Neuroactive steroid pregnenolone sulphate inhibits long-term potentiation via activation of \( \alpha_2 \)-adrenoreceptors at excitatory synapses in rat medial prefrontal cortex


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Abstract

Pregnenolone sulphate (PREGS) is one of the most important neuroactive steroids. Previous study showed that PREGS enhanced long-term potentiation (LTP) via activation of post-synaptic NMDA receptors at excitatory synapses in the hippocampus. The present paper studied the effect of PREGS on LTP at excitatory synapses in the pyramidal cells of layers V–VI of the medial prefrontal cortex (mPFC) using whole-cell patch-clamp in slices and made a comparison with that in the hippocampus. We also studied the mechanism of the effect of PREGS in the mPFC. We found that PREGS inhibited induction of LTP in the mPFC and had no influence on NMDA currents, which was different from its effect in the hippocampus. Moreover, the effect of PREGS on LTP in the mPFC was cancelled by \( \alpha_2 \)-adrenoreceptor antagonist, \( \alpha_2 \)-A-adrenoreceptor antagonist, G protein inhibitor, adenylate cyclase inhibitor and protein kinase A inhibitor. These results suggest that PREGS inhibits LTP via activation of the \( \alpha_2 \)-adrenoreceptor–G\( _i \) protein–adenylate cyclase–protein kinase A signalling pathway in the mPFC.

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Introduction

Pregnenolone sulphate (PREGS) is one of the most important neuroactive steroids (Corpechot et al., 1983) and has been found to have many important effects, e.g. cognitive enhancing, promnesic, anti-stress and antidepressant effects (Akwa et al., 2001; Flood et al., 1992; Mathis et al., 1994, 1996; Maurice et al., 2001; Mayo et al., 2001, 2003; Meziane et al., 1996; Noda et al., 2000; Strous et al., 2006; Urani et al., 1998; Vallee et al., 1997). Therefore, how PREGS affects brain function has received great attention.

It is known that the hippocampus is an important target of the actions of PREGS (Darnaudery et al., 2000; Martin-Garcia and Pallares, 2005; Meyer et al., 2002; Monnet et al., 1995; Schiess and Partridge, 2005; Schumacher et al., 1997; Vallee et al., 1997; Weaver et al., 1998). In addition, it is possible that some of the effects of PREGS on brain function could be mediated by the action of PREGS on the medial prefrontal cortex (mPFC) because this brain region has been shown to play an important role in a variety of higher-order brain functions and neuropsychiatric processes, e.g. emotion, cognition, stress, depression and schizophrenia (Castner et al., 2004; Drevets, 2000; Seamans et al., 1995). Moreover, in the mPFC there exists an enzyme system that synthesizes and breaks down PREGS (Aldred and Waring, 1999; Mellon and Griffin, 2002) and the level of PREGS in this brain region changes under various physiological, pathophysiological or pharmacological conditions (Caldeira et al.,...
2004; Higashi et al., 2003; Torres and Ortega, 2003; Vallee et al., 2000; Yan and Hou, 2004). However, the actions of PREGS on the mPFC remain to be studied.

In our previous works, modulation of glutamatergic synaptic transmission in the mPFC by PREGS was studied (Dong et al., 2005; Sun et al., 2005). These studies suggested that PREGS, at a concentration of \(\geq 20 \mu M\), promoted spontaneous glutamate release, but at a lower concentration (1 \(\mu M\)) it inhibited evoked glutamate release in this brain region. However, whether PREGS has actions on glutamatergic synaptic plasticity in the mPFC is unknown. This effect is as important, because the glutamatergic synaptic plasticity in the mPFC is thought to be important for the establishment, consolidation, and retrieval of permanent memory (Zhao et al., 2005) and plays an important role in shaping the function of the mPFC. Moreover, an enhancing effect of PREGS on long-term potentiation (LTP), a major form of synaptic plasticity, has been reported in the hippocampus (Sliwinski et al., 2004). This raises another interesting question involving the selectivity of the effect of PREGS on LTP, i.e. whether PREGS has a similar or different effect on LTP in the hippocampus and the mPFC. Therefore, in the present paper we studied the effect of PREGS on LTP in the mPFC using the whole-cell patch-clamp method in slices and made a comparison with that in the hippocampus. We also further studied the mechanism of the effect of PREGS on LTP in the mPFC.

