-dihydroxyphenylglycine (DHPG) significantly increased the spontaneous inhibitory postsynaptic current (sIPSC) amplitude, and this effect was protein kinase C (PKC) sensitive. Treatment with Aβ abolished the DHPG-induced enhancement of sIPSC amplitude. On the other hand, activation of group II mGluRs with (2R,4R)-4-aminopyrrolidine-2,4-dicarboxylate (APDC) significantly increased the NMDA receptor (NMDAR)-mediated currents via a PKC-dependent mechanism, and Aβ treatment also diminished the APDC-induced potentiation of NMDAR currents. In Aβ-treated slices, both DHPG and APDC failed to activate PKC. These results indicate that the mGluR regulation of GABA transmission and NMDAR currents is impaired by Aβ treatment probably due to the Aβ-mediated interference of mGluR activation of PKC. This study provides a framework within which the role of mGluRs in normal cognitive functions and AD can be better understood.

INTRODUCTION

Glutamate, the most abundant excitatory neurotransmitter in the CNS, activates both ligand-gated ion (ionotropic) channels and G-protein-coupled metabotropic (mGluRs) receptors (Nakanishi 1992). The former mediate fast glutamatergic transmission, whereas the latter exert slower and modulatory roles. The eight mGluR subtypes are divided into three groups based on the homology of their amino acid sequences and transduction mechanisms: group I mGluRs (mGluR1 and -5) stimulate phospholipase C and phosphoinositide hydroplysis, group II (mGluR2 and -3) and group III mGluRs (mGlu4, -6, -7, and -8) primarily couple to the inhibition of cAMP formation (Pin and Duvoisin 1995). The differential distribution of mGluR subtypes on pre- and/or postsynaptic terminals allows these glutamate receptors to play important roles in neuronal communication and signal processing underlying higher cognitive functions (Conn 2003). Many forms of synaptic plasticity rely on mGluR-mediated signaling (Anwyl 1999; Bashir et al. 1993; Cho and Bashir 2002). Mice lacking mGluR subtypes show impaired learning and altered synaptic plasticity (Aiba et al. 1994; Lu et al. 1997; Yokoi et al. 1996). Thus mGluRs have been implicated in regulating the molecular targets responsible for learning and memory (Conn and Pin 1997; Nakanishi 1994).

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by cognitive deficiency and memory loss. The pathogenesis of AD is believed to involve the accumulation of β-amyloid peptides (Aβ) (Price and Sisodia 1998; Selkoe 2001). Derived from β-amyloid precursor protein (APP) via proteolytic processing by β- and γ-secreteases, Aβ has the propensity to aggregate and form neuritic plaques—the main culprit of cognitive decline. In search for a safe and effective pharmacological strategy to ameliorate the Aβ-driven mental impairments, the attention has spread over a number of potential therapeutic targets. The mGluRs, because of their involvement in cognition and neurodegeneration (Nicoletti et al. 1996), offer exciting possibilities for AD drug development (Bruno et al. 2001; Conn and Pin 1997). Elucidating the function and dysfunction of mGluRs under normal and pathological conditions may therefore provide valuable insights into our knowledge of cognition and memory as well as our understanding of senile dementia and AD.

Prefrontal cortex (PFC), the primary brain region involved in cognitive control (Fuster 2001; Miller and Cohen 2001), is one of the main targets of Aβ deposits (Price and Sisodia 1998; Selkoe and Schenk 2003). To understand how the dysfunction of mGluRs contributes to the pathophysiology of AD (Lee et al. 2004), we need to determine whether Aβ interferes with the physiological functions of mGluRs in PFC neurons. PFC neural activity is controlled by GABAergic inhibition and glutamatergic excitation. The GABAergic transmission has been shown to play a key role in "working memory" by shaping the temporal flow of information during cognitive operations (Constantinidis et al. 2002; Rao et al. 2000), whereas the *N*-methyl-D-aspartate (NMDA)-type glutamate receptor has long been recognized as a major player in synaptic plasticity, learning, and memory (Malenka and Nicoll 1999). Thus the GABAergic system and the NMDA receptor are two potential targets of mGluR signaling involved in cognition. In this study, we examined the Aβ-induced alterations in the mGluR regulation of GABA transmission and NMDA receptor (NMDAR) currents in PFC pyramidal neurons.

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METHODS

Electrophysiological recordings in slices

Young adult rat slices containing PFC were prepared as described previously (Feng et al. 2001; Wang et al. 2003). All experiments were carried out with the approval of State University of New York at Buffalo Animal Care Committee. In brief, animals were anesthetized by inhaling 2-bromo-2-chloro-1,1,1-trifluoroethane (1 ml/100 g, Sigma) and decapitated, and brains were quickly removed, iced, and then blocked for slicing. The blocked tissue was cut in 300–400 μm slices with a Vibrotome while bathed in a low Ca^{2+}, HEPES-buffered salt solution (which contained, in mM: 140 sodium isethionate, 2 KCl, 4 $MgCl_2$, 0.1 $CaCl_2$, 23 glucose, 15 HEPES, and 1 kynurenic acid, pH = 7.4, 300–305 mosM). Slices were then incubated for 1–6 h at room temperature in a $NaHCO_3$-buffered saline bubbled with 95% O_2-5% CO_2 (containing, in mM): 126 NaCl, 2.5 KCl, 2 $CaCl_2$, 2 $MgCl_2$, 26 $NaHCO_3$, 1.25 NaH_2PO_4, 10 glucose, 1 pyruvic acid, 0.05 glutathione, 0.1 N^G-nitro-L-arginine, and 1 kynurenic acid, pH = 7.4, 300–305 mosM.

To evaluate the regulation of spontaneous inhibitory postsynaptic current (IPSC) by mGluRs in PFC slices, the whole cell voltage-clamp recording technique (Zhong et al. 2003) was used. Patch electrodes (5–9 MΩ) were filled with the following internal solution (in mM): 100 CsCl, 30 *N*-methyl-D-glucamine, 10 HEPES, 1 $MgCl_2$, 4 NaCl, 5 EGTA, 12 phosphocreatine, 2 MgATP, 0.2 Na_3GTP, and 0.1 leupeptin, pH = 7.2–7.3, 265–270 mosM. The slice (300 μm) was placed in a perfusion chamber attached to the fixed-stage of an upright microscope (Olympus) and submerged in continuously flowing oxygenated artificial cerebrospinal fluid. For blocking glutamate transmission, the α-amino-3-hydroxy-5-methyl-4-isoxazole proprionic acid/kainate (AMPA/KA) receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 10 μM) and N-methyl-D-aspartate receptor antagonist D(−)-2-amino-5-phosphonopetanoic acid (D-APV, 25 μM) were added to the recording solution. Cells were visualized with a ×40 water-immersion lens and illuminated with near infrared (IR) light, and the image was detected with an IR-sensitive CCD camera. A Multiclamp 700A amplifier (Axon instruments, Union City, CA) was used for these recordings. Tight seals (2–10 GΩ) from visualized pyramidal neurons were obtained by applying negative pressure. The membrane was disrupted with additional suction and the whole cell configuration was obtained. The access resistances (13–18 MΩ) were compensated 50–70%. Cells were held at −70 mV for the recording of spontaneous IPSCs. All experiments were performed side by side with cells from nontreated versus Aβ-treated slices.

