Pre- and Postsynaptic β-Adrenergic Activation Enhances Excitatory Synaptic Transmission in Layer V/VI Pyramidal Neurons of the Medial Prefrontal Cortex of Rats

Norepinephrine exerts an important influence on prefrontal cortical functions. The physiological effects of β-adrenoceptors (β-ARs) have been examined in other brain regions. However, little is known about β-AR regulation of synaptic transmission in the prefrontal cortex (PFC). The present study investigated β-AR modulation of glutamate synaptic transmission in layer V/VI pyramidal cells of the medial PFC (mPFC) of rats. Our results show that 1) isoproterenol (ISO), a selective β-AR agonist, increased the frequency of spontaneous and miniature excitatory postsynaptic currents (EPSC’s); 2) ISO enhancement of miniature EPSC’s (mEPSC’s) frequency no longer appeared in the presence of the voltage-gated Ca2+ channel blocker cadmium; 3) ISO enhanced the evoked excitatory postsynaptic currents (eEPSC’s) mediated by non-N-methyl-D-aspartic acid receptors (non-NMDA-Rs) and NMDA-Rs. The ISO facilitation of non-NMDA-R eEPSC’s was blocked by the membrane-permeable cyclic adenosine monophosphate (cAMP) inhibitor Rp-adenosine 3’,5’-cyclic monophosphorothioate triethylammonium salt (Rp-cAMPS); 4) ISO enhanced NMDA-induced current, with no effect on glutamate-induced non-NMDA-R current; 5) ISO enhancement of NMDA-R eEPSC and NMDA-induced current was blocked by intracellular application of Rp-cAMPS or the cAMP-dependent protein kinase (PKA) inhibitor PKI5-24; and 6) ISO suppressed the paired-pulse facilitation of non-NMDA-R and NMDA-R eEPSC’s. Taken together, these results provide the first electrophysiological demonstration that β-AR activation facilitates excitatory synaptic transmission in mPFC pyramidal cells through pre- and postsynaptic mechanisms, probably via cAMP or cAMP/PKA signaling.

Keywords: β-adrenoceptors, EPSC, isoproterenol, medial prefrontal cortex, rat

Introduction

The prefrontal cortex (PFC) plays a critical role in behaviors requiring high levels of mental integration (Goldman-Rakic 1987; Miller and Cohen 2001; Fuster 2003). The PFC guides behaviors using working memory (Goldman-Rakic 1987; Funahashi et al. 1989; Funahashi et al. 1993; Goldman-Rakic 1995; Baddeley 2003; Bodner et al. 2003). Animals or humans with lesions to the PFC exhibit a disorganized behavior, such as deficits in working memory, attention regulation, and response inhibition (Luria 1966; Fuster 1997).

Norepinephrine (NE) is a regulatory neurotransmitter distributed widely throughout the central nervous system (CNS) (Fuxe et al. 1968; Lindvall et al. 1984). Extensive behavioral and physiological studies have demonstrated that endogenously released NE of lower to moderate levels exerts an important and beneficial influence on PFC functions (Arnsten and Goldman-Rakic 1985; Arnsten and Li 2005; Rossetti and Carboni 2005). It is well documented that NE produces its beneficial influence on working memory through action at β2-ARs, especially β2,3-adrenoceptors (β2,3-ARs) in the PFC (Arnsten and Goldman-Rakic 1985; Arnsten et al. 1988; Li and Mei 1994; Franowicz and Arnsten 1998; Li et al. 1999; Mao et al. 1999; Franowicz et al. 2002; Arnsten and Li 2005; Wang et al. 2007). β-Adrenoceptors (β-ARs) (β1, β2, and β3) are G protein-coupled receptors that mediate physiological responses to NE. The physiological and cognitive behavioral effects of β-ARs have been extensively examined in other brain regions. For example, stimulation of β-ARs in the hippocampus lowers the threshold for induction of early long-term potentiation (LTP) (Thomas et al. 1996; Katsuki et al. 1997) and elicits a type of late-LTP that requires extracellular signal-regulated kinases activation and protein synthesis (Gelinas and Nguyen 2005). Similarly, activation of β-ARs in the amygdala enhances excitatory synaptic transmission (Huang et al. 1996; Huang, Wang, et al. 1998). β-ARs mediate NE-induced enhancement of γ-aminobutyric acid (GABA) inhibition in Purkinje cells of cerebellum and in layer V pyramidal cells of the barrel-field cortex (Waterhouse et al. 1982; Yeh and Woodward 1983; Sessler et al. 1995). Pharmacological blockade of β-ARs in the hippocampal CA1 region impairs consolidation of contextual fear memory and spatial memory in Morris water maze (Ji, Wang, et al. 2003; Ji, Zhang, et al. 2003), and blockade of β-ARs in the basolateral amygdala produces a severe deficit in fear memory (Debiec and Ledoux 2004; Hurlemann et al. 2005) and taste memory (Miranda et al. 2005).

In contrast, the behavioral and, especially, the physiological effects of prefrontal cortical β-ARs are still poorly understood. Tronel et al. (2004) reported that posttraining blockade of β-ARs in the medial PFC (mPFC) produces amnesia in rats, suggesting a role of β-ARs in the mPFC in late-phase memory consolidation. Ramos et al. (2005) reported that intra-mPFC infusion of the β-AR antagonist betaxolol in aged rats or systemic administration of betaxolol in aged monkeys improves working memory performance (Ramos et al. 2005). Thus, β-ARs could have a profound differential impact on cognitive processing depending on the brain regions.

Given the importance of prefrontal cortical β-ARs in cognitive processes, we sought to further characterize the electrophysiological effects of β-AR activation on excitatory synaptic transmission in layer V/VI pyramidal neurons of rat mPFC, using in vitro whole-cell patch-clamp recording technique. We examined the effects of the β-AR agonist isoproterenol (ISO) on electrophysiological measures that reflect the excitatory synaptic transmission, including spontaneous excitatory postsynaptic currents (sEPSC’s), miniature excitatory postsynaptic currents (mEPSC’s), evoked excitatory...
postsynaptic current (eEPSC), non-N-methyl-D-aspartic acid receptor (NMDA-R) and NMDA-R currents, and paired-pulse facilitation (PPF).

Materials and Methods

Brain Slice Preparation

Sprague-Dawley rats (14–24 days) were purchased from the Laboratory Animal Center, Fudan University Shanghai Medical School. All the experimental protocols used in the present study were in compliance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (1996). All efforts were made to minimize the number of animals used and their suffering. Rats were anesthetized with sodium pentobarbital (40 mg/kg, intraperitoneal) before decapitation. As sodium pentobarbital would produce some influence on synaptic responses mediated by glutamate receptors, we used ether and isoflurane in some experiments that have less long-term influence than sodium pentobarbital. The results using these approaches did not differ from each other (see Results).