Materials and methods

Slice preparation

20- to 30-d-old Sprague–Dawley rats were anesthetized with chloral hydrate (400 mg/kg i.p.). All experimental procedures conformed to Fudan University Ethics Committee as well as international guidelines on the ethical use of animals and all efforts were made to minimize the number of animals used and their suffering. Brain slices were prepared according to procedures described previously (Wang and Zheng, 2001). Briefly, following decapitation, the brain was quickly removed and submerged in ice-cold artificial cerebrospinal fluid (aCSF) saturated with 95\%O\(_2\)/5\%CO\(_2\). Cells were visualized with an upright microscope (Olympus BX50WI) using infrared-differential interference contrast video microscopy. Pyramidal cells in layers V–VI of the mPFC and in the middle part of the hippocampal CA1 were identified by their pyramidal shape and the presence of apical dendrites. Voltage and current signals were recorded in whole-cell recording mode with a patch-clamp amplifier (Axopatch 200B, Axon Instruments, Union City, CA, USA) that was connected to a Digidata 1200 interface (Axon). The data filtered at 2 kHz were digitized and stored on disks using pClamp (version 6, Axon). Patch electrodes were pulled from glass capillaries using a Narishige micropipetter puller (model PB-7, Narishige, Tokyo, Japan). They were filled with a solution containing (in mm): 140 K-gluconate, 0.1 CaCl\(_2\), 2 MgCl\(_2\), 1 EGTA, 2 ATP.K\(_2\), 0.1 GTP.Na\(_4\) and 10 Hepes (pH 7.25) and had a resistance of 4–6 MΩ. Cells were first recorded under a current-clamp mode to record resting membrane potential and action potentials. The recorded cells were considered to be healthy based on the criteria that the resting membrane potential was stable with a value more negative than \(-50 \text{ mV}\) and the action potential had an amplitude of more than 80 mV. Cells were then held at \(-70 \text{ mV}\) under a voltage-clamp mode to record excitatory post-synaptic currents (EPSCs). A bipolar stimulating electrode was placed in layer V, 10–50 \(\mu\)m laterally to the apical dendrite of the recorded cell (Figure 1a) and picrotoxin (50 \(\mu\)m) was incubating chamber (30–32 °C) where they remained for at least 1 h before recordings began.

**Whole-cell recording and stimulation**

The slice was continuously perfused with aCSF saturated with 95\%O\(_2\)/5\%CO\(_2\). Cells were visualized with an upright microscope (Olympus BX50WI) using infrared-differential interference contrast video microscopy. Pyramidal cells in layers V–VI of the mPFC and in the middle part of the hippocampal CA1 were identified by their pyramidal shape and the presence of apical dendrites. Voltage and current signals were recorded in whole-cell recording mode with a patch-clamp amplifier (Axopatch 200B, Axon Instruments, Union City, CA, USA) that was connected to a Digidata 1200 interface (Axon). The data filtered at 2 kHz were digitized and stored on disks using pClamp (version 6, Axon). Patch electrodes were pulled from glass capillaries using a Narishige micropipetter puller (model PB-7, Narishige, Tokyo, Japan). They were filled with a solution containing (in mm): 140 K-gluconate, 0.1 CaCl\(_2\), 2 MgCl\(_2\), 1 EGTA, 2 ATP.K\(_2\), 0.1 GTP.Na\(_4\) and 10 Hepes (pH 7.25) and had a resistance of 4–6 MΩ. Cells were first recorded under a current-clamp mode to record resting membrane potential and action potentials. The recorded cells were considered to be healthy based on the criteria that the resting membrane potential was stable with a value more negative than \(-50 \text{ mV}\) and the action potential had an amplitude of more than 80 mV. Cells were then held at \(-70 \text{ mV}\) under a voltage-clamp mode to record excitatory post-synaptic currents (EPSCs). A bipolar stimulating electrode was placed in layer V, 10–50 \(\mu\)m laterally to the apical dendrite of the recorded cell (Figure 1a) and picrotoxin (50 \(\mu\)m) was
included in aCSF to block GABA<sub>A</sub> receptors. The EPSCs were elicited by 0.1 Hz stimulating pulses with the stimulation intensity adjusted to evoke an EPSC that was ~30% of the maximum amplitude, usually ~500 pA. After at least a 10-min period of baseline EPSC collection, LTP was induced with tetanic stimuli consisting of four trains of 100 Hz burst, 25 pulses per train and a total of 100 pulses, which were delivered at 0.1 Hz under current-clamp mode. The voltage-clamp mode and the baseline stimulation were restored 10 s after the end of the last tetanic stimulation (Auclair et al., 2000). To avoid any contamination by the polysynaptic component, the initial slope of EPSC (<1 ms from its onset) that contained only the monosynaptic component of the response (Hirsch and Crepel, 1990) was measured (Figure 1b). To induce N-methyl-D-aspartic acid (NMDA) currents, NMDA (50 μM) was pressure-ejected from a pipette positioned near the soma of recorded cells. NMDA currents were recorded under voltage-clamp mode at a holding potential of −60 mV in the presence of 1 μM TTX to block Na<sup>+</sup> currents, 50 μM picrotoxin to block GABA<sub>A</sub> receptors and 0 Mg<sup>2+</sup> to reveal NMDA responses. To observe paired pulse facilitation (PPF), two synaptic responses were evoked by a pair of stimuli given at short intervals (50 ms) at 0.05 Hz. The series resistance was monitored by measuring the instantaneous current in response to a 5 mV voltage-step command. Series resistance compensation was not used, but cells where series resistance changed by >15% were discarded.