Mini Analysis Program (Synaptosoft, Decatur, GA) was used to analyze synaptic activity. Individual synaptic events with fast onset and exponential decay kinetics were captured with threshold detectors in Mini Analysis software. All quantitative measurements were taken 4–6 min after drug application. IPSCs of 60 s (200–1,000 events) under each different treatment were used for obtaining the cumulative distribution plots. The detection parameters for analyzing synaptic events in each cell in the absence or presence of mGluR agonists were the same. Statistical comparisons of the frequency and amplitude of synaptic currents were made using the Kolmogorov-Smirnov (K-S) test. Numerical values were expressed as means ± SE.

mGluR ligands DHPG, DCG-IV, and (2R,4R)-4-aminopyrrolidine-2,4-dicarboxylate (APDC), 2-methyl-6-(phenylethynyl)pyridine hydrochloride (MPEP), 7-(hydroxyimino)cyclopropa[b]chromen-1a-carboxylate ethyl ester (CPCCOEt), as well as second-messenger reagents calphostin C, myristoylated $PKI_{14–22}$, myristoylated $PKC_{20–28}$, phorbol 12-myristate 13-acetate (PMA), U1026, and SB203580 (Tocris) were made up as concentrated stocks in water and stored at −20°C. The β-amyloid peptide $Aβ_{25–35}$, $Aβ_{1–42}$, and the control peptide containing the reverse sequence $Aβ_{40–1}$ were obtained from Sigma. $Aβ_{25–35}$ was resuspended in sterile distilled water at a concentration of 2 mM and incubated at 37°C for 1 h to allow fibril formation (Terzi et al. 1994). $Aβ_{1–42}$ and $Aβ_{40–1}$ peptides were dissolved in DMSO (2 mM stock) and diluted prior to use.

Whole cell recordings in acutely dissociated neurons

Whole cell recordings of currents in dissociated neurons employed standard voltage-clamp techniques (Tyszkiewicz et al. 2004; Yan et al. 1999). The internal solution consisted of (in mM) 180 *N*-methyl-D-glucamine (NMG), 40 HEPES, 4 $MgCl_2$, 0.1 bis-(*o*-aminophenoxy)-*N,N,N′,N′*-tetraacetic acid (BAPTA), 12 phosphocreatine, 2 Na_2ATP, 0.2 Na_3GTP, and 0.1 leupeptin, pH = 7.2–7.3, 265–270 mosM. The external solution consisted of (in mM) 127 NaCl, 20 CsCl, 10 HEPES, 1 $CaCl_2$, 5 $BaCl_2$, 12 glucose, 0.001 TTX, and 0.02 glycine, pH = 7.3–7.4, 300–305 mosM. Recordings were obtained with an Axon Instruments 200B patch-clamp amplifier that was controlled and monitored with an IBM PC running pCLAMP (v. 8) with a DigiData 1320 series interface (Axon Instruments, Union City, CA). Electrode resistances were typically 2–4 MΩ in the bath. After seal rupture, series resistance (4–10 MΩ) was compensated (70–90%). Care was exercised to monitor the constancy of the series resistance, and recordings were terminated whenever a significant increase (>20%) occurred. The cell membrane potential was held at −60 mV. NMDA (100 μM) was applied for 2 s every 30 s. Drugs were applied with a gravity-fed "sewer pipe" system. The array of application capillaries (150 μm ID) was positioned a few hundred micrometers from the cell under study. Solution changes were effected by the SF-77B fast-step solution stimulus delivery device (Warner Instrument, Hamden, CT).

Data analyses were performed with AxoGraph (Axon Instruments), Kaleidagraph (Albeck Software, Reading, PA), and StatView (Abacus Concepts, Calabasas, CA). For analysis of statistical significance, Mann-Whitney *U* tests were performed to compare the current amplitudes in the presence or absence of agonists. Student *t*-test was performed to compare the differential degrees of current modulation between groups subjected to different treatment.

Western blot analysis

For detecting activated PKC, a phospho-PKC (pan) antibody that recognizes PKCα, $β_I$, $β_{II}$, ε, η, and δ isoforms only when phosphorylated at a carboxyl-terminal residue homologous to Ser^{660} of $PKCβ_{II}$ was used in the Western blot analysis (Gu et al. 2003; Tyszkiewicz et al. 2004). After incubation, slices were transferred to boiling 1% SDS and homogenized immediately. Insoluble material was removed by centrifugation (13,000 *g* for 10 min), and protein concentration for each sample was measured. 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 phospho-PKC (pan) antibody (Cell Signaling, 1:2,000) or anti-phospho-p44/42 MAP kinase (Thr202/Tyr204) antibody (Cell Signaling, 1:100) for 1 h at room temperature. After being rinsed, the blots were incubated with horseradish peroxidase-conjugated anti-rabbit or anti-mouse antibodies (Amersham Biosciences, 1:2,000) for 1 h at room temperature. After three 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 a PKC antibody recognizing the α, β, and γ isoforms (Santa Cruz, 1:2,000) or an antibody recognizing EKR1/2 (Cell Signaling, 1:2000). Quantification was obtained from densitometric measurements of immunoreactive bands on autoradiograms.

