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Stimulation of α_2-Adrenoceptors Suppresses Excitatory Synaptic Transmission in the Medial Prefrontal Cortex of Rat

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Stimulation of α_2-, especially α_{2A}-adrenoceptor (AR), in the prefrontal cortex (PFC) produces a beneficial effect on cognitive functions such as working memory. α_2-Adrenergic agonists like clonidine and guanfacine have been used experimentally and clinically for treatment of psychiatric disorders such as attention-deficit/hyperactivity disorder (ADHD) and schizophrenia. However, the neurophysiological actions of α_2-ARs in the PFC are poorly understood. In the present study, we recorded field excitatory post-synaptic potential (fEPSP) and evoked excitatory post-synaptic current (eEPSC) in the medial prefrontal cortex (mPFC) of rats, using *in vivo* field-potential recording and *in vitro* whole-cell patch-clamp recording techniques, and examined the effects of the α_2-AR agonist clonidine and the selective α_{2A}-AR agonist guanfacine on fEPSP and eEPSC. Systemic or intra-mPFC application of clonidine or guanfacine significantly reduced fEPSP in the mPFC, either in anesthetized or freely moving rats. Consistently, bath-application of guanfacine suppressed eEPSC in layer V/VI pyramidal neurons, and this effect was blocked by the α_2-AR antagonist yohimbine or the G_i inhibitor NF023. Moreover, treatment with guanfacine had no effect on paired-pulse facilitation (PPF) of fEPSP and eEPSC. The present study provides the first electrophysiological evidence that stimulation of α_{2A}-AR inhibits excitatory synaptic transmission in the mPFC through a post-synaptic mechanism.
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INTRODUCTION

The prefrontal cortex (PFC) is essential for cognitive functions such as working memory (Baddeley, 2003; Bodner *et al*, 2005; Funahashi *et al*, 1989, 1993; Fuster, 2003; Goldman-Rakic, 1995; Miller and Cohen, 2001), attention regulation (Dias *et al*, 1996; Luria, 1966) and response inhibition (Aron *et al*, 2004; Luria, 1966). Animals or humans with lesions to the PFC exhibit disorganized behaviors like poor working memory, distractibility, impulsivity and hyperactivity (Fuster, 1997; Luria, 1966).

It is well documented that α_2-, especially α_{2A}-adrenoceptors (ARs) in the PFC are involved in regulating working memory function (Arnsten *et al*, 1988; Arnsten and Goldman-Rakic, 1985; Arnsten and Li, 2005; Franowicz and Arnsten, 1998; Franowicz *et al*, 2002; Li and Mei, 1994; Mao *et al*, 1999). α_2-AR agonists such as clonidine and guanfacine administered systemically or locally into the PFC improve working memory performance in rats and monkeys (Arnsten *et al*, 1988; Arnsten and Goldman-Rakic, 1985; Franowicz and Arnsten, 1998; Mao *et al*, 1999; Tanila *et al*, 1996). It has been shown that the beneficial effect of clonidine or guanfacine on working memory performance is mediated by α_{2A}-ARs (Arnsten *et al*, 1988; Arnsten and Li, 2005; Franowicz *et al*, 2002). In addition to working memory performance, α_{2A}-AR stimulation in the PFC also enhances visuomotor association learning in monkeys (Wang *et al*, 2004a, b).

It has been reported that clonidine and guanfacine are effective for treatment of human psychiatric disorders that involve PFC dysfunctions, such as attention–deficit/hyperactivity disorder (ADHD) (Arnsten *et al*, 1996; Arnsten and Li, 2005; Chappell *et al*, 1995; Hunt *et al*, 1995; Scahill *et al*, 2001; Taylor and Russo, 2001), schizophrenia (Friedman *et al*, 2001, 2004) and post-traumatic stress disorder (Horrigan, 1996; Porter and Bell, 1999). Atomoxetine is a reuptake inhibitor of norepinephrine (NE) and has also been used for treatment of ADHD (Gilbert *et al*, 2007; Perwien *et al*, 2006). Although both involving a noradrenergic mechanism, atomoxetine increases NE concentration in synaptic cleft, whereas clonidine and guanfacine directly stimulate post-synaptic α_{2A}- receptor.

The effects of α_2-AR stimulation or blockade on working memory have been investigated at both behavioral and cellular levels. For example, previous studies show that stimulation of α_2-ARs enhances PFC neuronal activity that is related to working memory (Li *et al*, 1999; Wang *et al*,

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2007), while blockade of α_2-ARs inhibits the working memory-related PFC neuronal activity (Li *et al*, 1999; Sawaguchi and Yamane, 1999; Wang *et al*, 2007). However, it is poorly understood how α_2-ARs regulate synaptic transmission in the PFC. In the present study, we investigated the effects of clonidine and guanfacine on excitatory synaptic transmission in the medial PFC of rats, using *in vivo* field-potential recording and *in vitro* whole-cell patch-clamp recording. Paired-pulse facilitation (PPF) was recorded and used as a measure to analyze whether a pre- or post-synaptic mechanism is involved in α_2-AR regulation. We attempted to know how α_{2A}-AR stimulation, either by systemic or local administration of clonidine and guanfacine, regulates PFC excitatory synaptic transmission.

MATERIALS AND METHODS

Drugs

Clonidine hydrochloride, yohimbine hydrochloride, bicuculline methiodide (BMI), 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), DL-2-amino-5-phosphonovaleric acid (AP-5), K^+gluconate, ATP.Mg, GTP.Na^{+3}, and HEPES were purchased from Sigma Chemical Company Ltd. (St Louis, MO, USA). NF023 was purchased from Tocris Cookson Ltd. (Ellisville, Missouri, USA) and guanfacine hydrochloride from Wyeth-Ayerst Company Ltd. (Princeton, NJ, USA). Other reagents in AR grade were purchased from the Shanghai Chemical Plant (Shanghai, China). Drugs were dissolved in ultra-pure water produced by an untrapure system (Millipore Q-Gard 1, USA) or sterile saline.