**Offline data analysis**

Offline data analysis was performed using Clampfit 9.0 (Axon), Kyplot (Koichi Yoshioka), and SigmaPlot (Jandel Scientific, San Rafael, USA). Six successive EPSCs were averaged. Numerical data were expressed as mean ± S.E.M. (standard error of the mean). Statistical significance was determined using paired Student’s t test or unpaired Student’s t test.

**Drugs**

Picrotoxin, pregnenolone sulphate (PREGS), N-[2-(p-bromocinnamylamino) ethyl]-5-isouquinolinesulfonamide (H89), N-ethylmaleimide (NEM), 2-amino-6-ethyl-4,5,7,8-te-trahydro-6H-oxazo1o-[5,4-d]-azepine (B-HT 933), yohimbine hydrochloride, N-(cis-2-phenyl-cyclopentyl)azacyclotridecan-2-imine-hydrochloride (MDL-12330A), N-methyl-D-aspartic acid (NMDA), haloperidol and 2-[2 H-(1-methyl-1,3-di-hydrosoindole) methyl]-4,5-dihydroimidazole (BRL-44408) were purchased from Sigma (St Louis, MO, USA). Tetrodotoxin (TTX) was made in the Research Institute of Aquatic Products of Hebei, P.R. China. Other reagents in AR grades were products of Shanghai Chemical Plant. All drugs were dissolved in dH<sub>2</sub>O, except for picrotoxin, PREGS, MDL-12330A, yohimbine and H89, which were dissolved in DMSO. When DMSO was used as vehicle, drugs were initially dissolved in DMSO and then diluted with aCSF at a final DMSO concentration <0.1%. In vehicle control experiments, we confirmed that the final concentration of DMSO in aCSF or pipette solution had no detectable effects on the parameters we observed. PREGS, H89, TTX, picrotoxin, B-HT 933, yohimbine, MDL-12330A, BRL-44408, haloperidol and NEM were applied by bath perfusion.

**Results**

**PREGS inhibits induction of LTP in the mPFC**

As shown in Figure 2a, in control slices, robust LTP could be reliably induced by tetanus and always lasted throughout the recording period (n = 6), but after treating slices with 1 μM PREGS for 15 min, LTP induction was inhibited in all cells recorded (n = 6, Figure 2a). The tetanus-evoked increase in the slope of EPSCs (209.6 ± 25.0%, n = 6, Figure 1b) was inhibited to 106.3 ± 14.9% by PREGS (n = 6, Figure 2b, p < 0.05), compared to control, measured at 30–40 min post-tetanus. PREGS (1 μM) had no significant effect on the basal slope of EPSCs (86.4 ± 10.7%) at 30–40 min after PREGS, compared to control (n = 7, p > 0.05), although the basal amplitude of EPSCs was inhibited by 40.2 ± 6.5% (n = 7, p < 0.05) at 30–40 min after 1 μM PREGS, which was similar to that reported by Sun et al. (2005). The concentration dependence of the inhibitory effect of PREGS on the induction of LTP was also tested. The result showed that in the range of 0.1–3 μM, the effect of PREGS was significant (p < 0.05) at a concentration of 0.3 μM, increased with an increase in the concentration and appeared to reach a plateau after 1 μM (n = 6, Figure 2c). We also observed the effect of PREGS on the induction of LTP in the CA1 pyramidal neurons of the hippocampus. The result showed that at the same concentration (1 μM) to that in the mPFC, we did not find a significant influence of PREGS on the induction of LTP in the hippocampus (n = 6, p > 0.05, Figure 3b). This result was consistent with that reported by Sliwinski and colleagues, who also did not find a significant influence of PREGS at 1 μM on the induction of LTP in the hippocampus (Sliwinski et al., 2004). Moreover, here we repeated the experiment of the effect of PREGS at a concentration of 0.3 μM on the induction of LTP in the hippocampus.
and we again obtained a similar result to that reported by Sliwinski et al. (2004), i.e. 0.3 μM PREGS could enhance LTP in the hippocampus (n = 5, p < 0.05, Figure 3b).