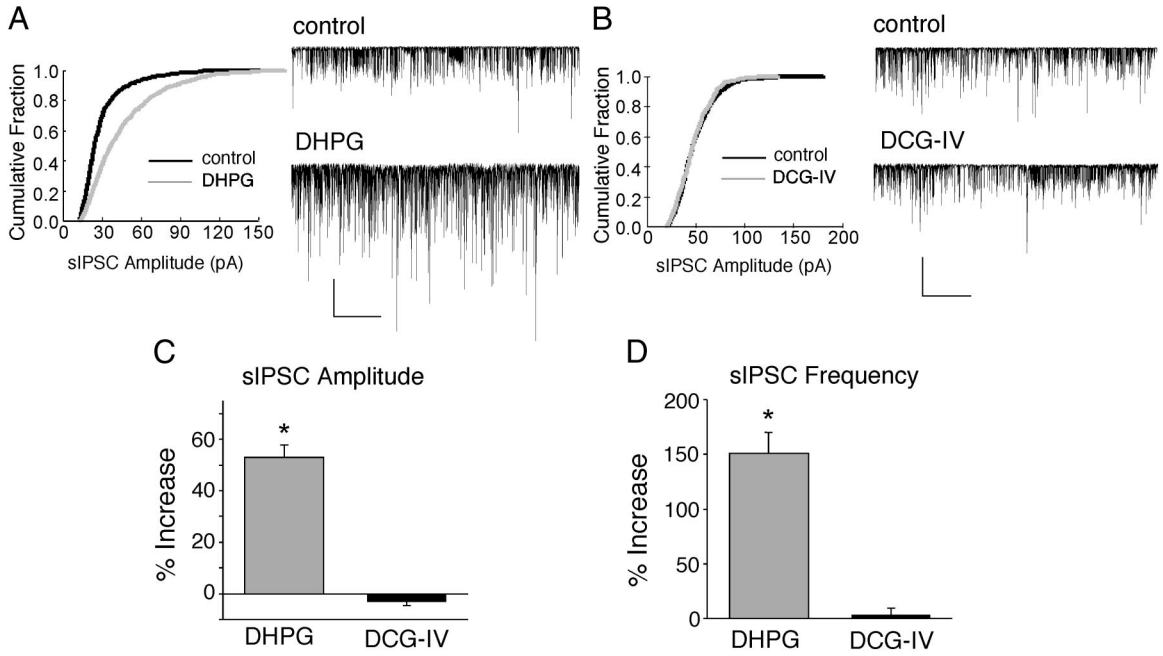

FIG. 1. Activation of group I, but not group II, metabotropic glutamate receptor (mGluR) enhances the amplitude and frequency of spontaneous inhibitory postsynaptic currents (sIPSC) in prefrontal cortex (PFC) pyramidal neurons. *A*: cumulative plots of the distribution of sIPSC amplitude (*left*) and representative traces (*right*) before (ctl) and during DHPG (50 μM) application. *B*: cumulative plots of the distribution of sIPSC amplitude (*left*) and representative traces (*right*) before (ctl) and during DCG-IV (500 nM) application. Scale bars: 30 pA, 10 s. *C* and *D*: summary histograms comparing the effect of DHPG and DCG-IV on sIPSC amplitude (*C*) and frequency (*D*). *, $P < 0.001$, *t*-test.

RESULTS

Activation of group I mGluRs enhances the spontaneous IPSC amplitude and frequency in rat PFC pyramidal neurons

To investigate the role of mGluRs on GABAergic inhibitory transmission in PFC, we examined the effects of group I and group II mGluR agonists on the spontaneous inhibitory postsynaptic currents (sIPSC) in deep layer PFC pyramidal neurons. Bath application of DHPG (50 μM), a selective group I mGluR agonist, resulted in a marked increase in the amplitude and frequency of sIPSC (Fig. 1*A*). In contrast, DCG-IV (500 nM), a highly potent group II mGluR agonist, was unable to regulate sIPSC (Fig. 1*B*). In a sample of cells we tested, DHPG caused a significant increase in the mean amplitude (52.7 $\pm$ 5.0%, $n = 21$; $P < 0.001$, K-S test) and mean frequency (150.4 $\pm$ 19.8%, $n = 21$; $P < 0.001$, K-S test) of sIPSC (Fig. 1, *C* and *D*), whereas DCG-IV had little effect on either the amplitude ($-3 \pm 1.5\%$, $n = 3$, $P > 0.05$, K-S test) or the frequency (3.7 $\pm$ 5.3%, $n = 3$; $P > 0.05$, K-S test) of sIPSC (Fig. 1, *C* and *D*). We further recorded the miniature IPSC (mIPSC) in the absence and presence of DHPG. In all cells tested, the amplitude and frequency of mIPSC (recorded in the presence of TTX) were not changed by DHPG (amplitude: $-0.3 \pm 2.3\%$; frequency: $-4.1 \pm 3.1\%$, $n = 8$, $P > 0.05$, K-S test). These results suggest that the modulation of sIPSC amplitude and frequency is specific to group I mGluRs.

To dissect which mGluR subtype contributes to the responses of DHPG, we applied selective antagonists of mGluR1 or -5. As shown in Fig. 2, *A–C*, the mGluR5 antagonist MPEP (5 μ) blocked the enhancing effect of DHPG on sIPSC amplitude, whereas the mGluR1 antagonist CPCCOEt (50 μM) failed to do so. As summarized in Fig. 2, *D* and *E*, in the presence of MPEP, DHPG had little effect on sIPSC mean amplitude ($-9.3 \pm 5.0\%$, $n = 7$; $P > 0.05$, K-S test) and mean frequency ($-11.4 \pm 9.5\%$, $n = 7$; $P > 0.05$, K-S test), which was significantly different from the effects of DHPG in the presence of CPCCOEt (amplitude: 67.3 $\pm$ 2.7%; frequency: 116.5 $\pm$ 43.4%; $n = 4$, $P < 0.001$, K-S test). These results suggest that mGluR5 mediates the effects of DHPG on GABA transmission.

Group I mGluR regulation of GABAergic transmission is PKC dependent

We next tried to determine the signaling mechanism underlying the mGluR regulation of sIPSC. Activation of group I mGluRs stimulates the PLC–PKC cascade (Conn and Pin 1997), thus we first examined whether PKC was involved in the regulation of GABA transmission by group I mGluRs.

PFC slices were incubated with the cell-permeable and specific PKC inhibitor, calphostin C (1 μM), for 1 h, followed by recordings of sIPSC in the absence and presence of DHPG. As shown in Fig. 3, *A* and *B*, the DHPG enhancement of sIPSC amplitude was abolished in slices treated with calphostin C, suggesting the involvement of PKC. To confirm the role of PKC, we also applied another mechanistically distinct PKC inhibitor, myristoylated PKC_{20-28} (20 μM) (Eichholtz et al. 1993). Similarly, the DHPG enhancement of sIPSC amplitude was abolished in slices treated with myristoylated PKC_{20-28} (Supplemental Fig. 3[1]). To determine whether the group I mGluR regulation of sIPSC amplitude is PKC-specific, we further examined the DHPG effect in PFC slices pretreated with the cell-permeable PKA inhibitor, myristoylated PKI_{14-22}

[1] The Supplementary Material for this article (3 figures) is available online at http://jn.physiology.org/cgi/content/full/00939.2004/DC1.