Animals

Spraque—Dawley (SD) rats (200–250 g) were used for *in vivo* field-potential recording and SD rats of 14–24 days for *in vitro* whole-cell patch-clamp recording. The animals 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 NIH *Guide for the Care and Use of Laboratory Animals* (1996). This study was approved and monitored by the Ethical Committee of Animal Experiments, Fudan University Institute of Neurobiology. All efforts were made to minimize the number of animals used and their suffering.

Implantation of Electrode

Rats were anesthetized with urethane (1250 mg/kg, i.p.; for field-potential recordings under anesthesia) or pentobarbital sodium (40 mg/kg, i.p; for implantation of stimulating and recording electrodes in the medial prefrontal cortex (mPFC)). The animals were fixed in a stereotaxic apparatus (Narishige SN-2, Japan). A stainless-steel guide cannula, with a teflon-coated stainless steel wire glued to it, was inserted to the mPFC (AP, 3.2 mm; ML, 0.6 mm). The guide cannula was used for drug infusion and the stainless-steel wire for field-potential recording. The tip of the stainless-steel wire was 1.0 mm beyond that of the guide cannula.

A concentric electrode was used as a stimulating electrode, which was inserted to the boundary area of the gray and white matters in the mPFC (AP, 2.7 mm; ML, 2.8 mm). A test pulse (50 μs in duration, 300–350 μA in intensity) was delivered, once every 30 s, through the stimulating electrode. The tip positions of the stimulating and recording electrodes were adjusted finely and slowly in order to obtain an optimal field excitatory post-synaptic potential (fEPSP).

Recordings of fEPSP in Anesthetized Rats

Recording of fEPSP under anesthesia began soon after the surgery had been completed. A current intensity that elicited a fEPSP with ~65% of maximal response was used as a stimulating current. fEPSP was evoked, once every 1 min, by delivering a single current pulse (50 μs in duration). After a baseline recording of fEPSP for 30 min, clonidine, guanfacine or saline was given systemically or infused into the mPFC through an injection needle. The injection needle was inserted into the guide cannula, with its tip 0.8 mm beyond that of the guide cannula. Infusion was 1 μl in volume and was completed with 3–5 min. The injection needle was left in place throughout a recording experiment. fEPSP was recorded for 120 min after drug infusion.

PPF of fEPSP was also recorded in the absence and presence of clonidine or guanfacine. A pair of current pulses (50 μs in pulse duration and 50 ms in inter-pulse interval) was delivered, once every 10 min. PPF was calculated as the ratio of the slope of second pulse-induced fEPSP to that of the first pulse-induced fEPSP.

Recordings of fEPSP in Freely Moving Rats

For recordings of fEPSP in freely moving rats, the stimulating and recording electrodes were implanted into the mPFC and fixed to the skull with dental cement. The animals were allowed 7 days to recover. A current intensity evoking a fEPSP with ~65% of maximal response was used as a stimulating current. fEPSP was induced, once every 1 min, by delivering a single current pulse (50 μs in duration). After a 30-min baseline recording, clonidine, guanfacine or saline was administered, either systemically or locally. For local infusion, the animals were handled gently and an injection needle was inserted in the guide cannula. The tip of the infusion needle was placed 0.8 mm beyond that of the guide cannula. Infusion was 1 μl in volume and was completed with 3–5 min. The infusion needle was left in place throughout a recording experiment. fEPSP was recorded for 120 min after drug infusion.

Whole-Cell Recordings of eEPSC in Slices

Rats were anesthetized with sodium pentobarbital (40 mg/kg, i.p.) before decapitation. The brains were quickly removed, submerged in ice-cold artificial cerebrospinal fluid (ACSF) containing (in mM): 119 NaCl, 2.5 KCl, 1 $CaCl_2$, 3 $MgSO_4$, 1 NaH_2PO_4, 26.2 $NaHCO_3$, 11 glucose, and saturated with 95% O_2–5% CO_2. Coronal slices (400 μm in thickness), which contained the mPFC, were cut on a Vibroslice (MA752, Campden Instruments, USA). The anatomic locations of these slices were assessed with reference to Paxinos and Watson (Paxinos and Watson, 1986). Brain slices were transferred and incubated in ACSF for at least 1 h at room temperature, while constantly oxygenated with 95% O_2–5% CO_2. After incubation, a single

slice was transferred to a submerged recording chamber. The perfusion ACSF, which was continuously oxygenated, was delivered with a pump (Peri-Star 291, World Precision Instruments, USA) at a rate of 2–3 ml per min. In the perfusion ACSF, the concentration of $CaCl_2$ was increased to 2.5 mM and $MgCl_2$ decreased to 1.5 mM. All experiments were performed at room temperature.

A slice was viewed with an upright microscope (Axioskopz Fsmot, Zeiss, Germany) equipped with infrared-differential interference contrast (IR-DIC) optics. The image was detected with an IR-sensitive CCD (C2400-79H, Hamamatsu, Japan) and displayed on a black-white TV monitor. Whole-cell patch-clamp recordings were made from layer V/VI pyramidal cells in the mPFC. Patch pipettes (3–7 MΩ) were fabricated from borosilicate tubing (1.5 mm OD, 0.86 mm ID, Sutter Instruments, USA) on a horizontal microelectrode puller (P-97, Sutter Instruments, USA). The internal pipette solution contained (in mM): 150 K^+gluconate, 0.4 EGTA, 8 NaCl, 2 ATP.Mg, 0.1 GTP.Na^{+3}, and 10 HEPES, with a pH adjusted to 7.2–7.4 and an osmolarity to 290–320 mOsm. The spiking pattern with frequency adaptation in response to a depolarization current was used as a measure for electrophysiological identification of pyramidal neurons. For recordings of evoked excitatory post-synaptic current (eEPSC), a bipolar stimulation electrode was positioned 150–200 μm subjacent to a recorded neuron. A current pulse with 50–120 μA in intensity and 100 μs in duration was used as a stimulating current. The current pulse was delivered at 0.033 Hz. eEPSC was recorded in the presence of BMI (20 μM) in the perfusion ACSF, which blocked GABAergic transmission in a recorded neuron. Guanfacine and/or yohimbine were bath administered. PPF of eEPSC was also recorded in the absence and presence of guanfacine. To do so, a pair of current pulses with an inter-pulse interval of 50 ms was delivered, once every 30 s.