The brains were quickly removed (within 1 min), submerged in ice-cold artificial cerebrospinal fluid (ACSF) containing (in mM): 119 NaCl, 2.5 KCl, 1 CaCl₂, 3 MgSO₄, 1 NaH₂PO₄, 26.2 NaHCO₃, and 11 glucose and saturated with 95% O₂–5% CO₂. Coronal bilateral slices (400 µm thick) were fabricated from the brains using a McIlwain tissue chopper (Bartels, Germany). The slices were then incubated at room temperature for at least 20 min before a single slice was transferred to an oxygenated (95% O₂–5% CO₂) ACSF bath and incubated at room temperature for at least 1 h before a single slice was transferred to a submerged recording chamber for recording.

The perfusion ACSF was delivered with a pump (Peri-Star 291, World Precision Instruments, Sarasota, FL) at a rate of 2–3 ml/min. In this perfusing ACSF, CaCl₂ was increased to 2.5 mM and MgCl₂ decreased to 1.5 mM. All experiments were performed at room temperature (approximately 23°C).

As described above, slice preparations were from young rats with postnatal age of 14–24 days. For in vitro patch-clamp recordings, young rats with this age have been widely used in literatures (Morisset and Urban 2001; Seamans et al. 2001; Gonzalez-Islas and Hablitz 2003; Kaneko and Takahashi 2004; Trantham-Davidson et al. 2004; Huang and Hsu 2006). The main reason is that cortical neurons from rats of this age can survive easier in vitro. Although the cerebral cortex is immature by postnatal 14 days and not yet completely mature by 24 days, there is evidence showing that the glutamate receptors and adrenergic receptors of the animals at this young age are functionally similar to that of the adults (Ernsdieck-Erns et al. 1991; Burgard and Hablitz 1993).

Visualization of Pyramidal Neurons

A slice was viewed with an upright microscope (Axioskop Fsmot, Zeiss, Germany) equipped with infrared-differential interference contrast (IR-DIC) optics. The image was detected with an infrared-sensitive camera (C2400-79H, Hamamatsu, Japan) and displayed on a black-white video monitor. Layer V/VI pyramidal cells of the mPFC were easily recognizable via 40× water-immersion lens. The spike-firing pattern that accompanied with spike frequency adaptation in response to a depolarization current was used to electrophysiologically identify pyramidal neurons. In some neurons examined, Lucifer yellow was added into internal pipette solution at a concentration of 0.05% (w/v), which could enter into the patched cells through diffusion. At the end of the experiments, the slices were fixed in 4% paraformaldehyde. The morphology of the neurons was confirmed using a confocal laser scanning microscope (SP2, Leica, Germany).

Patch-Clamp Recording

Whole-cell recordings were made in layer V/VI pyramidal cells of the mPFC. Patch pipettes (3–7 MΩ) were fabricated from borosilicate tubing (1.5 mm outside diameter, 0.86 mm inside diameter, Sutter Instruments, Novato, CA) on a horizontal microelectrode puller (P-97, Sutter Instruments). The internal pipette solution contained (in mM) 150 KCl, 0.4 ethyleneglycol-bis-aminoethyl ether-tetraacetate, 8 NaCl, 2 ATP, Mg, 0.1 GTP·Na₃, and 10 N-2-hydroxyethylpiperazine–N’-2-ethanesulfonic acid (HEPES), with pH adjusted to 7.2–7.4 by KOH, and had an osmolality of 290–320 mosm.

Voltage and current signals in current- and voltage-clamp modes were recorded with an HEKA EPC9 amplifier (Heka, Germany) connected to a Digidata interface (Molecular Devices, Union City, CA). The data were digitized and stored on disks using Pulse software (Heka, Lambrecht, Germany). Resting membrane potential and action potential were recorded under the current-clamp mode. The mode was then shifted to the voltage-clamp mode for recording of eEPSCs, mEPSCs, eEPSC, or NMDA-induced currents. The series resistance (Rs) was monitored at regular intervals throughout recording. The range of Rs was 10–20 MΩ. Data were discarded if the Rs of a recorded cell changed by 15%.

For recordings of eEPSC, a custom-made bipolar stimulation electrode was positioned 150–200 µm subjacent to a recorded neuron. Current pulses (50–120 µA in amplitude and 100 µs in pulse duration), generated and isolated by Master-8 (A.M.P. Instruments Ltd, Jerusalem, Israel), were used as stimuli and delivered at 0.033 Hz. In most cases, we used a stimulation intensity that could evoke an eEPSC of approximately 100–150 pA. All traces of synaptic currents shown in the figures are the average of 10 consecutive responses.

Drug application

(-)-Isoproterenol hydrochloride (ISO), 3,5-propanol (PRO) hydrochloride, bicuculline methiodide (BMI), L-glutamic acid potassium salt (glutamate), 6-cyano-7-nitroquinolinolinedione (CNQX), 3,5-di-amino-5-phosphonovaleric acid (AP-5), NMDA, Rp-Adenosine 3',5'-cyclic monophosphorothioate triethylammonium salt (Rp-cAMPS), K+ gluconate, ATP.Mg, GTP.Na₃, and HEPES were purchased from Sigma Chemical Company (Sigma, St Louis, MO). Tetrodotoxin (TTX) was purchased from the Research Institute of Aquatic Products, Hebei Province, China. The protein kinase A inhibitor fragment 5–24 (PKA, 5–24) and cyclohexylamine (CTZ) were purchased from Tocris Cookson Ltd (Ellisville, MO). Other reagents in analytical reagent (AR) grade were purchased from the Shanghai Chemical Company, Shanghai, China. All drugs were dissolved in ultrapure deionized water (Millipore Q-Gard 1, Billerica, MA). All drug solutions were kept in refrigerators (not more than 5 days prior to use). Stock solutions of PKA, 5–24 and Rp-cAMPS were stored in frozen aliquots at −70°C and those of AP-5 and CNQX in frozen aliquots at −20°C. Internal pipette solution was also stored at −20°C. Stock solutions for other drugs (such as TTX and BMI) were stored at 0–4°C. All stock solutions were diluted with ACSF before application. eEPSCs, mEPSCs, eEPSC, and glutamate- and NMDA-induced currents were recorded in the continuous presence of BMI (20 µM) that blocks feedforward or feedback GABAergic transmission in a recorded neuron. This was because that ionotropic glutamate receptors (including NMDA-R and non-NMDA-R) and adrenoceptors (including β-ARs) are present not only in pyramidal cells but also in GABAergic interneurons. Thus, it is necessary to block any possible and indirect influence from GABAergic interneurons when NMDA-R, non-NMDA-R, or β-ARs in a patched pyramidal neuron were studied.