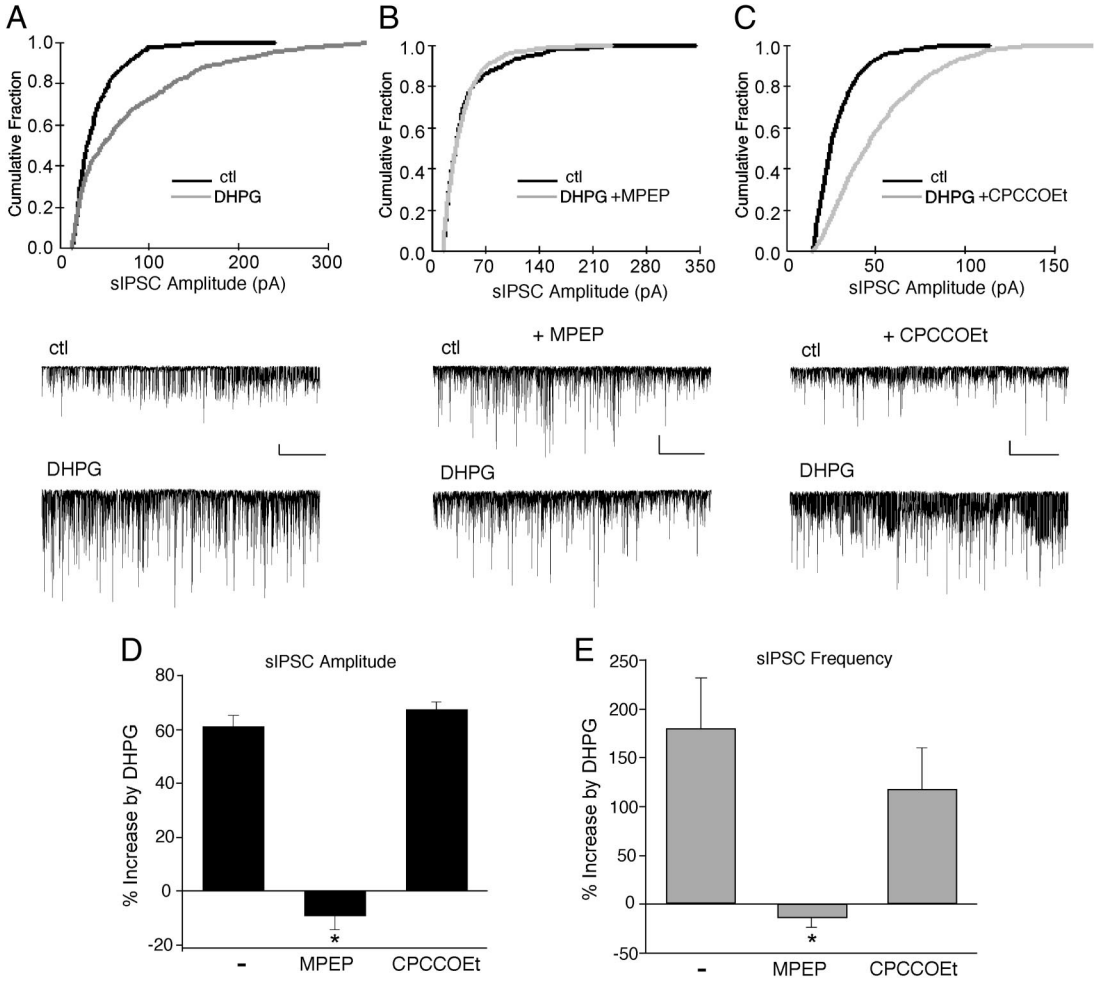

FIG. 2. The selective mGluR5 antagonist, but not mGluR1 antagonist, blocks the mGluR enhancement of sIPSC amplitude and frequency. *A–C*: cumulative plots of the distribution of sIPSC amplitude (*top*) and representative traces (*bottom*) before (ctl) and during DHPG (50 μM) application in the absence (*A*) or presence of the mGluR5 antagonist 2-methyl-6-(phenylethynyl)pyridine hydrochloride (MPEP; 5 μM, *B*) or the mGluR1 antagonist 7-(hydroxyimino)cyclopropa[b]chromen-1a-carboxylate ethyl ester (CPCCOEt; 50 μM, *C*). Scale bars: 50 pA, 10 s. *D* and *E*: summary histograms comparing the effect of DHPG on sIPSC amplitude (*D*) and frequency (*E*) in the absence (−) or presence of MPEP or CPCCOEt. *, $P < 0.001$, *t*-test.

(1 μM) (Glass et al. 1989). As shown in Fig. 3*C*, after incubation with $PKI_{14–22}$, DHPG still induced a robust increase in the amplitude of sIPSC. As summarized in Fig. 3*D*, DHPG caused little increase in sIPSC amplitude in calphostin-treated cells (5.3 ± 7.2%, $n = 7$, $P > 0.05$, K-S test) or $PKC_{20–28}$-treated cells (3.3 ± 6.1%, $n = 4$, $P > 0.05$, K-S test), which was significantly different from the effect of DHPG in non-treated cells (52.6 ± 6.5%, $n = 21$, $P < 0.001$, K-S test) or $PKI_{14–22}$-treated cells (67.2 ± 17.1%, $n = 4$; $P < 0.001$, K-S test).

Because previous studies have shown that mGluR-induced synaptic plasticity is mediated by the p42/44 or p38 MAP kinase (Bolshakov et al. 2000; Rush et al. 2002), we further explored the potential involvement of these MAP kinases in the mGluR regulation of GABA transmission. Blocking the activation of p42/44 MAP kinase (ERK) with U0126 (20 μM) (Favata et al. 1998), the specific inhibitor of MEK (the upstream kinase of ERK), failed to affect the DHPG-induced enhancement of sIPSC amplitude (Fig. 3*D*, 59.3 ± 5.8%, $n = 4$, $P < 0.001$, K-S test). Similarly, incubation with the specific p38 MAP kinase inhibitor SB203580 (1 μM) (Young et al. 1997) was also ineffective in blocking the DHPG-induced enhancement of sIPSC amplitude (Fig. 3*D*, 63.6 ± 16.3%, $n = 5$, $P < 0.001$, K-S test), suggesting the lack of involvement of p42/44 or p38 MAP kinase in the mGluR regulation of GABA transmission. The effect of group I mGluRs on the sIPSC frequency was not significantly altered by any of these kinase inhibitors (Fig. 3*E*, controls: 141.4 ± 28.2%, $n = 16$; calphostin C: 162.5 ± 53.7%, $n = 6$, $PKC_{20–28}$: 214.5 ± 48.1%, $n = 4$; $PKI_{14–22}$: 103.7 ± 34.1%, $n = 4$; U0126: 133 ± 52.1%, $n = 4$; SB203580: 122.2 ± 34.1%, $n = 5$; $P < 0.001$, K-S test). Taken together, these data suggest that the group I mGluR regulation of sIPSC amplitude, but not frequency, is mediated by a mechanism depending on the activation of PKC.

Treatment with β-amyloid peptides abolishes the group I mGluR regulation of sIPSC amplitude

β-Amyloid peptide (Aβ), derived from APP via proteolytic cleavage, is the primary component of neuritic plaques found in specific brain regions of AD patients (Selkoe and Schenk 2003). To investigate whether Aβ interferes with the group I mGluR modulation of GABAergic transmission in PFC, we incubated PFC slices with Aβ peptides and recorded sIPSC in