Signals were recorded with an HEKA EPC9 amplifier (Heka, Germany) connected to a Digidata interface. The data were digitized and stored on disks using Pulse software (Heka). The series resistance (Rs) was monitored at regular intervals throughout a recording. Data were discarded if Rs changed by 15%. In some neurons recorded, Lucifer yellow (0.05%) was filled into patch pipettes and the morphology of the neurons was post-examined using a confocal laser scanning microscope (SP2, Leica, Germany).

Data Analysis

All data are expressed as mean ± SEM in the text and figures. Data were statistically compared using paired *t*-test (for within-group comparison, between different time points) or unpaired *t*-test (for between-group comparison, at same time points). Asterisks in the figures indicate a significance level ($P<0.05$) and *n* refers to the number of rats or neurons. A trace of synaptic response shown in the figures represents the average of 10 consecutive recordings.

RESULTS

For systemic administration, three doses of clonidine (0.1, 1.0, and 2.5 mg/kg) or guanfacine (0.01, 0.1, and 1.0 mg/kg) were used. These dose ranges of clonidine or guanfacine could improve PFC function such as working memory (Arnsten and Goldman-Rakic, 1985; Arnsten *et al*, 1988; Franowicz and Arnsten, 1998, 1999). For local administration, we infused 2.67 μg clonidine (in 1 μl saline; 5 μM) or 1.3 μg guanfacine (in 1 μl saline; 5 μM) into the medial PFC.

Stimulation of α_2-ARs Reduces fEPSP in the mPFC of Anesthetized Rats

Figure 1 shows the effects of intramuscularly administered clonidine and guanfacine on fEPSP in the mPFC of anesthetized rats. While the slope of fEPSP was not affected by saline, it was significantly reduced following administration of clonidine or guanfacine. For example, the slope of the fEPSP at 90 min post-administration of clonidine was 88.6 ± 8.9% of baseline at the 0.1 mg/kg dose, 72.6 ± 4.1% at the 1.0 mg/kg dose, and 58.0 ± 8.1% at the 2.5 mg/kg dose (Figure 1a). Similar phenomena were seen following administration with guanfacine. The slope of the fEPSP at 90 min post-administration of guanfacine was 87.5 ± 6.6% of baseline at the 0.01 mg/kg dose, 79.1 ± 5.7% at the 0.1 mg/kg dose, and 87.6 ± 10.1% at the 1.0 mg/kg dose (Figure 1b).

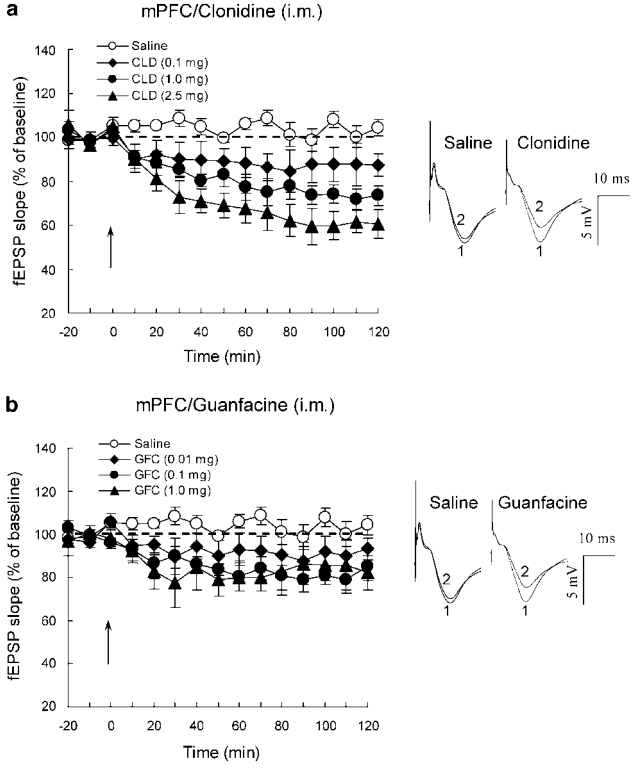

Figure 1 Systemic administration of the α_2-AR agonists clonidine and guanfacine suppresses fEPSP in the mPFC of anesthetized rats. (a) Clonidine inhibited fEPSP. The upward arrow indicates the injection time of clonidine (CLD). Saline, $n=6$ rats; CLD (0.1 mg/kg), $n=5$ rats; CLD (1.0 mg/kg), $n=6$ rats; CLD (2.5 mg/kg), $n=5$ rats. (b) Guanfacine inhibited fEPSP. The upward arrow indicates the injection time of guanfacine (GFC). Saline, $n=6$ rats; GFC (0.01 mg/kg), $n=5$ rats; GFC (0.1 mg/kg), $n=5$ rats; GFC (1.0 mg/kg), $n=5$ rats. fEPSP, field excitatory post-synaptic potential; i.m., intramuscular injection; mPFC, medial prefrontal cortex. Shown in the insets are representative fEPSPs recorded 10 min before (1) and 90 min after (2) the injection.

