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Pseudoginsenoside-F11 decreases morphine-induced behavioral sensitization and extracellular glutamate levels in the medial prefrontal cortex in mice

Yue Hao, Jing Yu Yang, Chun Fu Wu*, Min Fan Wu

Department of Pharmacology, Shenyang Pharmaceutical University, Shenyang 110016, P.R. China

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Abstract

Morphine produces a variety of behavioral and biochemical changes related to its abuse. Our previous studies showed that Pseudoginsenoside-F11 (PF11), an ocotillol-type saponin existing in American ginseng, can antagonize pharmacological effects of morphine. To further investigate the effects of PF11 on morphine abuse and the underlying mechanisms, we tested the effects of PF11 on morphine-induced development of behavioral sensitization and alterations in glutamate levels in the medial prefrontal cortex (mPFC) in freely moving mice by using *in vivo* microdialysis. As the results shown, PF11 antagonized the development of behavioral sensitization and decrease of glutamate in the mPFC induced by morphine. Therefore, these findings suggest that PF11 may block the development of morphine-induced behavioral sensitization via its effect, at least partially, on the glutamatergic system in the mPFC.

Keywords: Morphine; Pseudoginsenoside-F11; Behavioral sensitization; Medial prefrontal cortex; Glutamate; Microdialysis

1. Introduction

Morphine is commonly used clinically to relieve pain and treat chronic diarrhea. However, it produces a variety of behavioral and biochemical changes. Acute administration of morphine induces hyperactivity and stereotyped behavior in animals (Shuster et al., 1975; Li et al., 2004). Repeated morphine influences behaviors in the conditioned place preference test (Liu et al., 2005) and produces tolerance, reward, physical dependence and, upon drug cessation, physical withdrawal symptoms and relapse (Shalev et al., 2002).

Ginseng is the root of *Panax ginseng* C.A. Meyer which has been used for hundreds of years as a treatment for a wide variety of ailments. Numerous evidence have demonstrated that ginseng extracts or its principles exert some antagonistic effects on the behavioral and neurochemical toxicities of morphine. For example, ginseng extracts antagonize the morphine antinociception and morphine tolerance in rodents (Bhargava and Ramarao, 1991; Kim et al., 1990; Ramarao and Bhargava, 1990). Its saponins can inhibit hyperactivity and conditioned place preference induced by morphine (Kim et al., 1998) and modulate drug induced dopaminergic dysfunction (Tokuyama et al., 1992; Oh et al., 1997).

Our previous studies have shown that pseudoginsenoside-F11 (PF11), an ocotillol-type saponin isolated from leaves of *Panax pseudoginseng* subsp. *Himalaicus* H_{ARA} (Himalayan Panax) (Tanaka and Yahara, 1978) possesses the same antagonistic actions as ginseng on morphine. It can antagonize various pharmacological effects of morphine, such as memory impairment, hyperactivity, analgesia and analgesia tolerance (Li et al., 2000). Furthermore, PF11 markedly blocks morphine-induced conditioned place preference in mice (Li et al., 2000). These results suggest that PF11 has some neuroprotective effects against morphine-induced impairments and may attenuate morphine dependence.

It is well known that repeated morphine administration produces behavioral sensitization, which is characterized by an increased locomotor activity and/or stereotypic behavior (Giros et al., 1996) and hypothesized to underlie the craving associated with drug abuse (Kalivas et al., 1998). Alterations in dopamine transmission in the mesocorticolimbic system have been suggested to play a critical role in the development of sensitization (Banks and Gratton, 1995; Beyer and Steketee, 2002). As a part of the mesocorticolimbic system, the medial prefrontal cortex (mPFC) receives

* Corresponding author. Tel./fax: +86 24 2384 3567.
E-mail addresses: wucf@syphu.edu.cn, chunfuw@gmail.com (C.F. Wu).

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dopaminergic inputs from the ventral tegmental area (VTA) and nucleus accumbens (NAc) and accordingly provides a major glutamatergic projection to the NAc to regulate dopamine release (Conde et al., 1995; Montaron et al., 1996), which is crucial for the rewarding effect of morphine. Evidence also exists that excitatory glutamate neurotransmission in the mesocorticolimbic system may contribute to opiate dependence and withdrawal (Jacobs et al., 2005; Wang et al., 2005; Xi and Stein, 2002). Recently, we have found that both acute and chronic morphine decreased glutamate levels in the anterior cingulate cortex, a sub-region of the mPFC, in rats (Hao et al., 2005), suggesting that glutamatergic system in the mPFC may be closely involved in morphine dependence and withdrawal.