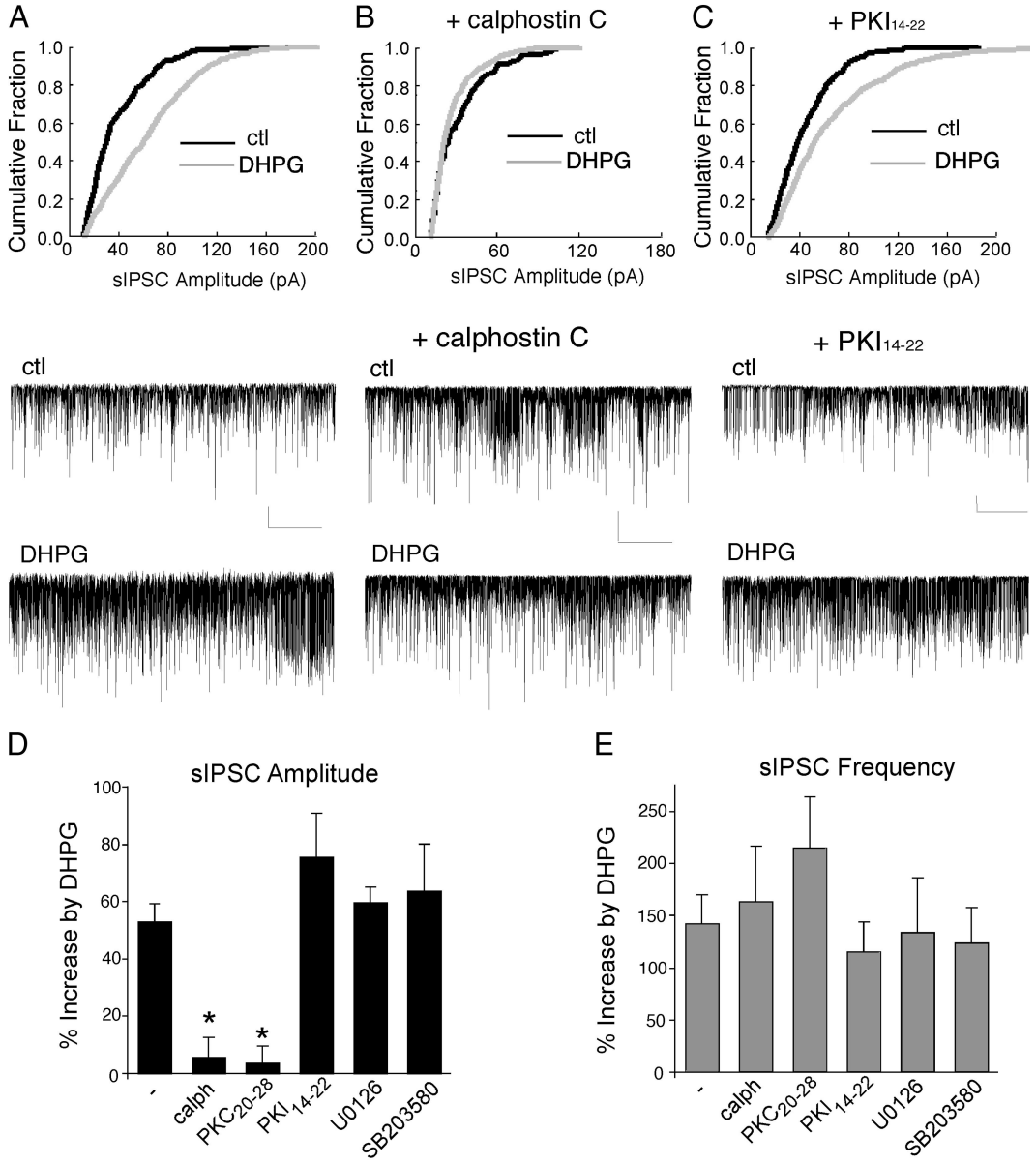

FIG. 3. The DHPG-induced increase in sIPSC amplitude is PKC dependent. *A–C*: cumulative plots of the distribution of sIPSC amplitude (*top*) and representative traces (*bottom*) before and during DHPG (50 μM) application in neurons from the nontreated slice (*A*) or the slice treated with calphostin C (1 μM, *B*) or PKI_{14-22} (1 μM, *C*). Scale bars: 20 pA, 10 s. *D* and *E*: summary histograms comparing the effect of DHPG on sIPSC amplitude (*D*) and frequency (*E*) in neurons under different treatments. *, $P < 0.001$, *t*-test.

the absence and presence of DHPG. We first compared the basal properties of sIPSCs in nontreated versus Aβ-treated slices (Supplemental Fig. 2). No significant difference was found between these groups (mean amplitude: control: 31.4 ± 1.9 pA, $n = 16$; $A\beta_{25-35}$-treated: 24.8 ± 3.0 pA, $n = 6$; $A\beta_{1-42}$-treated: 40.6 ± 5.5 pA, $n = 10$; $P > 0.05$, *t*-test; mean event number/min: control: 397.6 ± 50.3, $n = 16$; $A\beta_{25-35}$-treated: 296.7 ± 56.0, $n = 6$; $A\beta_{1-42}$-treated: 374.9 ± 76.5, $P > 0.05$, *t*-test). We then examined the effect of DHPG on sIPSCs in Aβ-treated slices.

As shown in Fig. 4, *A* and *B*, in the nontreated neuron, DHPG caused a potent enhancement of the sIPSC amplitude, whereas in the neuron treated with the aggregated $A\beta_{25-35}$ (1 μM), which represents the biologically active region of Aβ (Pike et al. 1995; Yankner et al. 1990), the effect of DHPG on sIPSC amplitude was abolished. Similarly, in the neuron treated with the full-length Aβ peptide, $A\beta_{1-42}$ (1 μM), DHPG failed to increase sIPSC amplitude (Fig. 4*C*). Lower concentration of $A\beta_{1-42}$ (0.5 μM), but not $A\beta_{1-42}$ (0.1 μM), also blocked the DHPG effect on sIPSC amplitude (Supplemental Fig. 1). In contrast, in the neuron treated with the control peptide containing the reverse sequence, $A\beta_{40-1}$ (1 μM), the enhancing effect of DHPG on sIPSC amplitude was intact (Fig. 4*D*).

The group I mGluR regulation of GABA transmission with or without Aβ treatment is summarized in Fig. 4, *E* and *F*. DHPG failed to increase sIPSC amplitude in $A\beta_{25-35}$-treated slices (−3.8 ± 5.3%, $n = 6$, $P > 0.05$, K-S test) or $A\beta_{1-42}$-treated slices (1 μM: −4.7 ± 8.2%, $n = 10$; 0.5 μM: 0.6 ± 5.3%, $n = 5$; $P > 0.05$, K-S test), which was significantly