For recording of mEPSCs, TTX (1 µM) was bath applied to block voltage-activated sodium channels and then eliminate spontaneous action potentials at presynaptic terminals that trigger glutamate releases. For recording of eEPSC, the NMDA-R antagonist AP-5 (50 µM) or the non-NMDA-R antagonist CNQX (20 µM) was bath applied to isolate non-NMDA-R- or NMDA-R-mediated current, respectively.

For recording of NMDA-induced current, both CNQX (20 µM) and TTX (1 µM) were applied. NMDA was pressure or bath applied. For pressure application, NMDA (300 µM, dissolved in ACSF) was delivered to the soma of a recorded cell from a distance of approximately 10 µm, using a patch pipette (tip diameter 3–8 µm). Pressure application was controlled by a microinfusion syringe pump (MD-1001, Bioanalytical Systems, West Lafayette, IN). Pulse duration for pressure application varied from 3 to 5 s but was constant for a given cell. The interpulse interval was at least 2 min to avoid cumulative effect of the drug. For bath application, NMDA (30 µM) was applied 3 times for 30 s each, with an interpulse interval of 10 min. The reason that...
The data was analyzed using a paired t-test. The frequency of the mEPSCs was compared using non-parametric Kolmogorov–Smirnov (KS) test. The frequency of mEPSCs was statistically compared to the number of neurons examined.

To examine the effects of β-AR activation on sEPSCs, mEPSCs, eEPSCs (including NMDA-R- and non-NMDA-R-mediated eEPSCs), and glutamate- and NMDA-induced currents, the β-AR agonist ISO (1, 10, and/or 100 μM) was bath applied. The threshold amplitude for detection of an event was 2.5 times of the root mean square noise level for sEPSCs and mEPSCs, successive spontaneous events recorded during ISO application. Complete exchange took less than 1 min. ISO treatment was limited to one neuron per slice. In the experiments with inhibition of postsynaptic intracellular cyclic adenosine monophosphate (cAMP) or cAMP-dependent protein kinase (PKA), Rp-cAMPS (100 μM) or PIK5-24(500 nM) was added into internal pipette solution. In the experiments that tested the inhibition of PKA in presynaptic terminals, the cell-permeable cAMP analogue Rp-cAMPS (20 μM) was bath applied.

Data Analysis
Effect of ISO was assessed 5 min after the start of ISO application. For sEPSCs and mEPSCs, successive spontaneous events recorded during a 3-min postdrug period were determined automatically with a threshold-crossing algorithm. The root mean square noise level for sEPSCs and mEPSCs, successive spontaneous events recorded during ISO application was statistically compared using non-parametric Kolmogorov–Smirnov (KS) test. The frequency was 2.5 times of the root mean square noise level for sEPSCs and mEPSCs.

Results
Whole-cell recordings were conducted in 221 layer V/VI pyramidal cells in slices of mPFC. These neurons were identified using IR-DIC by their pyramidal shaped cell bodies and the presence of a long apical dendrite extending toward superficial layers. They had a resting membrane potential of −64.71 ± 0.59 mV (mean ± SEM) and an action potential amplitude of 88.13 ± 0.77 mV, with no spontaneous discharge. These pyramidal cells exhibited a spike frequency adaptation in response to a depolarizing current pulse. Most of them could be characterized as “regular spiking” cells according to Yang et al. (1996).

Stimulation of β-ARs Increases Both the Frequency and Amplitude of sEPSCs
In the continuous presence of BMI, sEPSCs were recorded in 20 pyramidal cells under voltage-clamp at a holding potential of −70 mV. Bath application of the β-AR agonist ISO (100 μM) caused a significant increase in the frequency of sEPSCs in 17 of the 20 cells (Fig. 1A–D). The ISO facilitation could not be washed out within 20 min after termination of application (Fig. 1B). The cumulative probability curve of sEPSC frequency exhibited a significant shift to the left during ISO application (Fig. 1C; *P < 0.05, KS test). Analysis of pooled data from the 17 neurons showed that the sEPSC frequency was 2.2 ± 0.3 Hz in control and 3.7 ± 0.4 Hz during ISO application, with a percentage increase of 76.5 ± 7.3% (Fig. 1D; *P < 0.05, paired t-test). ISO produced no effect on the sEPSC frequency when the β-AR antagonist PRO was coadministered: the frequency was 2.3 ± 0.3 Hz in control and 2.4 ± 0.4 Hz during application of ISO + PRO (Fig. 1D; *P > 0.05, paired t-test). Of the 20 neurons examined, 3 neurons showed no change in sEPSC frequency during ISO application.

In 12 of the 20 neurons, ISO induced a significant increase in the amplitude of sEPSCs. The cumulative distribution of sEPSC amplitude showed that the cumulative curve displayed a significant shift to the right during ISO application (Fig. 1E; *P < 0.05, KS test). The peak amplitude was 16.2 ± 0.6 pA in control and 19.5 ± 0.7 pA during ISO application, with a percentage increase of 20.7 ± 2.4% (Fig. 1F; *P < 0.05, paired t-test). The ISO enhancement of sEPSC amplitude did not appear when PRO was coapplied: the peak amplitude was 17.2 ± 0.6 pA in control and 17.5 ± 0.6 pA during application of ISO + PRO (Fig. 1F; *P > 0.05, paired t-test). Of the remaining 8 neurons, 7 neurons showed no change and one showed a decrease in sEPSC amplitude during ISO application.

Stimulation of β-ARs Increases the Frequency but not Amplitude of mEPSCs
mEPSCs were recorded in 10 pyramidal cells under voltage-clamp at a holding potential of −70 mV and in the presence of the voltage-activated sodium channel blocker TTX (1 μM). The concentration of TTX can completely eliminate action potential-mediated synaptic activity (Morisset and Urban 2001). The mEPSCs were completely blocked by bath application of the non-NMDA-R antagonist CNQX (20 μM) but not by the NMDA-R antagonist AP-5 (50 μM), indicating that these spontaneous inward currents were mediated primarily by postsynaptic non-NMDA-Rs.