**PREGS has no influence on NMDA currents induced by local application of NMDA**

To study the role of the inhibition of NMDA receptors in the effect of PREGS on LTP in the mPFC, we observed the effect of 1 μM PREGS on NMDA currents induced by local application of NMDA (50 μM, for 5 s). The result showed that infusion of 1 μM PREGS for 20 min did not significantly influence the NMDA currents (Figure 4a, b, n = 6). The change in percentage of the amplitude of NMDA currents in the presence and absence of PREGS was 95.3 ± 10.9% and 102.6 ± 7.9%, which was not statistically significant (p > 0.05, n = 6, Figure 4c). This result suggests that the inhibitory effect of PREGS on the induction of LTP in the mPFC may not be due to an inhibition of post-synaptic NMDA receptors.

**Effect of PREGS on induction of LTP is cancelled by G_i protein inhibitor**

To check the role of the activation of G_i proteins in the effect of PREGS on LTP in the mPFC, we studied the influence of the Gi protein inhibitor NEM (Riorden et al., 1972) on the effect of PREGS. Slices were incubated with 50 μM NEM for at least 30 min and then LTP was recorded. The results showed that treatment of slices with NEM alone had no influence on the induction of LTP (Figure 5a, n = 6). The tetanus-evoked increase in the slope of EPSCs with NEM (227.9 ± 32.6%, n = 6, Figure 5b) was not statistically significant compared to that without NEM (209.6 ± 25.0%, n = 6, Figure 5b), measured at 30–40 min post-tetanus. However, in slices pretreated with NEM, the inhibitory effect of 1 μM PREGS on the induction of LTP disappeared (Figure 5c, n = 6). The tetanus-evoked increase in the slope of EPSCs in the presence of NEM after PREGS was 217.7 ± 33.8% (Figure 5d, n = 6, measured at 30–40 min post-tetanus), showing that the inhibitory effect of PREGS on the induction of LTP was completely blocked.