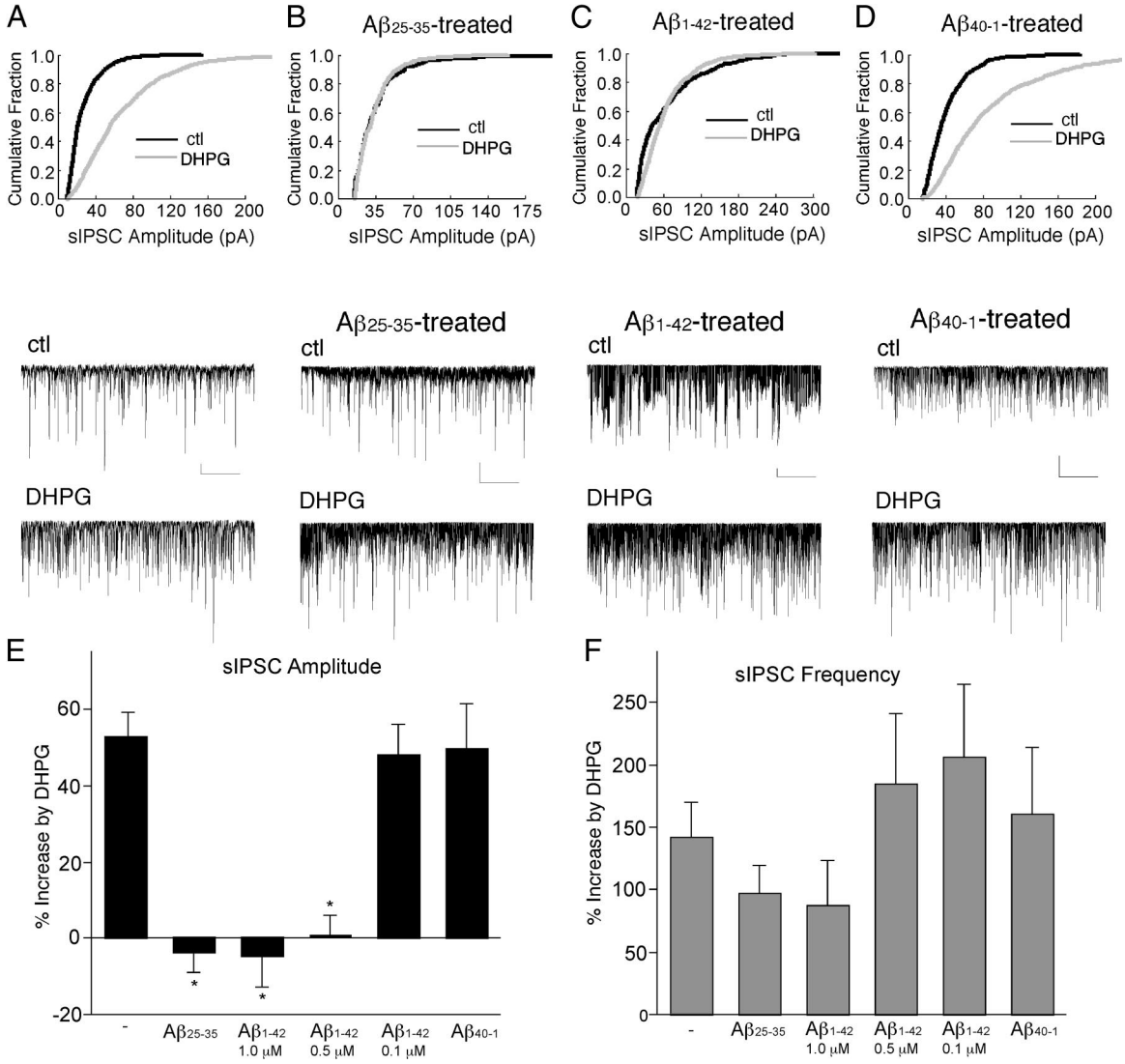

FIG. 4. Activation of group I mGluRs fails to increase the sIPSC amplitude in slices treated with Aβ. *A–D*: cumulative plots of the distribution of sIPSC amplitude (*top*) and representative traces (*bottom*) before and during DHPG (50 μM) application in neurons from the nontreated slice (*A*) or the slice treated with aged $A\beta_{25-35}$ (1 μM, *B*) or $A\beta_{1-42}$ (1 μM, C) or the control peptide $A\beta_{40-1}$ (1 μM, *D*). Scale bars: 30 pA, 10 s. *E* and *F*: summary histograms comparing the effect of DHPG on sIPSC amplitude (*E*) and frequency (*F*) in neurons under different treatments. *, $P < 0.001$, *t*-test.

different from the effect of DHPG in nontreated slices (52.5 ± 3.5%, $n = 16$, $P < 0.001$, K-S test), $A\beta_{1-42}$ (0.1 μM)-treated slices (48 ± 7.9%, $n = 5$, $P < 0.001$, K-S test), or $A\beta_{40-1}$-treated slices (49.5 ± 11.9%, $n = 6$; $P < 0.001$, K-S test). The DHPG-induced increase in sIPSC frequency was not affected by Aβ treatment [Fig. 4*F*, controls: 141.4 ± 28.2%, $n = 16$; $A\beta_{25-35}$: 96.7 ± 22.2%, $n = 6$; $A\beta_{1-42}$ (1 μM): 86.8 ± 35.5%, $n = 10$; $A\beta_{1-42}$ (0.5 μM): 184.2 ± 56.3%, $n = 5$; $A\beta_{1-42}$ (0.1 μM): 205.8 ± 58.7%, $n = 5$; $A\beta_{40-1}$: 159.7 ± 53.5%, $n = 6$; $P < 0.001$, K-S test]. Collectively, these results suggest that β-amyloid interferes with the group I mGluR regulation of sIPSC amplitude.

β-Amyloid peptides impair the group II mGluR-mediated potentiation of NMDAR currents in PFC neurons

We have recently reported that activation of group II mGluRs increases NMDAR currents in PFC pyramidal neurons via a PKC-dependent mechanism (Tyszkiewicz et al. 2004). Considering that β-amyloid peptides impair the group I mGluR-mediated, PKC-dependent regulation of GABAergic transmission, we hypothesized that the group II mGluR-mediated, PKC-dependent regulation of NMDAR currents may also be impaired by Aβ exposure. To investigate this, we incubated PFC slices with $A\beta_{1-42}$ (1 μM) for 4 h and recorded NMDAR currents from acutely isolated neurons. We first compared the basal NMDAR currents in nontreated versus Aβ-treated slices (Supplemental Fig. 2). No significant difference on the amplitude of NMDAR currents was found between these groups (control: 1.07 ± 0.19 nA, $n = 9$; $A\beta_{1-42}$-treated: 1.22 ± 0.18 nA, $n = 13$; $A\beta_{40-1}$-treated: 1.24 ± 0.22 nA, $n = 4$; $P > 0.05$, *t*-test). We then examined the effect of group II mGluRs on NMDAR currents in Aβ-treated slices. As shown in Fig. 5, *A* and *B*, application of APDC, a selective agonist for group II mGluRs, caused a reversible enhancement of NMDAR currents in the neuron from a nontreated slice but failed to enhance NMDAR currents in the neuron from an $A\beta_{1-42}$-treated slice. In contrast, the enhancing effect of APDC on NMDAR cur-