As systemically administered clonidine and guanfacine could act at the whole central nervous system (CNS), we then investigated the effects of locally administered clonidine and guanfacine on fEPSP in the mPFC of anesthetized rats. As shown in Figure 2, intra-mPFC clonidine or guanfacine significantly reduced fEPSP, consistent with the results from systemic administration. The slope of the fEPSP at 90 min post-infusion of clonidine (2.67 μg/1 μl) was 66.3 ± 9.0% of baseline, and it was 61.5 ± 9.5% of baseline at 90 min post-infusion of guanfacine (1.3 μg/1 μl). Thus, stimulation of α_2-ARs inhibits excitatory synaptic transmission in the mPFC in anesthetized rats.

The clonidine- or guanfacine-inhibition of fEPSP may involve pre- and/or post-synaptic mechanisms. Thus, we tested the effect of intra-mPFC guanfacine on the PPF of fEPSP. PPF of synaptic transmission is thought to be a pre-synaptically mediated phenomenon (Zucker and Regehr, 2002). A change in PPF by a drug reflects a pre-synaptic site of drug action (Hajos *et al*, 2001). As shown in Figure 2c, although guanfacine significantly suppressed the fEPSFs induced by the two-paired pulses, the ratio of the second fEPSP to the first fEPSP (ie the PPF of fEPSP) was not changed, suggesting that the guanfacine inhibition of fEPSP did not involve a pre-synaptic mechanism. In other words, the guanfacine inhibition of fEPSP was not due to a decrease in glutamate release from pre-synaptic terminals.

Stimulation of α_2-ARs Reduces fEPSP in the mPFC of Freely Moving Rats

The effect of α_2-AR stimulation on fEPSP of the mPFC was examined in freely moving rats. As shown in Figure 3a, intramuscularly administered clonidine significantly decreased the slope of fEPSP. The slope of fEPSP at 90 min after injection of clonidine was 81.2 ± 10.1% of baseline at the 0.1 mg/kg dose, 64.2 ± 6.0% at the 1.0 mg/kg dose, and 53.4 ± 8.3% at the 2.5 mg/kg dose. Similar effect was observed following intramuscular administration of guanfacine. As shown in Figure 3b, guanfacine significantly suppressed fEPSP: the slope of fEPSP at 90 min after injection of guanfacine was 84.3 ± 6.2% of baseline at the 0.01 mg/kg dose, 79.7 ± 9.0% at the 0.1 mg/kg dose, and 71.7 ± 4.9% at the 1.0 mg/kg dose.

We also investigated the effects of locally administered clonidine and guanfacine on the fEPSP of mPFC in freely moving rats. As shown in Figure 4, intra-mPFC clonidine (2.67 μg/1 μl) or guanfacine (1.3 μg/1 μl) significantly inhibited the fEPSP. The slope of fEPSP at 90 min post-administration was 63.8 ± 6.2% of baseline for clonidine (Figure 4a) and 58.7 ± 10.6% for guanfacine. Thus, stimulation of α_2-ARs suppresses excitatory synaptic transmission in the mPFC of freely moving rats.

Stimulation of α_{2A}-AR Suppresses Evoked-EPSC of mPFC in Slices

As shown above, *in vivo* field-potential recordings indicate that stimulation of α_2-ARs or α_{2A}-AR suppresses excitatory synaptic transmission in the mPFC, most likely via a post-synaptic mechanism. However, because of the technical limitation of the field-potential recording, it was not clear