With the consideration that PF11 can antagonize morphine-induced behavioral adaptations, it is well worthy to investigate whether the antagonistic effects of PF11 on morphine have some relations to the regulation of glutamatergic system in the mPFC. Therefore, in the present study we tested the effects of PF11 on morphine-induced behavioral sensitization and alterations in the extracellular glutamate levels in the mPFC in mice.

2. Materials and methods

2.1. Subjects

Male Swiss–Kunming mice, weighing 24–28 g at the beginning of the experiments, were supplied by the Experimental Animal Centre of Shenyang Pharmaceutical University. The mice were maintained under standard housing conditions in 12L:12D light/dark of cycle with *ad libitum* access to water and food. Experiments were performed during the daytime and each animal was tested only once. All experiments were conducted according to the NIH Guide for the Care and Use of Laboratory Animals (NIH publications no. 80–23, revised 1996). The experimental procedures were approved by the local Committee on Animal Care and Use.

2.2. Drugs and treatments

PF11, provided by the Department of Chemistry for Natural Products of Shenyang Pharmaceutical University, was given intragastrically at dose of 4 and 8 mg/kg. In repeated treatments, PF11 was given once a day for 7 days.

Morphine hydrochloride, purchased from Shenyang First Pharmaceutical Factory, China, was administrated intraperitoneally (10 mg/kg, i.p.) in the acute treatment. In the chronic treatments, morphine was given at dose of 10 mg/kg once a day for 7 days to produce behavioral sensitization. To test the effects of PF11 on morphine, PF11 was administrated 60 min before each treatment of morphine. All the drugs were dissolved in the 0.9% saline. Control groups were treated with saline instead of corresponding drugs. The control and morphine groups are the same in the acute PF11 and repeated PF11 tests.

2.3. Behavioral sensitization

Locomotor activity was measured by an ambulometer with four activity chambers (JIL-2, Institute of Materia Medica, Chinese Academy of Medical Sciences, China). Activity chambers of 25 cm × 15 cm (diameter × height) consisted of opaque perspex walls and floors and transparent perspex lids. Each chamber was equipped with infrared photobeams connected to a computer to quantify locomotor activity.

Behavioral sensitization was tested based on the method previously described (Li et al., 2004) with slight modifications. Briefly, morphine (10 mg/kg, i.p.) was injected once a day for 7 days and PF11 was administrated 60 min before each injection of morphine. After the injection of morphine, mice were immediately placed in the activity cage for a 60 minute activity recording period on day 1 and day 7 to test the development of behavioral sensitization. The experimental time for the 7 days remained approximately at the same time everyday during the daytime.

2.4. Surgery

Mice were anesthetized with chloral hydrate (400 mg/kg, i.p.) and mounted in a stereotaxic frame with the incisor bar lowered to a 15° angle (nose down). A U-shape dialysis probe consisted of cellulose hollow fiber (0.25 mm inside diameter; molecular weight cutoff 50,000 Da) was implanted vertically into the mPFC. The coordinates for placement of the tip of the dialysis probe were: AP + 2.3 mm, ML − 0.3 mm, DV − 4.0 mm from the bregma and dural surface according to the mouse brain atlas of Franklin and Paxinos (1997). The active region of the dialysis probe was 2.5 mm in length. The orientation of the probe loop in the cortex was sagittal. Mice were used in the experiments about 24 h after the implantation.

2.5. In vivo microdialysis

On the day of the experiment, mice were perfused with Ringer 's solution (in mM: NaCl 147, KCl 4, $CaCl_2$ 2.3) through the dialysis probe at a constant flow rate of 2.5 μl/min using a microinfusion pump (Hao et al., 2005). After a 60 min washout period, the perfusate fractions were collected every 20 min successively. The drugs were administrated when a stable basal value (the levels of glutamate in the last three samples with the variation no more than 10%) was obtained. Additional samples were collected for another 3 h. To test the effect of PF11 on acute morphine, PF11 (4 or 8 mg/kg, i.g.) was given 60 min before morphine (10 mg/kg, i.p.) injection. To test the effects of repeated PF11 on the basal levels of glutamate in chronic morphine-treated mice. PF11 (4 or 8 mg/kg, i.g.) was given 1 h before each morphine treatment (10 mg/kg, i.p.) for 7 days. On day 7, the basal levels of glutamate were recorded as ng/sample in each group in the microdialysis study same as the method described above.