**Effect of PREGS on induction of LTP is blocked by α_2-adrenoreceptor antagonist**

To study the role of two kinds of G_i protein-coupled receptors, D_2 receptors and α_2-adrenoreceptors, on the effect of PREGS, we observed the influence of the D_2 receptor antagonist and the α_2-adrenoreceptor
antagonist on the effect of PREGS on LTP in the mPFC. The result showed that the D<sub>2</sub> receptor antagonist haloperidol (0.3 µM) alone had no influence on the induction of LTP (200.8 ± 5.6% with haloperidol, n = 4, and 209.6 ± 25.0% without haloperidol, n = 6, p > 0.05) and it had no influence on the inhibitory effect of 1 µM PREGS on the induction of LTP (101.8 ± 2.5% after PREGS in the presence of haloperidol, n = 5, and 106.3 ± 14.9% after PREGS in the absence of haloperidol, n = 6, p > 0.05). Moreover, we also checked the effect of a higher concentration of haloperidol (3 µM) on PREGS actions on LTP. The result showed that 3 µM haloperidol still had no influence on the inhibitory effect of 1 µM PREGS on the induction of LTP (107.5 ± 12.6% after PREGS in the presence of haloperidol, n = 5, 106.3 ± 14.9% after PREGS in the absence of haloperidol, n = 6, measured at 30–40 min post-tetanus). However, interestingly, after treatment of slices with the a<sub>2</sub>-adrenoreceptor inhibitor yohimbine (10 µM), the inhibitory effect of PREGS on the induction of LTP was completely cancelled (Figure 6a, n = 6). Yohimbine (10 µM) alone had no influence on the induction of LTP (176.1 ± 9.9% with yohimbine, n = 4, and 209.6 ± 25.0% without yohimbine, n = 6, p > 0.05), but the tetanus-evoked increase in the slope of EPSCs in the presence of yohimbine after PREGS was 200.1 ± 21.0% (Figure 6b, n = 6, measured at 30–40 min post-tetanus), showing that the inhibitory effect of PREGS on the induction of LTP was completely blocked. We also observed the effect of the a<sub>2</sub>-adrenoreceptor agonist B-HT 933 on the induction of LTP. The result showed that 10 µM B-HT 933 could mimic the inhibitory effect of PREGS on the induction of LTP. After treatment of slices with B-HT 933 for 15 min, the tetanus-evoked increase in the slope of EPSCs (209.6 ± 25.0% without B-HT 933, n = 6) was inhibited to 109.7 ± 7.7% (n = 6, p < 0.05, measured at 30–40 min post-tetanus), which was similar to that produced by 1 µM PREGS (106.5 ± 15.4%, n = 6). We also studied the role of a<sub>2</sub>A-adrenoreceptors in the inhibitory effect of PREGS on the induction of LTP in the mPFC by examining the influence of the a<sub>2</sub>A-adrenoreceptor antagonist BRL-44408 on the effect of PREGS. The result showed that 2 µM BRL-44408 alone had no influence on the induction of LTP (208.2 ± 19.9% with BRL-44408, n = 4, and 209.6 ± 25.0% without BRL-44408, n = 6, measured at 30–40 min post-tetanus). However, in the presence of BRL-44408, the inhibitory effect of 1 µM PREGS on the induction of LTP was blocked (Figure 6c, n = 6). The tetanus-evoked increase in the slope of EPSCs in the presence of BRL-44408 after PREGS was 205.5 ± 11.1% (Figure 6d, n = 6, measured at 30–40 min post-tetanus), showing that the inhibitory effect of PREGS on the induction of LTP was blocked.

**Effect of PREGS on induction of LTP is cancelled by adenylyl cyclase (AC) and cAMP-dependent protein kinase (PKA) inhibitor**

To study the role of AC and PKA in the inhibitory effect of PREGS on the induction of LTP in the mPFC, we examined the influence of the AC inhibitor MDL-12330A and the PKA inhibitor H89 on the effect of PREGS. The results showed that pretreatment of slices with 10 µM MDL-12330A alone had no influence on the...
induction of LTP (196.9 ± 12.1% with MDL-12330A, n = 4, and 209.6 ± 25.0% without MDL-12330A, n = 6, p > 0.05, measured at 30–40 min post-tetanus). However, in the presence of MDL-12330A, the inhibitory effect of 1 μM PREGS on the induction of LTP was reversed (Figure 7a, n = 6). The tetanus-evoked increase in the slope of EPSCs in the presence of MDL-12330A after PREGS was 202.4 ± 17.6% (Figure 7a, n = 6, measured at 30–40 min post-tetanus), showing that the inhibitory effect of PREGS on the induction of LTP was blocked. Moreover, pretreatment of slices with H89 could completely occlude the inhibitory effect of 1 μM PREGS on the induction of LTP (Figure 7b, n = 6). H89 (1 μM) alone had no influence on the induction of LTP (199.5 ± 12.1% with H89, n = 4, and 209.6 ± 25.0% without H89, n = 6, p > 0.05, measured at 30–40 min post-tetanus). However, the tetanus-evoked increase in the slope of EPSCs in the presence of H89 after PREGS was 186.1 ± 13.3% (Figure 7b, n = 6, measured at 30–40 min post-tetanus), showing that the inhibitory effect of PREGS on the induction of LTP was blocked.