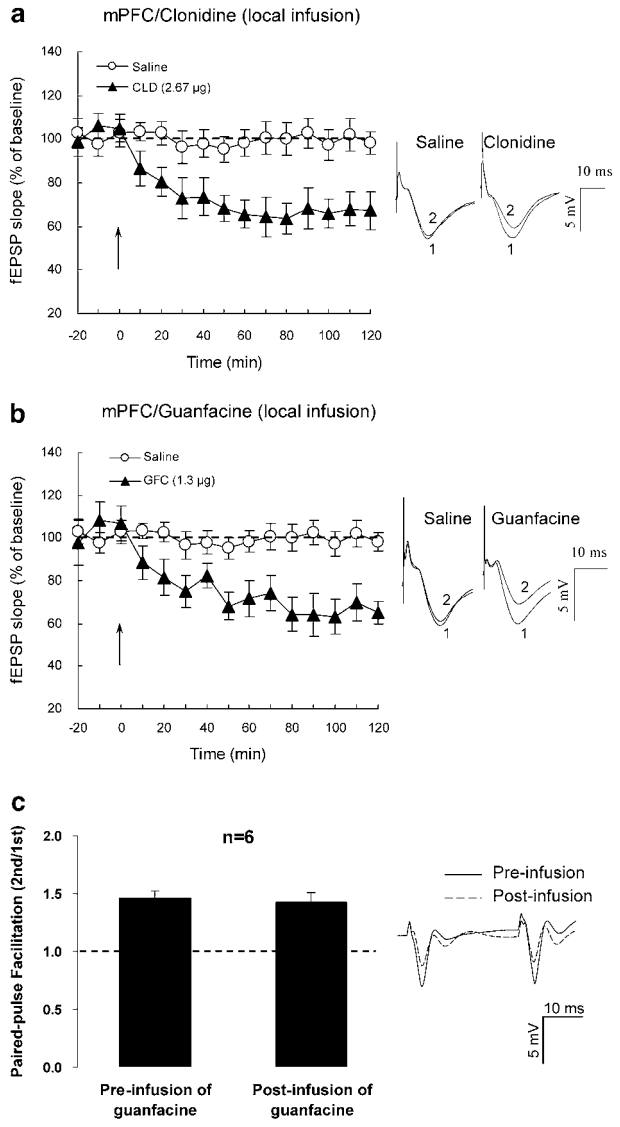

Figure 2 Local administration of the α_2-AR agonists clonidine and guanfacine suppresses fEPSP in the mPFC of anesthetized rats. (a) Clonidine with the 2.67 μg dose (in 1 μl saline) inhibited fEPSP. The upward arrow indicates the injection time of clonidine (CLD). Saline, $n = 5$ rats; CLD (2.67 μg), $n = 5$ rats. (b) Guanfacine with the 1.3 μg dose (in 1 μl saline) inhibited fEPSP. The upward arrow indicates the injection time of guanfacine (GFC). Saline, $n = 5$ rats; GFC (1.3 μg), $n = 4$ rats. (c) Guanfacine had no effect on the paired-pulse facilitation of fEPSP. fEPSP, field excitatory post-synaptic potential; mPFC, medial prefrontal cortex. Shown in the left panels are representative fEPSPs recorded 10 min before (1) and 90 min after (2) the injection.

whether the inhibitory effect of α_2-AR stimulation came from direct action of clonidine and guanfacine at α_2-ARs at pyramidal neurons, or from indirect action of the drugs at α_2-ARs at inhibitory neurons. Thus, we performed whole-cell patch-clamp recordings *in vitro* to address this question.

Whole-cell recordings were conducted in layer V/VI pyramidal cells in mPFC. The pyramidal neurons were identified using IR-DIC by their pyramidal-shaped cell bodies and the presence of long apical dendrite extending toward superficial layers. The pyramidal neurons examined in the present study had a resting membrane potential

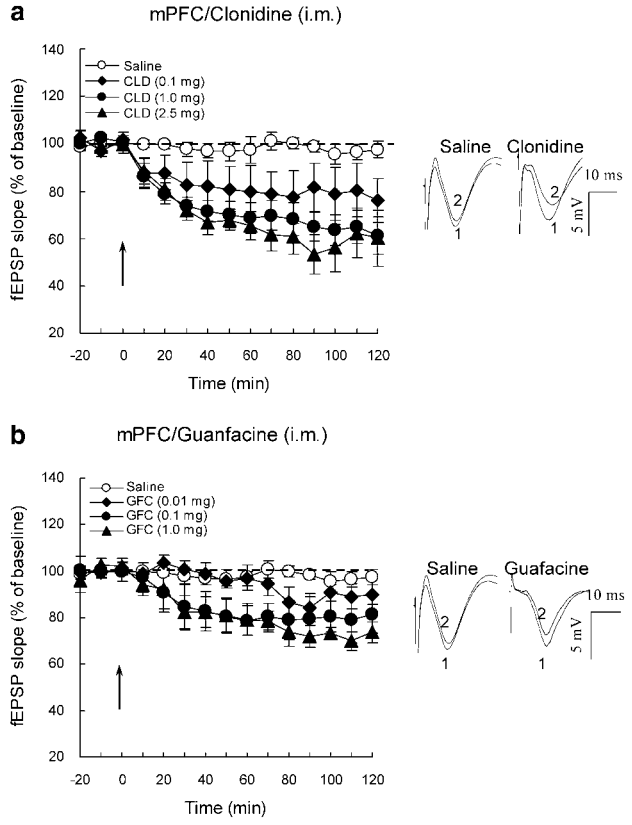

Figure 3 Systemic administration of the α_2-AR agonists clonidine and guanfacine suppresses fEPSP in the mPFC of freely moving rats. (a) Clonidine inhibited fEPSP. The upward arrow indicates the injection time of clonidine (CLD). Saline, $n=7$ rats; CLD (0.1 mg/kg), $n=6$ rats; CLD (1.0 mg/kg), $n=10$ rats; CLD (2.5 mg/kg), $n=5$ rats. (b) Guanfacine inhibited fEPSP. The upward arrow indicates the injection time of guanfacine (GFC). Saline, $n=7$ rats; GFC (0.01 mg/kg), $n=5$ rats; GFC (0.1 mg/kg), $n=6$ rats; GFC (1.0 mg/kg), $n=6$ rats. fEPSP, field excitatory post-synaptic potential; i.m., intramuscular injection; mPFC, medial prefrontal cortex. Shown in the left panels are representative fEPSPs recorded 10 min before (1) and 90 min after (2) the injection.