Glutamate contents in the samples were determined using a high-performance liquid chromatography with fluorescence detection system, which was consisted of two pumps (LC-10A, Shimadzu, Japan), a reverse-phase column (5 μ, C_{18}, 200 × 4.6 mm, Diamonsil™), a column oven (CTO-10A, Shimadzu, Japan) and a fluorescence detector (RF-10AXL, Shimadzu, Japan). Twenty microliters of sample was derivatized by incubation for 90 s at 25 °C with 10 μL derivatizing reagent (27 mg *o*-phthalaldehyde, purchased from Sigma, USA, dissolved in 500 μL ethanol, 20 μL

2-mercaptoethanol and 4.5 ml, 0.4 mol/L borate buffer, pH 9.3, stored at 4 °C for 24 h before use). A binary methanol gradient was run with two pumps, A: 50 mM phosphate buffer containing 5% methanol, pH 6.7 and B, methanol containing 2.5 tetrahydrofuran, pH 3.5. The gradient started at 30% B, rose linearly to 60% during the next 7 min, maintained at 60% B for 5 min, and returned to 30% B during the next 3 min, with a flow rate of 1 ml/min. The excitation wavelength of the fluorescence detector was 340 nm and emission wavelength was 450 nm. The column temperature was kept at 35 °C. Standards of glutamate were assayed before and after the dialysis samples.

2.6. Histology

After the final sample was collected, the mouse was sacrificed and histological verification of probe placement was made via frozen coronal sections (30 μm in thickness) using a freezing microtome (AS-620, Shandon, USA). Mice with the correct probe placements were included in the final analysis (Fig. 1).

2.7. Statistical analysis

In the behavioral sensitization test, data were expressed as mean±S.E.M. and analyzed by repeated measures ANOVA with group as between factor (six levels: saline, morphine, PF11 4 mg/kg, PF11 8 mg/kg, PF11 4 mg/kg+morphine, PF11 4 mg/kg+morphine) and day as within factor (two levels: day 1 and day 7) ($n=8$). If significant main effects or interactions were confirmed, individual one-way ANOVAs or Fishers LSD *post hoc* tests were used to further assess the nature of the effect. Within each group, treatment days were compared by one-way