Bath application of 100 μM ISO significantly increased the frequency of mEPSCs in all the 10 neurons examined (Fig. 2A–D), suggesting that ISO acts at presynaptic β-ARs to increase glutamate release. This facilitation could not be washed out within 20 min after termination of application (Fig. 2B). The cumulative probability curve of the frequency of mEPSCs was shifted to the left during ISO application (Fig. 2C; *P < 0.05, KS test). Analysis of pooled data from the 10 neurons showed that the frequency of mEPSCs was 1.8 ± 0.2 Hz in control and 2.5 ± 0.2 Hz during ISO application, with a percentage increase of 46.1 ± 6.8% (Fig. 2D; *P < 0.05, paired t-test). The ISO enhancement of the mEPSC frequency was blocked by PRO: the frequency was 1.9 ± 0.2 Hz in control and 1.8 ± 0.3 Hz during application of ISO + PRO (Fig. 2D; *P > 0.05, paired t-test).

Although the frequency of mEPSCs was increased, the amplitude of mEPSCs was unchanged during application of 100 μM ISO, suggesting that β-ARs do not regulate the activity of postsynaptic non-NMDA-Rs. The cumulative distribution curve of mEPSC amplitude was not changed during ISO application (Fig. 2E; *P > 0.05, KS test). The mEPSC amplitude was 14.4 ± 0.5 pA in control and 14.7 ± 0.5 pA during ISO application (Fig. 2F; *P > 0.05, paired t-test). The mEPSC amplitude was also unchanged in the presence of PRO: the peak amplitude was 13.8 ± 0.5 pA in control and 13.6 ± 0.6 pA during application of ISO + PRO (Fig. 2F; *P > 0.05, paired t-test).
Figure 1. Stimulation of β-ARs increases both the frequency and amplitude of sEPSC’s. (A) Whole-cell recordings of sEPSC’s in a pyramidal cell in the absence (left) and in the presence of ISO (right). (B) Time course of the ISO facilitation of the sEPSC frequency in the pyramidal cell. (C) Cumulative distribution of interevent intervals of sEPSC’s in control (without ISO) and in the presence of ISO. (D) Histograms showing the ISO-induced increase in the sEPSC frequency in the pyramidal cells examined. The ISO facilitation was blocked by the β-AR antagonist PRO. (E) Cumulative distribution of the sEPSC amplitude in control and in the presence of ISO. (F) ISO enhancement of the sEPSC amplitude in the pyramidal cells examined. This enhancement was blocked by coadministered PRO. Holding potential was −70 mV. The concentration of ISO was 100 μM. *P < 0.05 versus control.
Figure 2. Stimulation of β-ARs increases the frequency but not amplitude of mEPSCs. (A) Whole-cell recordings of mEPSCs in a pyramidal cell in the absence (left) and in the presence of ISO (right). (B) Time course of the ISO facilitation of the mEPSC frequency in the pyramidal cell. (C) Cumulative distribution of interevent intervals of mEPSCs in control (without ISO) and in the presence of ISO. (D) Histograms showing the ISO-induced increase in the mEPSC frequency in the pyramidal cells examined. The ISO potentiation was blocked by coapplied PRO. (E) Cumulative distribution of the mEPSC amplitude in control and in the presence of ISO. (F) ISO had no effect on the mEPSC amplitude nor did it when PRO was coadministered. Holding potential was −70 mV. The concentrations of ISO were 10 and 100 μM, respectively. *P < 0.05 versus control.
We also examined the effect of 10 μM ISO on mEPSCs in 6 pyramidal cells. Similarly, 10 μM ISO increased the mEPSC frequency but did not affect the mEPSC amplitude: the frequency was 1.5 ± 0.2 Hz in control and 2.1 ± 0.1 Hz during application of ISO, with a percentage increase of 44.0 ± 12.0% (Fig. 2D; P < 0.05, paired t-test), and the amplitude was 14.1 ± 0.5 pA in control and 15.5 ± 0.4 pA during ISO application (Fig. 2F; P > 0.05, paired t-test). An one-way ANOVA revealed no significant difference between the enhancement effects of the 10 and 100 μM doses of ISO (Fig. 2D, F1,14 = 0.03, P = 0.86).

To determine whether the ISO-induced increase in mEPSC frequency involves voltage-gated Ca2+ channels, we conducted similar experiments but adding cadmium (200 μM) in the perfusion ACSF. Cadmium is known as a broad-spectrum blocker for voltage-gated Ca2+ channels. Cadmium per se did not alter mEPSC frequency: it was 1.6 ± 0.2 Hz in control (in the absence of cadmium) and 1.7 ± 0.2 Hz in the presence of cadmium (Fig. 3B; P > 0.05, paired t-test). In the presence of cadmium, ISO (100 μM) did not induce an increase in mEPSC frequency (Fig. 3B, 1.7 ± 0.3 Hz in control and 1.6 ± 0.3 Hz in the presence of cadmium + ISO; P > 0.05, paired t-test) or in mEPSC amplitude (Fig. 3C). This result indicates that the ISO-induced increase in mEPSC frequency requires presynaptic voltage-gated Ca2+ channels.

**Stimulation of β-ARs Enhances the Amplitude of eEPSC**

We then examined the effects of β-AR stimulation on eEPSC. Under voltage-clamp at a holding potential of −70 mV, eEPSCs were evoked at a stimulation rate of 0.033 Hz, which could be completely blocked by coapplication of AP-5 (50 μM) and CNQX (20 μM) (figure not shown), indicating that the currents were mediated by ionotropic glutamate receptors.

Bath-application of ISO (1, 10, and 100 μM) dose dependently enhanced the amplitude of eEPSC (Fig. 4A). For 1 μM ISO, the amplitude of eEPSC was 94.4 ± 4.8 pA in control and 108.4 ± 7.5 pA during ISO application, with a percentage increase of 12.9 ± 4.5% (Fig. 4A, left; P < 0.05, paired t-test).

For 10 μM ISO, the amplitude of eEPSC was 130.7 ± 14.5 pA in control and 173.1 ± 21.9 pA during ISO application, with a percentage increase of 33.0 ± 9.8% (Fig. 4A, middle; P < 0.05, paired t-test). For 100 μM ISO, the amplitude of eEPSC was 85.3 ± 10.4 pA in control and 112.3 ± 8.8 pA during ISO application, with a percentage increase of 31.8 ± 13.0% (Fig. 4A, right; P < 0.05, paired t-test). An one-way ANOVA revealed no significant difference in the facilitation effects between 10 and 100 μM ISO (F1,17 = 4.72, P = 0.044) but no difference between 10 and 100 μM ISO (F1,16 = 0.30; P = 0.59). The ISO enhancement was completely blocked when PRO (50 μM) was coadministered (Fig. 4A, middle).