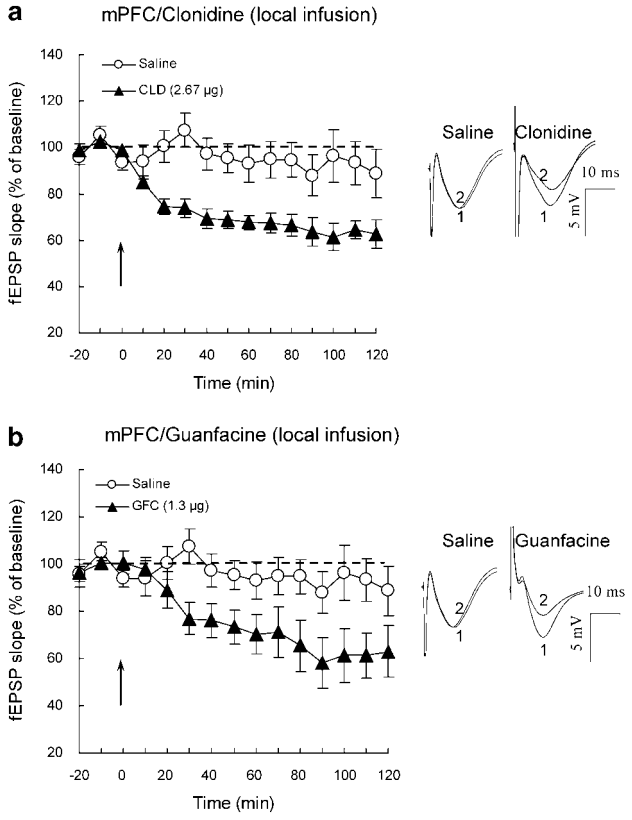

Figure 4 Local administration of the α_2-AR agonists clonidine and guanfacine suppresses fEPSP in the mPFC of freely moving rats. (a) Clonidine with the 2.67 μg dose (in 1 μl saline) significantly inhibited fEPSP. The upward arrow indicates the injection time of clonidine (CLD). Saline, $n=5$ rats; CLD (2.67 μg), $n=6$ rats. (b) Guanfacine with the 1.3 μg dose (in 1 μl saline) significantly inhibited fEPSP. The upward arrow indicates the injection time of guanfacine (GFC). Saline, $n=5$ rats; GFC (1.3 μg), $n=6$ rats. fEPSP, field excitatory post-synaptic potential; mPFC, medial prefrontal cortex. Shown in the left panels are representative fEPSPs recorded 10 min before (1) and 90 min after (2) the injection.

negative than −55 mV, an action potential amplitude larger than 80 mV, with no spontaneous discharge. These neurons exhibited a spike frequency adaptation in response to depolarizing inward current injection ('regular spiking' neurons; Yang *et al*, 1996). Evoked excitatory post-synaptic current (eEPSC) were recorded in 25 pyramidal cells, under voltage-clamp at a holding potential of −70 mV. The eEPSC could be completely inhibited by co-application of the NMDA-receptor antagonist AP-5 (50 μM) and the non-NMDA-receptor antagonist CNQX (20 μM), indicating that it was mediated by glutamate receptors.

BMI (20 μM), which blocks feed-forward or feedback GABAergic transmission in a recorded pyramidal cell, was bath-applied when eEPSC was recorded. As ionotropic glutamate receptors and/or adrenoceptors (including α-ARs) are present not only in pyramidal cells, but also GABAergic interneurons, it is thus necessary to block any possible and indirect influence from GABAergic interneurons when glutamate receptors and adrenoceptors in a patched cell was studied. Hence, in the continuous presence of BMI, the change of eEPSC induced by α-AR stimulation occurs in the recorded cell, but not via GABAergic interneurons.

As shown in Figure 5a, bath application of guanfacine (20 μM) significantly inhibited eEPSC: the amplitude of eEPSC was 99.2 ± 6.1 pA before guanfacine application, and it was 71.7 ± 4.3 pA during guanfacine application, with a percentage reduction of 27.5 ± 3.5%. This inhibition was blocked when the non-selective α_2-AR antagonist yohimbine (20 μM) was co-administered.

Figure 5b shows the PPF of eEPSC in the absence (control) and presence of guanfacine. As shown, guanfacine suppressed eEPSC induced by the two-paired pulses, but the ratio of the second eEPSC to the first eEPSC (ie the PPF of eEPSC) was not altered: it was 122.4 ± 8.1% in control and 133.1 ± 12.2% during guanfacine application, again suggesting that the guanfacine-inhibition of excitatory synaptic transmission was not due to a decrease in glutamate release from pre-synaptic terminals, but involved a post-synaptic mechanism in the recorded cells.

It is known that α_{2A}-AR is a G_i-coupled metabotropic receptor. We added the G_i inhibitor NF023 (10 μM) into the internal pipette solution in order to block the intracellular