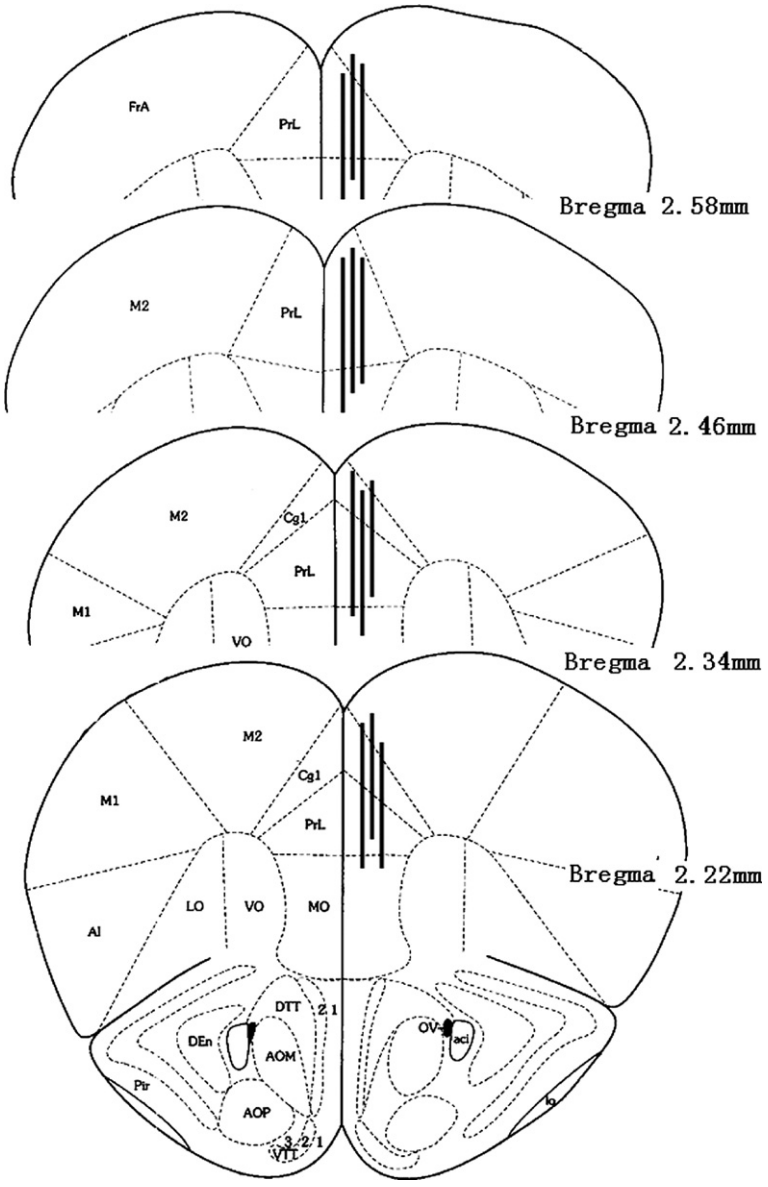

Fig. 1. Location of microdialysis probe in the medial prefrontal cortex. Silhouettes of probe tracks were drawn onto representative section of the mouse brain redrawn from the atlas of Franklin and Paxinos (1997).

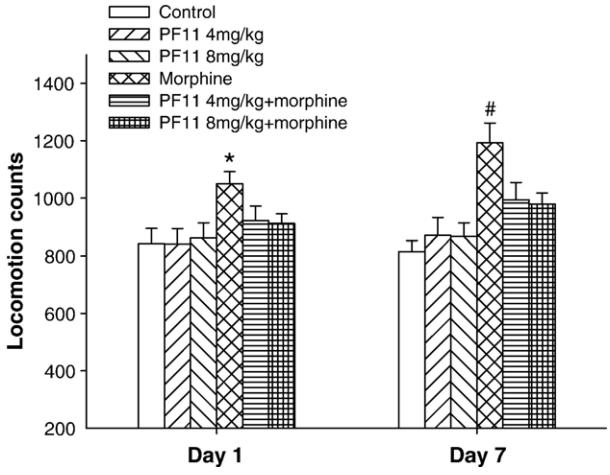

Fig. 2. Effects of pseudoginsenoside-F11 on morphine-induced locomotor sensitization. Morphine (10 mg/kg, i.p.) was given daily for 7 days. Pseudoginsenoside-F11 (4 or 8 mg/kg, i.g.) was given 60 min before morphine injection. Each point represents the locomotor activity (mean±SEM) of 8 mice. *$P<0.05$ compared to saline group on day 1. #$P<0.05$ compared to day 1 of the same group. Repeated measures ANOVA followed by a LSD *post hoc* test.

ANOVA to compare morphine-induced hyperactivity on day 1 with that on day 7 in mice.

In the microdialysis study of acute morphine, the levels of extracellular glutamate were shown in figures as percentages of basal values that were averaged from last three or four pre-drug fractions. Statistical analysis of the effect of drugs on extracellular glutamate levels was performed using repeated measures ANOVA with one between factor group (six levels: saline, morphine, PF11 4 mg/kg, PF11 8 mg/kg, PF11 4 mg/kg+morphine, PF11 4 mg/kg+morphine) and one within factor time (ten levels: 0, 20, 40, 60, 80, 100, 120, 140, 160 and 180) ($n=30$). Individual between-group comparisons were performed by LSD *post hoc* multiple test when appropriate. Simple effects were assessed by one-way ANOVA for each time point. In the microdialysis study of chronic morphine, the levels of glutamate (mean±S.E.M.) were expressed as ng/sample (uncorrected from the probe recovery) and the basal values after chronic treatments were analyzed using one-way ANOVA. Individual between groups comparisons were performed by LSD *post hoc* multiple test.

The levels of significant were taken as $P<0.05$. All statistical procedures were performed using the SPSS 13.0 software for windows (SPSS Inc., USA).

3. Results

3.1. Effects of PF 11 on the development of morphine-induced locomotor sensitization

As seen in Fig. 2, either acute or repeated treatment of PF11 alone had no significant effect on locomotor activity. However, acute morphine 10 mg/kg caused a marked increase in locomotor activity compared to the control group ($P<0.05$). After 7 days treatments, only morphine group obtained behavioral sensitization [$F(1,15)=7.29$, $P<0.05$)]. Nether PF11 4 mg/kg+morphine group [$F(1,15)=0.77$, $P>0.05$)] nor PF11 8 mg/kg+morphine group [$F(1,15)=3.66$, $P>0.05$)] induced behavioral sensitization, indicating morphine-induced hyperactivity and the development of behavioral sensitization were significantly antagonized by pretreatment with both 4 and 8 mg/kg PF11. Repeated measures ANOVA revealed a significant effect of group [$F(5,42)=8.02$; $P<0.001$], but no significant effects of day [$F(1,42)=3.59$; $P>0.05$] and group×day [$F(5,42)=0.81$; $P>0.05$].