In the experiments described above, the animals were anesthetized with sodium pentobarbital before decapitation. One may argue that sodium pentobarbital would have some influence on the glutamate receptor-mediated responses. We therefore did additional control experiments, using the more volatile anesthetics, that is, ether or isoflurane, to anesthetize rats. Ether and isoflurane have less long-term influence than sodium pentobarbital and can easily be washed out in brain slices. As shown in Figure 4B, ISO (100 μM) significantly enhanced eEPSC under anesthesia with ether (left) or isoflurane (right), with a percentage increase of 44.3 ± 19.9% for ether and 37.0 ± 10.0% for isoflurane. These control experiments in turn suggest that the ISO potentiation of eEPSC was not due to a possible influence of sodium pentobarbital on the glutamate receptor-mediated responses.

**Stimulation of β-ARs Enhances Non-NMDA-R-Mediated eEPSC**

To characterize whether the ISO enhancement of eEPSC was due to a modulation of non-NMDA-R component of eEPSC, we pharmacologically isolated non–NMDA-R-mediated eEPSC by bath applying AP-5 (50 μM) to block NMDA-R. Membrane potential was held at −70 mV.

As shown in Figure 5, ISO (1, 10, and 100 μM) dose dependently enhanced the amplitude of non–NMDA-R eEPSC. For 1 μM ISO, the amplitude of non–NMDA-R eEPSC was...
101.0 ± 4.0 pA in control and 111.5 ± 6.0 pA during ISO application, with a percentage increase of 10.4 ± 3.1% (Fig. 5A, left; *P < 0.05, paired t-test). For 10 μM ISO, the amplitude of non-NMDA-R eEPSC was 129.0 ± 10.6 pA in control and 156.7 ± 8.9 pA during ISO application, with a percentage increase of 23.1 ± 8.1% (Fig. 5A, middle; *P < 0.05, paired t-test). For 100 μM ISO, the amplitude of non-NMDA-R eEPSC was 103.0 ± 15.2 pA in control and 139.0 ± 17.8 pA during ISO application, with a percentage increase of 39.6 ± 6.1% (Fig. 5A, right; *P < 0.05, paired t-test). An one-way ANOVA revealed significant difference in the facilitation effects between 10 and 1 μM ISO (F_{1,11} = 4.71; *P = 0.048) but no difference between 100 and 10 μM ISO (F_{1,17} = 2.33; *P = 0.15).

Although ISO enhanced non-NMDA-R eEPSC, it was not clear whether this enhancement came from a presynaptic mechanism or a postsynaptic one. As the previous experiments had showed that ISO increased mEPSC frequency but not amplitude, it was most likely that ISO acted at presynaptic β-ARs to facilitate, via enhancing glutamate release, the non-NMDA-R eEPSC. To confirm this, we then investigated the effect of ISO (100 μM) on the PPF of non-NMDA-R eEPSC. As shown in Figure 5B, ISO significantly reduced the PPF of non-NMDA-R eEPSC. The PPF was 130.0 ± 10.3% in control and 107.1 ± 5.6% during ISO application (Fig. 5B; *P < 0.05, paired t-test), again suggesting a presynaptic mechanism for the ISO potentiation of non-NMDA-R eEPSC.

In order to know whether the ISO enhancement of non-NMDA-R eEPSC involves presynaptic cAMP signaling, we bath applied the membrane-permeable cAMP inhibitor Rp-AMPS (20 μM). Under this condition, ISO enhancement no longer appeared: the non-NMDA-R eEPSC was 117.6 ± 9.6 pA in control and 128.5 ± 12.7 pA during application of 100 μM ISO (Fig. 5C; *P > 0.05, paired t-test), suggesting an involvement of presynaptic cAMP signaling.

To further confirm that β-ARs do not modulate the activity of postsynaptic non-NMDA-Rs, we bath applied AP-5 to block...
NMDA-R, along with TTX and BMI, and pressure administered glutamate to induce non-NMDA-R current. As shown in Figure 6, ISO (100 μM) produced no effect on the glutamate-induced current (i.e., non-NMDA-R current): the current was $666.9 \pm 75.6$ pA in control and $624.9 \pm 59.0$ pA during ISO application (Fig. 6A, C; $P > 0.05$, paired t-test). Similar experiments were done in the presence of CTZ that could block desensitization of a-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor that might occlude subtle change in non-NMDA-R current. Under this condition, ISO (100 μM) still did not affect the glutamate-induced current: the current was $595.8 \pm 25.6$ pA in control and $600.9 \pm 24.7$ pA during ISO application (Fig. 6B, C; $P > 0.05$, paired t-test).

Stimulation of β-ARs Enhances NMDA-R-Mediated eEPSC

We then examined whether the ISO enhancement of eEPSC was due to a modulation of NMDA-R component of eEPSC. We recorded NMDA-R-mediated eEPSC by bath applying CNQX (20 μM) to block non-NMDA-Rs. Membrane potential was held at −40 mV to partially relieve the voltage-dependent Mg$^{2+}$ blockade of NMDA-R channel.

As shown in Figure 7, ISO (1, 10, and 100 μM) dose dependently enhanced the amplitude of NMDA-R eEPSC. For 1 μM ISO, the amplitude of NMDA-R eEPSC was $70.1 \pm 3.1$ pA in control and $83.7 \pm 3.6$ pA during ISO application, with a percentage enhancement of 20.0 ± 3.8% (Fig. 7A, left; $P < 0.05$, paired t-test). For 10 μM ISO, the amplitude of NMDA-R eEPSC was $99.3 \pm 15.2$ pA in control and $139.0 \pm 25.2$ pA during ISO application, with a percentage enhancement of 38.6 ± 9.3% (Fig. 7A, middle; $P < 0.05$, paired t-test). For 100 μM ISO, the amplitude of NMDA-R eEPSC was $40.4 \pm 5.2$ pA in control and $61.8 \pm 7.4$ pA during ISO application, with a percentage enhancement of 55.3 ± 7.8% (Fig. 7A, right; $P < 0.05$, paired t-test). An one-way ANOVA revealed significant difference in the facilitation effects between 10 and 1 μM ISO ($F_{1,13} = 5.30; P = 0.03$) but no difference between 100 and 10 μM ISO ($F_{1,13} = 1.88; P = 0.19$).

In addition, we investigated the effect of ISO on the PPF of NMDA-R eEPSC. ISO (100 μM) significantly reduced the PPF of NMDA-R eEPSC. The PPF was $143.3 \pm 2.3\%$ in control and $116.7 \pm 4.6\%$ during ISO application (Fig. 7B; $P < 0.05$, paired t-test), again suggesting that ISO acts at presynaptic β-ARs to increase glutamate release.