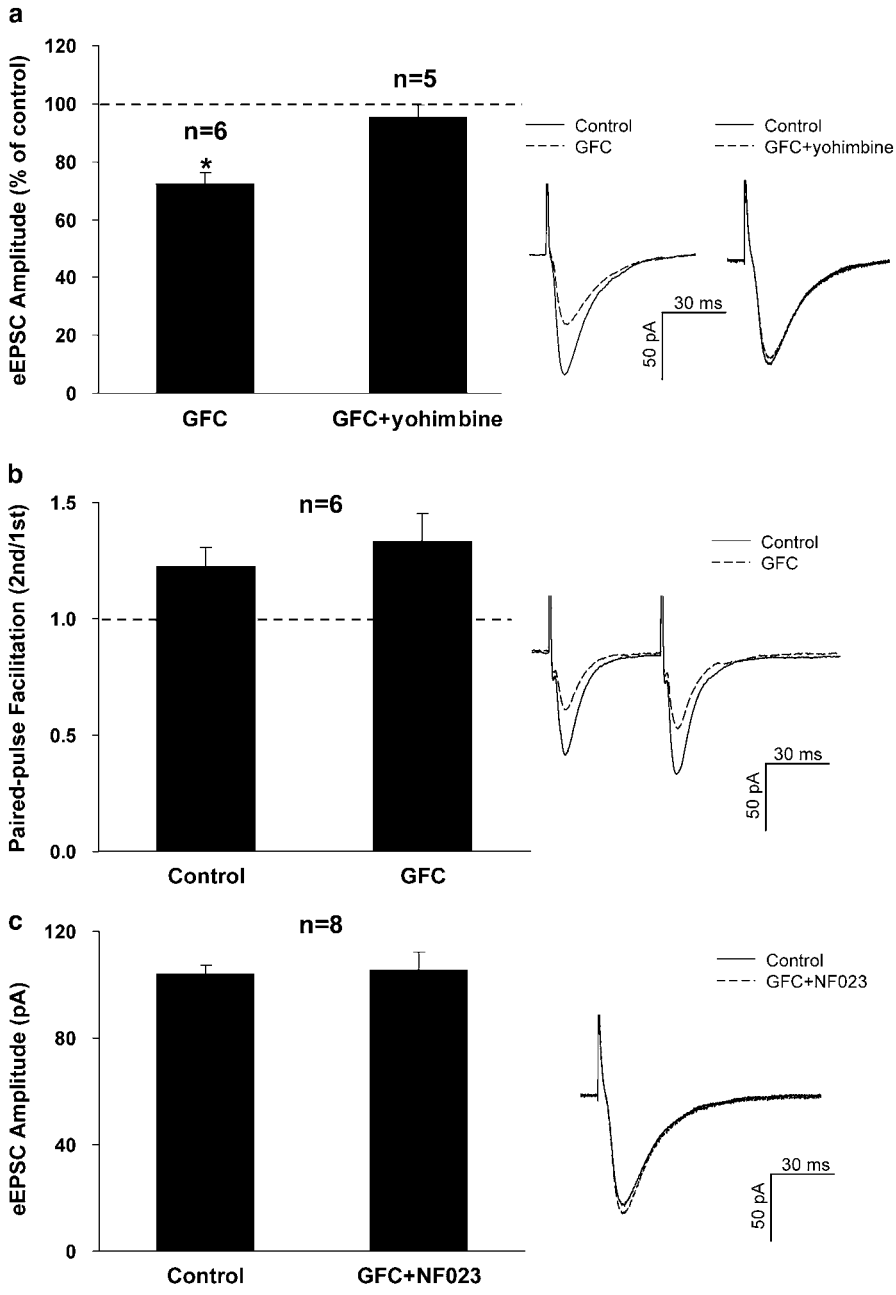

Figure 5 Stimulation of α_{2A}-AR suppresses eEPSC in layer V/VI pyramidal cells in the mPFC. (a) The α_{2A}-AR agonist guanfacine reduced eEPSC, and this inhibitory effect was blocked by the α_2-AR antagonist yohimbine. $^*P<0.05$ *vs* control. (b) Guanfacine had no effect on paired-pulse facilitation of eEPSC. (c) Guanfacine was without effect on eEPSC in the presence of the G_i inhibitor NF023. Holding potential was −70 mV. The concentration of guanfacine and yohimbine was 20 μM and that of NF023 (10 μM). eEPSC, evoked excitatory post-synaptic current; GFC, guanfacine. Shown in the left panels are representative eEPSCs.

signaling pathway of α_{2A}-AR. NF023 could enter into the cytoplasm of a patched cell through the pipette. As shown in Figure 5c, guanfacine did not suppress eEPSC in the presence of NF023: the amplitude of eEPSC was 103.0 ± 3.3 pA in control and 105.4 ± 6.9 pA in the presence of NF023.

DISCUSSION

NE, acting on α_2-AR, has been reported to produce a beneficial effect on PFC cognitive functions. It has been reported that α_2-ARs are localized post-synaptic to NE terminals in the PFC (MacDonald *et al*, 1997). In the monkey PFC, the post-synaptic α_{2A}-ARs are positioned at the dendritic spines of pyramidal neurons (Aoki *et al*, 1998), where glutamate receptors are concentrated. Thus, it is possible that α_{2A}-ARs may exert an influence on glutamate synaptic transmission.

The present study shows that stimulation of α_2-ARs by clonidine reduced *in vivo* fEPSP and *in vitro* eEPSC in layer V/VI pyramidal cells. Stimulation of α_{2A}-AR by guanfacine produced a similar effect on fEPSP and eEPSC. Guanfacine had no effect on the PPF of fEPSP and eEPSC, nor when

intracellular G_i was blocked. The present results indicate that the α_{2A}-AR has an inhibitory regulation on excitatory synaptic transmission in the mPFC, probably through a post-synaptic mechanism. The present study provides electrophysiological evidence that stimulation of α_2-AR suppresses glutamate synaptic transmission in mPFC.

As systemic administration of clonidine or guanfacine has a beneficial effect on prefrontal cortical cognitive functions, we wanted to know how systemically administered clonidine or guanfacine affects excitatory synaptic transmission in the mPFC. Our results show that intramuscular clonidine and guanfacine both suppressed fEPSP in the mPFC in anesthetized or freely moving rats, indicating that the α_2-AR agonists could pass the blood-brain barrier and act at α_2-ARs in the CNS. As systemically administered clonidine and guanfacine could act at the whole CNS, one may argue that their inhibitory effects may be from the actions of the agonists at α_2-ARs in other brain areas instead of the mPFC *per se*. Thus, we observed the effects of direct intra-mPFC infusion of clonidine and guanfacine on fEPSP. We found that locally applied clonidine and guanfacine suppressed fEPSP, either in anesthetized or freely moving rats, as systemically administered clonidine or guanfacine did. The guanfacine inhibition of fEPSP was not mediated by a pre-synaptic mechanism because the PPF of fEPSP was not altered during the application of guanfacine. Thus, a post-synaptic mechanism may account for the inhibitory regulation of α_{2A}-AR on excitatory synaptic transmission in the mPFC.

In the present study, the inhibitory effect of systemically administered clonidine on fEPSP was stronger than that of guanfacine (Figures 1 and 3). It is known that α_2-AR has three subtypes: α_{2A}, α_{2B} and α_{2C}-ARs. α_{2A}-AR is predominately distributed in the PFC, while α_{2B}- and α_{2B}-ARs mainly in other brain regions. While guanfacine is a selective agonist for α_{2A}-AR, clonidine is affinitive for all the three subtypes. It is possible that clonidine also acts at α_{2B}- and α_{2C}-ARs located in other brain regions and produces an indirect influence on PFC excitatory synaptic transmission. It would be also possible that clonidine has a higher permeability than guanfacine to pass the blood-brain barrier.