However, when we added 1 μM H89 into the pipette solution and allowed H89 to be perfused into the post-synaptic cell by the pipette, we did not find any influence of H89 on the effect of PREGS on LTP. H89 (1 μM) added to the pipette solution alone had no significant influence on the induction of LTP (162.1 ± 5.7%, with H89, n = 5, and 209.6 ± 25.0% without H89, n = 6, p > 0.05, measured at 30–40 min post-tetanus). The tetanus-evoked increase in the slope of EPSCs with H89 after PREGS was still inhibited to 109.5 ± 11.0% (n = 6, Figure 8), measured at 30–40 min post-tetanus. This result suggests that the site of the inhibitory effect of PREGS on LTP may not be at a post-synaptic site, but at a presynaptic site. This view was further supported by the following PPF experiment. PPF is an enhancement of the synaptic response to the second of two closely spaced action potentials and is caused by presynaptic accumulation of Ca2+ after an initial stimulation, that leads to increased transmitter release during a second stimulus applied after a short interval (Zucker, 1989). Thus, PPF is a presynaptically mediated phenomenon (Zucker and Regehr, 2002), and an alteration in PPF by a drug indicates a presynaptic site of drug action (Hajos et al., 2001). Moreover, it is generally accepted that an increase in PPF indicates a decrease in presynaptic glutamate release, whereas a decrease in PPF indicates an increase in presynaptic glutamate release (Thomson, 2000). PPF was given as 0.05 Hz with a 50-ms interstimulus interval. After a stable PPF was obtained, we collected 10-min data as baseline and then LTP was induced with tetanus. The result showed that PPF decreased significantly after tetanus stimulation in the control group, especially during the initial 10 min after tetanus (Figure 9, n = 6). The averaged PPF during the initial 10 min after tetanus was decreased to 74.2 ± 5.4% (Figure 9, n = 6) in the control group. This result suggests that presynaptic glutamate release increases during LTP, especially during the initial 10 min after tetanus. However, when 1 μM PREGS was applied, the decrease in PPF during LTP was reversed (Figure 9, n = 5). The averaged percentage of PPF during the initial 10 min after tetanus with PREGS was reversed to 107.3 ± 7.9% of baseline...
suggesting that PREGS inhibited the increase in presynaptic glutamate release during LTP. This result was consistent with our previous result that showed the inhibitory effect of PREGS on presynaptic glutamate release was cancelled by PKA inhibitor (Sun et al., 2005).

Discussion

Previous studies examined the effect of PREGS on LTP in CA1 in rat hippocampal slices. Sliwinski et al. (2004) found that PREGS at 0.1 μM and 0.3 μM, but not at 0.6 μM and 3 μM, could enhance LTP in the hippocampus. Sabeti et al. (2007) reported that PREGS at 5 μM could strengthen both NMDA receptor-dependent and -independent (but L-type calcium channel-dependent) LTP, while at a higher concentration (15 μM) it depresses NMDA receptor-dependent LTP under conditions in which sigma-receptor function was blocked. The present study extended the observation to the mPFC, but, interestingly, found that PREGS, at concentrations which were reported to have an enhancing effect or no effect on LTP in the hippocampus (Sliwinski et al., 2004), inhibited LTP in the mPFC. To confirm this regional difference, here, as a control, we also observed the effect of PREGS on LTP in the hippocampus and obtained a similar result to that reported by Sliwinski et al. (2004). This finding suggests that PREGS may have a different action on LTP, depending on the brain region.

Regarding the mechanism of the enhancing effect of PREGS on LTP in the hippocampus, a previous study has proposed that it might be through the modulation of post-synaptic NMDA receptors (Sliwinski et al., 2004). This view was supported by our experiment that 1 μM PREGS could increase

Figure 5. Influence of the G protein inhibitor N-ethylmaleimide (NEM) on the effect of PREGS on the induction of LTP in pyramidal cells of layers V–VI of the medial prefrontal cortex. (a) Time-course of the effect of 50 μM NEM on the induction of LTP (n=6). (b) Averaged result of the effect of 50 μM NEM on the induction of LTP (p >0.05), compared to control. (c) Time-course of the effect of 50 μM NEM alone (n=6) and the effect of 1 μM PREGS on the induction of LTP in the presence of 50 μM NEM (n=6). (d) Averaged result of the effect of 50 μM NEM alone and the effect of 1 μM PREGS on the induction of LTP in the presence of 50 μM NEM, measured at 30–40 min post-tetanus (n=6, p >0.05), compared to NEM-alone group. EPSC, Excitatory post-synaptic current.
NMDA currents in the hippocampus (data not shown). However, interestingly, in the mPFC PREGS at a concentration of 1 μM that already significantly inhibited LTP had no effect on NMDA currents, suggesting that the inhibitory effect of PREGS on the induction of LTP in the medial cortex might not be due to an inhibition of post-synaptic NMDA receptors. This point was consistent with the study of Shirakawa et al. (2002) who showed that PREGS even at 100 μM did not have an effect on NMDA currents in rat cortical neurons.