In order to determine whether the ISO facilitation of NMDA-R eEPSC involves a postsynaptic mechanism, Rp-cAMPS (100 μM) was added in internal pipette solution to inhibit...
postsynaptic cAMP signaling. Under this condition, the NMDA-R eEPSC was 52.0 ± 7.7 pA in control and 63.5 ± 9.0 pA during ISO application, with a percentage potentiation of 23.5 ± 7.5% (Fig. 7C; \( P < 0.05 \), paired \( t \)-test). However, this enhancement was significantly smaller than that in the absence of Rp-cAMPS (compare the effects of 100 \( \mu \)M ISO in Fig. 7A-C; \( F_{1,14} = 8.32, P = 0.01 \), one-way ANOVA). This result suggests that the ISO-induced enhancement of NMDA-R eEPSC partly involves postsynaptic PKA.

**Figure 6.** Stimulation of \( \beta \)-ARs has no effect on glutamate-induced non-NMDA-R current. (A) Non-NMDA-R current was induced by puff application of glutamate in the presence of the NMDA-R antagonist AP-5 (left); ISO (100 \( \mu \)M) did not enhance the non-NMDA-R current (right). (B) Non-NMDA-R current was induced by puff application of glutamate in the presence of AP-5 and CTZ (left); ISO (100 \( \mu \)M) did not enhance the non-NMDA-R current (right). (C) Histograms showing the amplitudes of the non-NMDA-R currents in control (without ISO) and in the presence of ISO or CTZ + ISO. Holding potential was \(-70\) mV.

As Rp-cAMPS could leak out of a patched cell and influence presynaptic processes in possibly subtle ways, we conducted similar experiments with the PKA inhibitor PKI \(_{5,24}\) to probe postsynaptic PKA involvement. PKI is membrane impermeable and has been successfully used in previous studies on hippocampal neurons to implicate postsynaptic PKA in synaptic plasticity (Duffy and Nguyen 2003). PKI \(_{5,24}\) (500 nM) was added in internal pipette solution to inhibit the activity of postsynaptic PKA. Under this condition, the NMDA-R eEPSC was 56.4 ± 4.8 pA in control and 71.5 ± 6.5 pA during application of ISO (100 \( \mu \)M), with a percentage enhancement of 26.8 ± 3.7% (Fig. 7C; \( P < 0.05 \), paired \( t \)-test). However, the enhancement was significantly smaller than in the absence of PKI \(_{5,24}\) (compare the effects of 100 \( \mu \)M ISO in Fig. 7A,C; \( F_{1,14} = 8.00, P = 0.01 \), one-way ANOVA). This result suggests that the ISO-induced enhancement of NMDA-R eEPSC partly involves postsynaptic PKA.

**Stimulation of \( \beta \)-ARs Enhances NMDA-Induced Inward Current**

To further determine the existence of a postsynaptic mechanism underlying the ISO enhancement of NMDA-R–mediated synaptic transmission, we directly applied NMDA, through pressure pipette or bath perfusion, to induce NMDA-R current. Brain slices were submerged in Mg\(^{2+}\)-free solution containing CNQX (20 \( \mu \)M), TTX (1 \( \mu \)M), and BMI (20 \( \mu \)M). A patched cell under this condition could be completely isolated pharmacologically. Membrane potential was held at \(-40\) mV for pressure application of NMDA (Cepeda et al. 1998) or at \(-60\) mV for bath application of NMDA (Wirkner et al. 2002). The NMDA-induced current could be eliminated by AP-5 (50 \( \mu \)M), indicating that the current was mediated by NMDA-R.

Figure 8 shows the currents induced by pressure-applied NMDA (300 \( \mu \)M) before and during application of ISO (100 \( \mu \)M). The NMDA-induced current was 170.4 ± 24.4 pA in control (without ISO) and 251.5 ± 36.7 pA in the presence of ISO, with a percentage increase of 51.2 ± 15.5% (Fig. 8A,C; \( P < 0.05 \), paired \( t \)-test). The ISO enhancement no longer existed when Rp-cAMPS (100 \( \mu \)M) was added in the internal pipette solution: the NMDA-induced current was 188.7 ± 28.0 pA in control and 226.6 ± 37.4 pA in the presence of Rp-cAMPS (Fig. 8B,C; \( P > 0.05 \), paired \( t \)-test).

Figure 9 shows the currents induced by bath-applied NMDA (30 \( \mu \)M) before and during application of ISO (100 \( \mu \)M). We first performed a pilot experiment in 6 pyramidal cells. In these neurons, NMDA was applied 3 times for 30 s each (T1, T2, and T3), with an interapplication interval of 10 min. There was a dramatic decrease of the current response from T1 (279.1 ± 84.4 pA) to T2 (165.6 ± 42.1 pA; \( P < 0.05 \) vs T1, paired \( t \)-test) and a slight decrease from T2 (165.6 ± 42.1 pA) to T3 (156.6 ± 40.2 pA; \( P > 0.05 \) vs T2, paired \( t \)-test). Therefore, we selected T2 and T3 responses to assess ISO effect (also see Wirkner et al. 2002; Wirkner et al. 2004). T2 response was recorded as control (without ISO) and T3 response in the presence of ISO.

The NMDA-induced current was 145.6 ± 27.0 pA in control and 236.6 ± 49.6 pA in the presence of ISO, with a percentage increase of 65.1 ± 12.1% (Fig. 9A,C; \( P < 0.05 \), paired \( t \)-test). The ISO enhancement was blocked when Rp-cAMPS (100 \( \mu \)M) was administered into the internal pipette solution: the NMDA-induced current was 190.2 ± 42.5 pA in control and 197.2 ± 39.6 pA in the presence of Rp-cAMPS (Fig. 9B,C; \( P > 0.05 \), paired \( t \)-test).

**Discussion**

The present study systematically examined the roles of \( \beta \)-ARs in regulating excitatory synaptic transmission in layer V/VI pyramidal neurons of the mPFC. We found that \( \beta \)-AR activation facilitates excitatory synaptic transmission through both pre- and postsynaptic mechanisms, probably via CAMP or CAMP/PKA signaling.