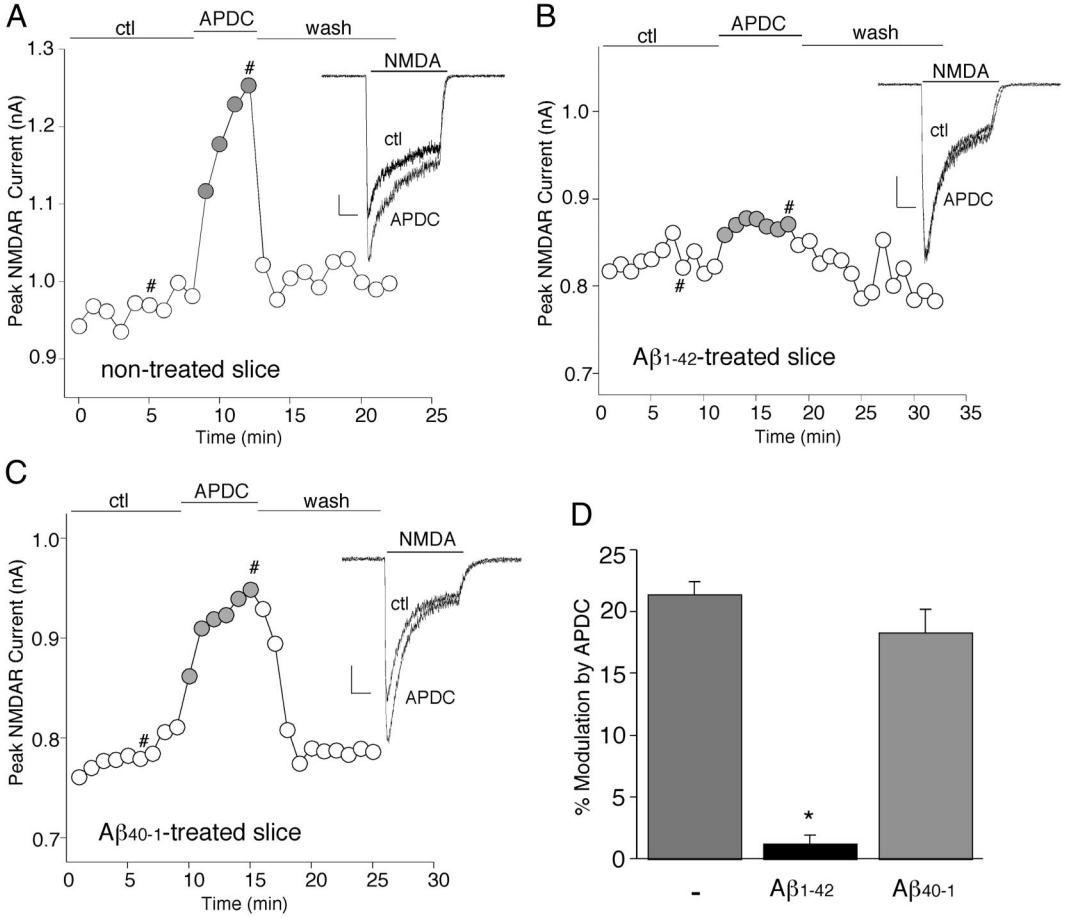

FIG. 5. The group II mGluR-mediated enhancement of NMDAR current is abolished by Aβ treatment. *A–C*: plots of peak NMDAR currents as a function of time and APDC (50 μM) application in PFC pyramidal neurons isolated from the nontreated slice (*A*) or the slice treated with $A\beta_{1-42}$ (1 μM, *B*) or $A\beta_{40-1}$ (1 μM, *C*). Inset: representative NMDA (100 μM)-evoked current traces before and during APDC (50 μM) application (at time points denoted by #). Scale bars: 0.15 nA, 0.5 s. *D*: cumulative data showing the percentage modulation of NMDAR currents by APDC in neurons under different treatments. *, $P < 0.001$, *t*-test.

rents was intact in the neuron from a slice treated with the control peptide $A\beta_{40-1}$ (Fig. 5*C*). As summarized in Fig. 5*D*, APDC had little effect on NMDAR currents in $A\beta_{1-42}$-treated neurons (1.2 ± 0.8%, $n = 13$; $P > 0.05$, Mann-Whitney), which was significantly different from the effect of APDC in nontreated neurons (21.3 ± 1.1%, $n = 9$, $P < 0.001$, Mann-Whitney) or $A\beta_{40-1}$-treated cells (18.3 ± 1.9%, $n = 4$; $P < 0.001$, Mann-Whitney). These data indicate that the group II mGluR regulation of NMDAR currents is disrupted by Aβ.

β-Amyloid peptide treatment inhibits the mGluR activation of PKC

We then tried to determine the mechanism underlying the Aβ-induced impairment of mGluR functions. Because both the group I mGluR enhancement of sIPSC amplitude and the group II mGluR potentiation of NMDAR currents are PKC-dependent, we speculated that the impairment of these mGluR functions by Aβ may be caused by the loss of mGluR activation of PKC after Aβ treatment. To examine this, we compared DHPG- and APDC-induced activation of PKC in PFC slices treated with or without $A\beta_{1-42}$ (1 μM). As shown in Fig. 6*A*, application of the group I mGluR agonist DHPG (100 μM) caused a strong increase in PKC activity in the nontreated control slice but failed to do so in the $A\beta_{1-42}$ -treated slice. The total level of PKC was not changed by DHPG with or without $A\beta_{1-42}$ treatment. As summarized in Fig. 6*B*, DHPG significantly increased the PKC activity in nontreated slices (1.7 ± 0.03-fold, $n = 6$, $P < 0.001$, *t*-test), but not in $A\beta_{1-42}$-treated slices (0.9 ± 0.05-fold, $n = 4$, $P > 0.05$, *t*-test). Similarly, as shown in Fig. 6*C*, application of the group II mGluR agonist APDC (50 μM) also potently increased PKC activity in the nontreated control slice, but this effect was abolished in the $A\beta_{1-42}$-treated slice. Summarized data (Fig. 6*D*) show that APDC significantly increased PKC activity in control slices (1.9 ± 0.11-fold, $n = 6$, $P < 0.001$, *t*-test) but lost the capability to activate PKC in $A\beta_{1-42}$-treated slices (0.8 ± 0.02-fold, $n = 4$; $P > 0.05$, *t*-test). Similarly, the capability of phorbol ester PMA to activate PKC was also impaired in $A\beta_{1-42}$-treated slices (Fig. 6*E*, $n = 4$), consistent with the previous finding that Aβ directly inhibits PKC activation (Lee et al. 2004).

To test whether the impairment of mGluR activation of PKC is due to a selective change in the signaling mechanisms or a general dysfunction of mGluRs caused by β-amyloid peptides, we examined the effect of group I mGluRs on ERK activation in Aβ-treated PFC slices. As shown in Fig. 6*F*, application of DHPG (100 μM) induced a strong increase in ERK phosphorylation (Thr202/Tyr204) and activation, and this effect was not altered in slices pretreated with $A\beta_{1-42}$ (1 μM). Similar results

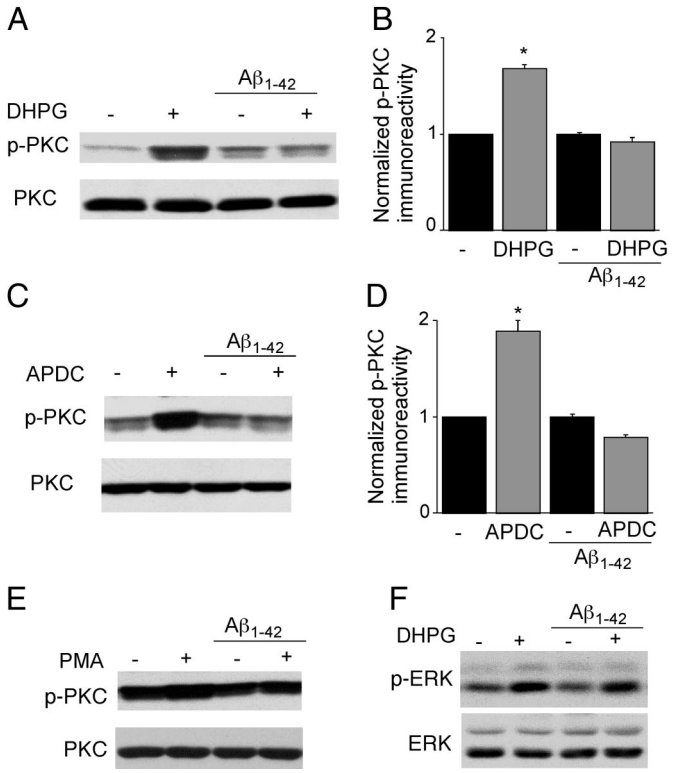

FIG. 6. Aβ treatment blocks the activation of PKC by group I and II mGluRs. *A* and *C*: immunoblots of activated PKC autophosphorylated at carboxyl terminus (*top*) and total PKC (*bottom*) incubated without or with DHPG (100 μM, 10 min; *A*) or APDC (50 μM, 10 min; *C*) in nontreated or $A\beta_{1-42}$ (1 μM)-treated PFC slices. *B* and *D*: quantification of the activated PKC in response to DHPG (*B*) or APDC (*D*) in nontreated or $A\beta_{1-42}$-treated slices. *, $P < 0.001$, *t*-test. *E*: immunoblots of activated PKC (*top*) and total PKC (*bottom*) incubated without or with PMA (0.1 μM, 5 min) in nontreated or $A\beta_{1-42}$ (1 μM)-treated PFC slices. *F*: immunoblots of p42/44 MAP kinase (p-ERK; *top*) and total ERK (*bottom*) incubated without or with DHPG (100 μM, 10 min) in nontreated or $A\beta_{1-42}$ (1 μM)-treated PFC slices.

were obtained in four to five experiments. These findings indicate that the activation of PKC by both group I and II mGluRs is selectively disrupted by Aβ treatment, which may explain the Aβ-induced impairment of mGluR regulation of GABA transmission and NMDAR currents.