The stimulation of layer V of the mPFC can elicit a complex synaptic response which may involve polysynaptic components in pyramidal cells of layers V–VI of this brain region. However, when the initial slope of EPSCs (a 1-ms period from its onset; pA/ms) evoked by this stimulation is used to measure excitatory synaptic transmission efficacy, this initial slope is considered to be a monosynaptic excitatory response (Hirsch and Crepel, 1990; Pavlidis et al., 2000). Therefore, to study the effect of PREGS on the monosynaptic excitatory response in pyramidal cells of layers V–VI of the mPFC, we used the initial slope of EPSCs as the parameter of excitatory synaptic transmission efficacy. The result showed that PREGS had no significant effect on basal initial slope of EPSCs, but could inhibit tetanus-evoked LTP of the initial slope of EPSCs, suggesting that PREGS might selectively inhibit LTP at the excitatory monosynaptic level. However, it should be noted that in our previous study when we used the amplitude of EPSCs, which might be contaminated by polysynaptic components

Figure 6. Influence of the α2-adrenoreceptor inhibitor yohimbine and the α2A-adrenoreceptor inhibitor BRL-44408 on the effect of PREGS on the induction of LTP in pyramidal cells of layers V–VI of the medial prefrontal cortex. (a) Time-course of the effect of 10 μM yohimbine alone (n = 6) and the effect of 1 μM PREGS on the induction of LTP in the presence of 10 μM yohimbine (n = 6). (b) Averaged result of the effect of 10 μM yohimbine alone (n = 6) and the effect of 1 μM PREGS on the induction of LTP in the presence of 10 μM yohimbine (n = 6), measured at 30–40 min post-tetanus (p > 0.05), compared to the yohimbine alone group. (c) Time-course of the effect of 2 μM BRL-44408 alone (n = 4) and the effect of 1 μM PREGS on the induction of LTP in the presence of 2 μM BRL-44408 (n = 6). (d) Averaged result of the effect of 2 μM BRL-44408 alone (n = 4) and the effect of 1 μM PREGS on the induction of LTP in the presence of 2 μM BRL-44408 (n = 6), measured at 30–40 min post-tetanus (p > 0.05), compared to the BRL-44408-alone group. EPSC, Excitatory post-synaptic current.
(Akaneya et al., 1997; Vickery et al., 1997), as the parameter of synaptic transmission, we found that PREGS could significantly inhibit the amplitude of EPSCs. Moreover, here, if we examined the effect of PREGS on the amplitude of EPSCs, we obtained a similar result to that of Sun et al. (2005). These results suggest that in addition to an inhibitory effect on LTP at the excitatory monosynaptic level, PREGS may have another inhibitory effect on basal excitatory synaptic transmission at the polysynaptic level. Of course, the present finding is that PREGS can selectively inhibit LTP at excitatory monosynaptic afferents impinging on pyramidal cells of layers V–VI of the mPFC.

Gi protein, as an inhibitory G protein, is one of the key signalling molecules in the system that is coupled to the inhibition of LTP (DeBock et al., 2003; Pineda et al., 2004). To explore the signal transduction mechanism underlying the inhibitory effect of PREGS on LTP, we examined the role of Gi protein in the effect of PREGS on LTP. The result showed that the Gi protein inhibitor NEM cancelled the effect of PREGS on LTP, suggesting that the activation of Gi proteins played a key role in the inhibitory effect of PREGS on LTP.

It is known that there are many different receptors coupled to Gi proteins. To study the upstream receptor mechanism for the activation of Gi proteins by PREGS, we investigated the role of two kinds of Gi protein-coupled receptors, D2 receptors and α2-adrenoreceptors, on the effect of PREGS on LTP. The results showed that the D2 receptor antagonist...
block the effect of PREGS on LTP. This evidence further supported the involvement of the $a_2$-adrenoceptor–$G_i$ protein signalling pathway in the effect of PREGS on LTP. This was also supported by the result that the $a_2$-adrenoceptor agonist B-HT 933 could mimic the inhibitory effect of PREGS on LTP. Moreover, we further studied the role of the subtype of the $a_2$-adrenoceptors in the effect of PREGS and demonstrated that the $a_2A$-adrenoceptors played a key role in the effect of PREGS because the $a_2A$-adrenoceptor antagonist could completely cancel the effect of PREGS. However, how, directly or indirectly, PREGS activates $a_2$-adrenoceptors remains to be studied. The possibility that PREGS produced its effect by promoting intrinsic norepinephrine release seemed unlikely because some reports showed that PREGS not only had no promoting effect, but even had some extent of inhibitory effect, on intrinsic norepinephrine release (Cannizzaro et al., 2003; Monnet et al., 1995). In addition, the fact that PREGS promoted spontaneous glutamate release via $a_1$-adrenoceptors at concentrations of $\geq 20 \mu M$ (Dong et al., 2005), but inhibited LTP via $a_2$-adrenoceptors at lower concentrations ($\leq 1 \mu M$) appeared not to support the view that PREGS produced its effect by promoting intrinsic norepinephrine release, because if it were through intrinsic norepinephrine, it should act at similar concentrations to the actions on $a_1$- and $a_2$-adrenoceptors.