In the present study, we examined the effects of 3 doses of ISO (1, 10, and 100 \( \mu \)M) on eEPSC. Our results showed that
10 μM ISO induced a significantly stronger enhancement in eEPSC than 1 μM ISO did, whereas 100 μM ISO produced no further enhancement. It was possible that 10 μM ISO was the dose inducing maximal effect, and therefore, 100 μM ISO could not produce a further facilitation. The selection of 10 and 100 μM ISO was based on some previous studies examining the effects of β-AR activation on synaptic transmission in the hippocampus and cerebral cortex. For example, Thomas et al. (1996) and Gelinas and Nguyen (2005) investigated the effects of β-AR activation on LTP of field excitatory postsynaptic potential in hippocampal slices. These studies showed that 10 μM ISO did not alter basal synaptic transmission mediated by AMPA receptors, while enabling LTP induction (Thomas et al. 1996; Gelinas and Nguyen 2005). On the other hand, Hillman et al. (2005) systematically examined the dose-effect relationship of ISO on spiking frequency in CA1 pyramidal cells. They found that 10 and 100 μM ISO produced an equal and maximal increase in spiking frequency of CA1 pyramidal cells (Hillman et al. 2005). Moreover, Sessler et al. (1995) also used 10–100 μM ISO to examine the effect of β-AR stimulation on GABA-induced input resistance in layer V pyramidal neurons of the somatosensory cortex of rats (Sessler et al. 1995).

Previous studies that explored the question on β-AR regulation of cortical glutamatergic transmission have specifically shown that NE or ISO dramatically enhances, via β-ARs, glutamate- and NMDA-induced spiking responses, with no effect on AMPA-induced spiking response in cerebral cortical tissue obtained from children undergoing surgery for intractable epilepsy or in the frontal cortical neurons of adult rats (Radisavljevic et al. 1994). Using intracellular recording from neurons of the barrel-field cortex of rats, Sessler et al. (1995) found that β-AR activation by ISO facilitates GABAergic inhibition in the pyramidal cells and this facilitation involves cAMP signaling (Sessler et al. 1995). However, using iontophoresis coupled with extracellular recordings, NE was shown to enhance glutamate-evoked firing response in rat sensory-motor cortex (area 3) (Mouradian et al. 1991). Interestingly, this enhancement was mediated by α-ARs instead of β-ARs because the β-AR antagonist PRO did not block the facilitation.

Figure 7. Stimulation of β-ARs enhances NMDA-R-mediated eEPSC. (A) NMDA-R eEPSC in the absence (control) and in the presence of 1, 10, and 100 μM ISO. (B) PPF of NMDA-R eEPSC in the absence (control) and in the presence of 100 μM ISO. (C) ISO enhancement of NMDA-R eEPSC was partly blocked by intracellular application of the cAMP inhibitor Rp-cAMPS or the PKA inhibitor PKI 5-24. The ISO potentiation in the presence of Rp-cAMPS or PKI 5-24 was significantly smaller than in the absence of Rp-cAMPS (compare the effects of 100 μM ISO in C and A). Holding potential was −40 mV. *P < 0.05 versus control.
It should be pointed out that, due to the limitation of the extracellular recording technique used in that study, it was difficult to identify whether the recorded units were pyramidal cells or interneurons. Moreover, the iontophoretically applied drugs could spread locally to act on various cell types and local circuits and produce both direct and indirect influences on the recorded units.

In the present study, we aimed to examine the electrophysiological regulation by \( \beta \)-ARs of glutamate synaptic transmission in layer V/VI pyramidal cells in a brain slice preparation, using whole-cell patch-clamp recording. We found that \( \beta \)-ARs are involved in regulating glutamate release from presynaptic terminals. Bath application of ISO resulted in a significant increase in sEPSC frequency and amplitude. As spontaneous action potentials were not blocked when sEPSC's were recorded, it was difficult to know whether a presynaptic or postsynaptic mechanism, or both, underlay the ISO facilitation of sEPSC's. We therefore investigated ISO effect on mEPSC's in the presence of TTX, which blocks voltage-activated sodium channel and eliminates spontaneous action potential invasion of presynaptic terminals. Under this condition, ISO significantly enhanced mEPSC frequency, with no effect on mEPSC amplitude, suggesting that ISO may act at presynaptic \( \beta \)-ARs and facilitate spontaneous release of glutamate. Although there is no direct anatomical evidence showing that \( \beta \)-ARs exist in presynaptic terminals of glutamate synapses in the mPFC, a previous study by Nicholas et al. 1993 shows that \( \beta \)-AR mRNA is present in the intralaminar thalamic nuclei and hippocampus (Nicholas et al. 1993). Because neurons from both these sites project to the mPFC, it is possible that \( \beta \)-ARs synthesized in cellular bodies of these projection neurons are transported to the presynaptic terminals in the mPFC.

The facilitation effects of ISO on sEPSC's and mEPSC's could not be washed out within 20 min after stopping of ISO application. There might be 2 reasons. First, ISO activated long-lasting intracellular signaling cascade responses. Second, the 20-min washout time was not long enough. Because it was difficult to keep whole-cell recording in a cell for a long period

![Figure 8](http://cercor.oxfordjournals.org/)  
![Figure 9](http://cercor.oxfordjournals.org/)
of time, we could not wait long enough for a complete washout.

The ISO-induced increase in mEPSC frequency may involve voltage-dependent calcium channels in presynaptic terminals because this facilitation was blocked in the presence of cadmium. Activation of presynaptic β-ARs might induce a phosphorylation of these calcium channels and enhance spontaneous quantal release of glutamate from presynaptic terminals. Huang et al. (1996) and Huang, Wang, et al. (1998) reported that ISO induces a long-term enhancement of excitatory glutamate transmission in the amygdala through a presynaptic mechanism that involves P- and/or Q-type calcium channels.

To further determine the existence of a presynaptic action of ISO, we pharmacologically isolated non-NMDA-R- and NMDA-R-mediated eEPSC’s and investigated the effects of ISO on PPF of these eEPSC’s. PPF of synaptic transmission is thought to be a presynaptically mediated phenomenon (Zucker and Regehr 2002). An alteration in PPF by a drug reflects a presynaptic site of drug action (Hajos et al. 2001). Our result showed that ISO inhibited the PPF of both non-NMDA-R- and NMDA-R-mediated eEPSC’s. It was hypothesized that a decrease in PPF indicates an increase in presynaptic glutamate release (Thomson 2000). Thus, these results further suggest a presynaptic action of ISO in regulating evoked release of glutamate and are consistent with previous studies showing that β-AR activation facilitates glutamate synaptic transmission in the cerebral cortex (Herrero and Sanchez-Prieto 1996), hippocampus, (Gereau and Conn 1994), and amygdala (Huang et al. 1996) through a presynaptic mechanism.