Field-potential recording technique has its limitation or disadvantage. For instance, we did not know whether the clonidine- and guanfacine-induced suppression of fEPSP came from the direct actions of clonidine and guanfacine at α_2-ARs at the pyramidal neurons recorded, or from the indirect action of the drugs at α_2-ARs at inhibitory neurons. To address this problem, we performed whole-cell patch-clamp recordings in the slices of mPFC. Our data clearly indicate that guanfacine could act at post-synaptic α_{2A}-ARs and produce an inhibitory modulation on glutamate synaptic transmission in the pyramidal cells, because guanfacine inhibition was blocked by the α_2-AR antagonist yohimbine, guanfacine was without effect on the PPF of eEPSC, and guanfacine inhibition no longer existed when the intracellular G_i was inhibited.

It is known that α_{2A}-AR is negatively coupled to adenylyl cyclase by G_i protein. Activation of α_{2A}-ARs results in a decrease in the level of intracellular cAMP and thus reduces cAMP-dependent protein kinase (PKA) activity, leading to the activation of protein phosphatase 1 (PP1). PP1 is localized to post-synaptic dendrites (Ouimet *et al*, 1995) and plays an inhibitory role in synaptic transmission and plasticity through modifying AMPA receptors (Mulkey *et al*, 1994; Yan *et al*, 1999), reducing autophosphorylation of CaMKII (Miller and Kennedy, 1986; Miller *et al*, 1988), and decreasing phosphorylation of GluR1 subunit (Mammen *et al*, 1997; McGlade-McCulloh *et al*, 1993). The present study shows that stimulation of α_{2A}-ARs inhibits excitatory synaptic transmission in the mPFC, probably involving G_i-cAMP-PKA-PP1 signaling pathway.

Behavioral electrophysiological studies in monkeys have shown that systemically or iontophoretically applied clonidine enhanced PFC neuronal activity related to working memory (Li *et al*, 1999; Sawaguchi and Yamane, 1999) or go/no-go performances (Li and Kubota, 1998). Behavioral pharmacological studies in rodents, monkeys, and humans have demonstrated that systemically or locally administered clonidine or guanfacine could improve PFC cognitive performances (Arnsten, 1997; Arnsten *et al*, 1996; Arnsten and Li, 2005). Brain imaging studies have shown that systemically administered guanfacine enhances regional cerebral blood flow in the PFC in both monkeys and humans (Avery *et al*, 2000; Swartz *et al*, 2000). Most recently, Arnsten and colleague have elaborated a model explaining the beneficial effect of α_{2A}-AR stimulation on PFC working memory (Wang *et al*, 2007). In their model, activation of α_{2A}-ARs inhibits the production of cAMP and

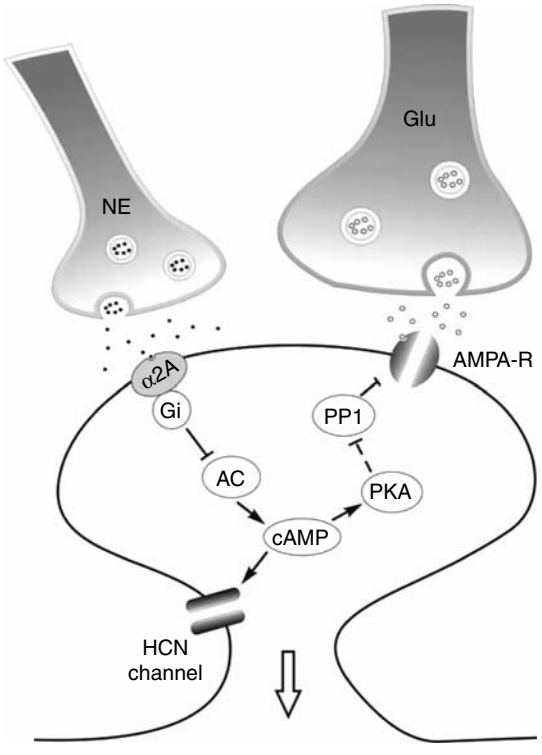

Figure 6 A hypothesized model for bidirectional regulation of PFC excitatory synaptic transmission by α_{2A}-ARs. The G_i–cAMP–HCN channel signaling facilitates excitatory cortical inputs on one hand, while the G_i–cAMP–PKA–PP1–AMPA receptor signaling restricts cortical inputs on the other hand. Through this balanced regulation, α_{2A}-ARs tune the synaptic output to an optimal state for working memory. AC, adenylyl cyclase; cAMP, cyclic adenosine monophosphate; Glu, glutamate; HCN, hyperpolarization-activated cyclic nucleotide-gated channel; NE, norepinephrine; PKA, cAMP-dependent protein kinase; PP1, protein phosphatase 1.

shuts down cAMP-dependent HCN channel, therefore enhancing the efficacy of excitatory synaptic inputs in PFC pyramidal cells and strengthening working memory function (Wang *et al*, 2007).

Thus, there may be a balance mechanism for α_{2A}-ARs to tune excitatory inputs in PFC pyramidal cells to an optimal state for working memory (Figure 6): α_{2A}-AR stimulation enhances excitatory inputs through G_i–cAMP–HCN pathway on one hand, and restricts them via G_i–cAMP–PKA–PP1 pathway on the other hand. The bidirectional regulations determine the strength of synaptic output. The negative regulation by α_{2A}-ARs may serve as a protective mechanism for working memory, especially when α_{2A}-ARs are overstimulated by high levels of NE released under stress (α_{2A}-ARs have a high affinity for NE). Indeed, stimulation of α_{2A}-ARs does not always enhance working memory function. For example, iontophoresis of a low dose of guanfacine (at 5 nA) enhances delay-related firing, while a high dose (at 50 nA) does not (see Figure 2b of Wang *et al*, 2007).

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DISCLOSURE/CONFLICT OF INTEREST

The authors declare that, except for income received from our primary employer, no financial support or compensation has been received from any individual or corporate entity over the past 3 years for research or professional service and there are no personal financial holdings that could be perceived as constituting a potential conflict of interest.

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