DISCUSSION

The activation of mGluRs has diverse cellular actions that lead to alterations in excitability and synaptic transmission (Anwyl 1999; Mannaioni et al. 2001; Semyanov and Kullmann 2000; Valenti et al. 2003). Because of the important role of GABAergic transmission in "working memory" subserved by PFC (Constantinidis et al. 2002), we first examined the mGluR regulation of GABA transmission in PFC pyramidal neurons. Activation of group I mGluRs produced a strong increase in the amplitude and frequency of sIPSC, which could be caused by the group I mGluR-induced depolarization of GABAergic interneurons as found in the hippocampus (McBain et al. 1994; Miles and Poncer 1993; van Hooft et al. 2000). The DHPG-induced increase in sIPSCs is probably mediated by mGluR5 receptors in PFC neurons. While group II mGluRs have no effect on GABAergic transmission, we found that activation of group II mGluRs significantly potentiated the currents through NMDAR channels in acutely dissociated PFC pyramidal neurons (Tyszkiewicz et al. 2004). It suggests that group II mGluRs not only inhibit evoked glutamate release in PFC via presynaptic mechanisms (Cartmell and Schoepp 2000; Marek et al. 2000) but also regulate postsynaptic NMDA receptor channels, one of the major substrates involved in cognition.

Because mGluRs have been implicated in memory acquisition, learning, and some neurodegenerative disorders (Conn and Pin 1997), growing effort is being directed to understand the relation between mGluR signaling and amyloid accumulation in AD. Aβ strongly inhibits the induction of long-term potentiation (LTP) (Walsh et al. 2002), a synaptic model of learning and memory, and this effect is prevented by a group I/II mGluR antagonist and a selective mGluR5 antagonist, suggesting the involvement of mGluR5 in the Aβ-mediated inhibition of LTP (Wang et al. 2004). Moreover, pharmacologic blockade or activation of different mGluRs produces neuroprotection in models for chronic neurodegenerative disorders. For example, prolonged exposure of cultured cortical cells to $A\beta_{25-35}$ induces neuronal apoptosis, and the apoptosis is substantially attenuated by group II or III mGluR agonists (Copani et al. 1995). Selective antagonism of mGluR5 also exhibits neuroprotective function against Aβ toxicity in cortical cultures (Bruno et al. 2000). These results have demonstrated that mGluR signaling exerts an important impact on the cellular actions of Aβ; however, the impact of Aβ deposits on mGluR functions is largely unknown.

The most important finding of the present study is that Aβ treatment impairs two important functions of mGluRs involved in cognition: the group I mGluR regulation of GABA transmission and group II mGluR regulation of NMDA receptor currents. Emerging evidence has suggested that AD is a synaptic failure, with functional—not structural—synaptic changes being responsible for the cognitive deficits prior to frank neuronal degeneration (Hsia et al. 1999; Selkoe 2002). The accumulation of diffuse deposits of Aβ in the brain is an early event in the development of AD, which emphasizes the importance of elucidating the neuronal response to Aβ before clinical symptoms arise. Strong correlations between soluable Aβ levels and the extent of synaptic loss and cognitive impairment have been found in AD brains (Lue et al. 1999; McLean et al. 1999), suggesting that synapses are the initial target in AD (Small et al. 2001). We speculate that the Aβ-induced dysregulation of GABAergic and glutamatergic synapses will cause subtle alterations of cortical synaptic efficacy, leading to impairment of memory as the disease progresses. The aberrant synaptic functions of mGluRs in neurons after Aβ treatment, along with our previous finding on the impaired synaptic functions of muscarinic receptors in Aβ-treated PFC slices and in APP transgenic mice (Zhong et al. 2003), provide a cellular mechanism showing how Aβ could alter the complex regulation of synapses, which may result in the deficient cognition and memory.

Several mechanisms could account for the DHPG effects on GABA transmission. First, mGluRs increase the excitability of GABAergic interneurons by suppressing potassium conductances or potentiating cation currents, therefore leading to the elevated probability of GABA release. Second, mGluRs enhance the probability of action potential-dependent GABA release from axon terminals, therefore leading to the increase

of the contribution of large-size (multiquantal) sIPSCs to the overall population of synaptic events. One possible underlying mechanism for this mGluR action is that the mGluRI/phospholipid/PKC signaling regulates the presynaptic protein synaptotagmine that functions as a calcium sensor to trigger synchronous vesicle fusion events, thus facilitating the Ca^{2+} cooperativity of transmitter release. The signaling mechanism mediating the DHPG increase in sIPSC frequency awaits to be elucidated.

Given the PKC dependence of both the group I mGluR enhancement of sIPSC amplitude (this study) and the group II mGluR potentiation of NMDAR currents (Tyszkiewicz et al. 2004), one possible mechanism underlying the Aβ-induced impairment of these mGluR functions is the loss of mGluR activation of PKC after Aβ treatment. Considerable evidence has suggested that many postreceptor signal transduction processes are severely compromised in AD, including the impaired G protein regulation of phospholipase C, the decreased receptor sites for the second messenger IP_3, and the reduced PKC level and activity (Cowburn et al. 2001; Fowler et al. 1995). PKC, the key enzyme in memory processes (Tanaka and Nishizuka 1994), shows deficiency in its translocation and activation in aged rat cortex (Pascale et al. 1998). Anchoring proteins that are involved in PKC signal transduction seem to contribute to this PKC activation deficit (Battaini et al. 1997). Because the stimulation of PKC by mGluRs increases the nonamyloidogenic, secretory pathway of APP processing (Lee et al. 1995), the disrupted mGluR activation of PKC in AD may inhibit the nonamyloidogenic APP processing pathway and lead to increased β-amyloid production. Enhanced Aβ generation, in turn, exacerbates mGluR abnormalities, causing the dysfunction of glutamatergic synapses.

Our data have demonstrated that Aβ peptides inhibit only the PKC limb of the mGluR pathway and not the ERK limb, suggesting that mGluR function in general is not affected by Aβ. These data also suggest that other neurotransmitter systems that activate PKC may be inhibited by Aβ as well, like the muscarinic receptors (Zhong et al. 2003). Thus Aβ may have a selective effect on PKC-dependent processes as opposed to general mGluR functioning.

Taken together, this study has revealed that the mGluR regulation of two important targets involved in cognition, GABA transmission and NMDA receptors, are impaired by Aβ treatment, which may be attributable to the Aβ-induced loss of mGluR activation of PKC. Given the important role of mGluRs, GABA transmission, NMDA receptors and PKC in cognition, this finding provides a potentially important clue to the possible ways in which Aβ could act to cause cognitive decline. It supports the notion that elucidation of the effects of Aβ on synaptic function rather than on cell death is critical for understanding the pathogenesis of AD and for finding novel therapeutic targets (Small et al. 2001).

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