The present results showed that ISO enhancement of non-NMDA-R-mediated eEPSC no longer appeared when Rp-cAMPS was bath applied, which is membrane permeable and can inhibit presynaptic cAMP signaling. It has been reported that PKA can phosphorylate N- and L-type voltage-gated calcium channels in rat hippocampal neurons (Hell et al. 1995), enhance L-type currents in rat neostriatal neurons (Surmeier et al. 1995), and increase glutamate release in cerebrocortical nerve terminals (Millán et al. 2003). Thus, it was likely that β-AR activation regulates presynaptic glutamate release through cAMP/PKA pathway. Nevertheless, because we did not inhibit presynaptic PKA per se, we cannot exclude the possibility that other presynaptic targets downstream of cAMP could also contribute to β-AR modulation of glutamate release. Taken together, our finding of a presynaptic β-AR regulation of glutamate synaptic transmission in the mPFC is consistent with that reported in previous studies in other brain regions (Hell et al. 1995; Surmeier et al. 1995; Huang et al. 1996; Huang, Wang, et al. 1998; Millán et al. 2003).

It has been shown that regulation of presynaptic glutamate release involves different mechanisms in different brain regions. For example, Schoppa and Westbrook (1997) reported that presynaptic mGluR1 (group I metabotropic glutamate receptors) enhances glutamate release and increases mEPSC frequency in cultural olfactory bulb mitral cells, whereas mGluR2 (group II metabotropic glutamate receptors) and mGluR6 and 7 (group III metabotropic glutamate receptors) have an opposite effect (Schoppa and Westbrook 1997). Contractor et al. (2000) reported that presynaptic GluR5 containing kainite receptors inhibit glutamate release, reduce mEPSC frequency, and suppress eEPSC in pyramidal neurons of the CA3 region (Contractor et al. 2000). We do not know whether there exist similar mechanisms in the mPFC. However, the present study provides evidence for the existence of a presynaptic β-AR regulation of glutamate release in the mPFC.

β-ARs do not seem to regulate the activity of postsynaptic non-NMDA-Rs in the mPFC because ISO produced no effect on mEPSC amplitude. This is further confirmed by the fact that ISO did not enhance glutamate-induced current in the presence of AP-5 (i.e., non-NMDA-R current). These results are consistent with the previous study by Huang, Lin, et al. (1998) who show that ISO does not affect AMPA-induced current in acutely dissociated amygdalar neurons. Thus, the ISO enhancements of eEPSC amplitude and non-NMDA-R eEPSC were probably due to an increase in glutamate release from presynaptic terminals.

To address whether β-AR activation directly regulates the activity of postsynaptic NMDA-R in the mPFC, we used TTX, CNQX, and BMI to pharmacologically isolate an inward NMDA-R current that was induced by pressure- and bath-applied NMDA. Our data showed that ISO significantly enhanced NMDA-induced current, indicating a direct modulation of postsynaptic NMDA-R activity by β-AR activation. The ISO facilitation of NMDA-induced current was eliminated by intracellular application of the cAMP inhibitor Rp-cAMPS, indicating the involvement of cAMP signaling. This is consistent with the finding by Huang, Lin, et al. (1998) who show that β-AR activation enhances NMDA-induced currents in amygdalar neurons.

As NMDA-Rs are distributed in both synaptic and extrasynaptic membranes (Hardingham and Bading 2003), it was difficult to identify action sites of pressure- or bath-applied NMDA. Therefore, we observed the effect of ISO on NMDA-R-mediated current induced by synaptic stimulation. As expected, ISO significantly enhanced the synthaptically activated NMDA-R eEPSC. This enhancement was partly attenuated when the recording pipette contained Rp-cAMPS or the PKA inhibitor PKI$_{5-24}$, indicating the involvement of postsynaptic cAMP/PKA signaling, which may mediate PKA-dependent NMDA-R phosphorylation and thereby enhance NMDA-R synaptic current. This result is consistent with previous studies showing that β-AR activation facilitates excitatory synaptic transmission in the amygdala (Huang et al. 1993; Huang et al. 1996; Huang, Lin, et al. 1998) and the hippocampus (Raman et al. 1996) through increasing the phosphorylation of postsynaptic NMDA-R and, as a result, enhancing the NMDA-R-mediated current. It is known that PKA can directly phosphorylate NMDA-R NR1 subunit (Snyder et al. 1998; Bird et al. 2005). Thus, it is possible that β-ARs regulate NMDA-R activity through cAMP/PKA/NR1 signaling pathway.

Although a great many studies have focused on the role of prefrontal cortical α$_2$-ARs, especially the α$_{2A}$-AR, in cognitive functions such as working memory, attention regulation, and response inhibition (Li and Mei 1994; Arnsten et al. 1996; Arnsten 1997; Arnsten et al. 1998; Li and Kubota 1998; Li et al. 1999; Gibbs and Summers 2002; Ma et al. 2003; Wang et al., 2007), the functional significance of β-ARs in the PFC are poorly understood. β-AR stimulation triggers cAMP production and activates PKA. Ramos et al. (2003) reported that the cAMP/PKA signaling becomes hyperactivated in the PFC with advancing age. Inhibition of cAMP/PKA signaling in the PFC of aged rats and monkeys improves working memory (Taylor et al. 1999; Ramos et al. 2003; Arnsten et al. 2005; Ramos et al. 2005). The present study provides electrophysiological evidence that stimulation of β-ARs enhances excitatory synaptic transmission in the mPFC. However, our data help to understand the
mechanisms underlying the β-AR regulation of PFC cognitive functions. It is possible that a normal expression of PFC functions requires an optimal level of β-AR/cAMP signaling. A moderate level of β-AR/cAMP signaling may exert, most likely via PKA, a beneficial influence on PFC cognitive functions, whereas hypo- or hyperactivation of this pathway may cause cognitive deficits.

PRO, a lipophilic β-AR blocker that can cross the blood-brain barrier and enter the CNS, has been used clinically to treat hypertension (Cruickshank et al. 1980). Given the findings in the present study, PFC excitatory synaptic transmission is likely to be reduced acutely following treatment with PRO and patients could show cognitive impairments. However, this seems not to be the case as clinical treatment of hypertension with PRO produces limited adverse effects on cognitive and psychological functions in patients (Perez-Stable et al. 2000).

In conclusion, the present study provides the first electrophysiological demonstration that β-ARs facilitate excitatory synaptic transmission in layer V/VI pyramidal cells in the mPFC by increasing the glutamate release from presynaptic terminals, probably via cAMP signaling, or by enhancing the responsiveness of postsynaptic NMDA-Rs, likely via cAMP/PKA signaling.

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Notes
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