The reason why PREGS has no inhibitory effect on LTP in the hippocampus remains unknown. One possible reason may be related to the different expression of $a_2$-adrenoceptors in different brain regions. Happe and colleagues examined the expression and the functional activity of $a_2$-adrenoceptors in different brain regions and found both the expression and the functional activity of $a_2$-adrenoceptors were high in the full extent of the cerebral cortex, but only found in a portion of the caudal hippocampus CA1 lacunosum moleculare layer (Happe et al., 2000). This might be one reason why PREGS had no inhibitory effect on LTP in the hippocampus because the region we recorded here was the middle part of the CA1 region where no expression and functional activity of $a_2$-adrenoceptors were detected (Happe et al., 2000). In addition, although the present observation that PREGS had no effect on NMDA currents in the mPFC, but did so in the hippocampus was consistent with reports in the literature (Bowley, 1993; Shirakawa et al., 2002), the reason for this difference also remains unknown. Recent evidence that PREGS exhibited both stimulatory and inhibitory effects on NMDA receptors, depending on the compositions of receptor

Figure 8. Influence of intra-pipette application of the PKA inhibitor H89 on the effect of PREGS on the induction of LTP in pyramidal cells of layers V–VI of the medial prefrontal cortex. (a) Time-course of the effect of the intra-pipette application of 1 $\mu M$ H89 alone ($n = 5$) and the effect of 1 $\mu M$ PREGS on the induction of LTP with the intra-pipette application of 1 $\mu M$ H89 ($n = 6$). (b) Averaged result of the effect of the intra-pipette application of 1 $\mu M$ H89 alone ($n = 5$) and the effect of 1 $\mu M$ PREGS on the induction of LTP with the intra-pipette application of 1 $\mu M$ H89 ($n = 6$, $^* p < 0.05$), compared to the H89-alone group. EPSC, Excitatory postsynaptic current.
subunits (Malayev et al., 2002) suggested that the relatively weak effect of PREGS on NMDA currents in the mPFC might be attributable to the composition of NMDA receptors expressed in these cells. In addition, it was also possible that, because NMDA receptor functions were strongly regulated by a variety of receptor-associated proteins (Scannevin and Huganir, 2000), differences in expression patterns of these associated proteins, rather than that of NMDA receptor subunits themselves, might determine the potency of the effects of PREGS on NMDA receptors (Shirakawa et al., 2002) in different brain regions.

Since LTP at the excitatory synapses between glutamate afferents and pyramidal cells of the mPFC has special roles in cognition, neuropsychiatric disorders and psychostimulant abuse, the inhibition of LTP by PREGS in the mPFC may have significant functional consequences on cognition, neuropsychiatric disorders and psychostimulant abuse. It has been reported that PREGS, especially, is an enhancer of...
cognition (Akwa et al., 2001; Flood et al., 1992; Mathis et al., 1994, 1996; Maurice et al., 2001; Mayo et al., 2001, 2003; Meziane et al., 1996; Noda et al., 2000; Strous et al., 2006; Urani et al., 1998; Vallee et al., 1997). The inhibitory effect of PREGS on LTP in the mPFC demonstrated in this study, is therefore unexpected since typically, LTP, as the substrate for long-lasting information storage, should be enhanced if an agent has a cognition-enhancing effect. However, recent studies showed that the inhibition of LTP also could enhance cognitive function because this inhibition could decrease saturation of the intrinsic neuronal pathways and thus benefit new memory formation (Pineda et al., 2004). Therefore, it is possible that the inhibition of LTP by PREGS in the mPFC may contribute to its cognition-enhancing effect in vivo. Of course, inhibition of LTP by PREGS in the mPFC may be an adverse factor in cognition. Therefore, the role of inhibition of LTP in the mPFC in cognition remains to be studied further. In addition, the opposite effect of PREGS on LTP in the hippocampus and the mPFC also suggests that the mechanism underlying the effect of PREGS on cognition is probably more complicated than originally anticipated. It may involve a delicate balance among LTP in different brain regions.

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Statement of Interest

None.

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