3.2. Effects of PF11 on acute morphine-induced decrease of glutamate

The results showed that acute morphine 10 mg/kg decreased extracellular glutamate levels by nearly 20–40% of baseline within 40–120 min after administration. Although 4 mg/kg PF11 neither influenced the basal value of glutamate nor antagonized morphine-induced glutamate decrease. It showed some intendancy to inhibit morphine-induced glutamate decrease (Fig. 3A). At the dose of 8 mg/kg, PF11 could antagonist morphine-induced glutamate decrease. Repeated measures ANOVA revealed a significant effects of group [$F(5,24)=14.74$; $P<0.001$], time [$F(9,216)=14.69$; $P<0.001$] and a significant interaction of group×time [$F(45,216)=5.78$; $P<0.001$]. LSD *post hoc* test indicated PF11 8 mg/kg+morphine group differed from morphine group ($P<0.001$). Notably, basal glutamate level was slightly elevated by 8 mg/kg PF11, but there was no significant difference to control group (Fig. 3B). One-way ANOVA on each time point revealed significant effects of 8 mg/kg PF11 on morphine-induced decrease of glutamate on 160 and 180 min ($P<0.05$).

3.3. Effects of repeated PF11 on acute morphine-induced decrease of glutamate

After repeated administrations of PF11, extracellular glutamate levels did no show any significant differences but a slight

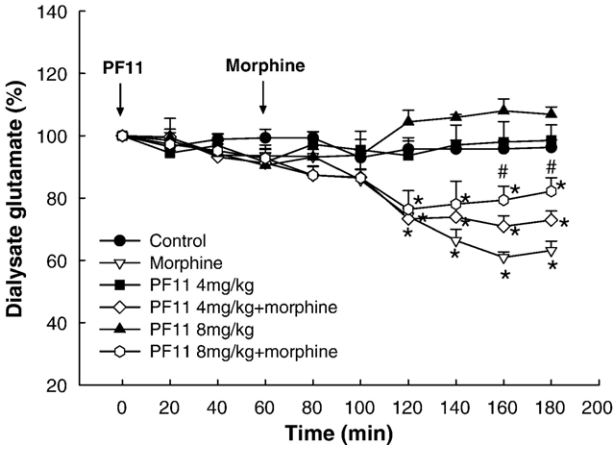

Fig. 3. Effects of pseudoginsenoside-F11 on acute morphine-induced decrease of extracellular glutamate in the medial prefrontal cortex in mice. Pseudoginsenoside-F11 (4 or 8 mg/kg, i.g.) was given 60 min before morphine (10 mg/kg, i.p.) injection. Results are expressed as percentage changes (mean±SEM) from the basal value. $n=5$; *$P<0.05$ compared to saline group on each time point; #$P<0.05$ compared to morphine group on each time point. Repeated measures ANOVA followed by a LSD *post hoc* test.

increase to the control group (11.86±1.88 ng/sample). The levels of glutamate were 14.42±1.41 ng/sample (4 mg/kg PF11) and 14.46±2.08 ng/sample (8 mg/kg PF11), respectively. It is similar as the results obtained in acute treatment, repeated PF11 at the dose of 4 mg/kg had on effect on morphine-induced glutamate decrease. However, the percentage decrease of glutamate is smaller than morphine group (Fig. 4A). Repeated 8 mg/kg PF11 could antagonize morphine-induced glutamate decrease in the mPFC (Fig. 4B). Repeated measures ANOVA revealed significant effects of group [$F(5,24)=12.86$; $P<0.001$], time [$F(9,216)=11.70$; $P<0.001$] and a significant interaction of group×time [$F(45,216)=5.78$; $P<0.001$]. LSD *post hoc* test indicated PF11 8 mg/kg+morphine group differed from morphine group ($P<0.001$). One-way ANOVA on each time point revealed significant effects of repeated 4 mg/kg PF11 on morphine-induced decrease of glutamate on 160 min ($P<0.05$) and repeated 8 mg/kg PF11 on morphine-induced decrease of glutamate during 140 to 180 minute ($P<0.05$).

3.4. Effects of repeated PF11 on the basal level of glutamate in chronic morphine treated mice

Chronic morphine treatment, once a day for 7 days at the dose of 10 mg/kg, decreased the basal levels of glutamate in the mPFC to 5.94±1.35 ng/sample, which were significant lower than the control group (11.86±1.88 ng/sample). Although in PF11+morphine groups glutamate levels did not deduced too sharply with the pretreatments of PF11 compare with the morphine group, 4 mg/kg PF11 could not prevent the decrease of glutamate, only 8 mg/kg PF11 significantly blocked the decrease of glutamate basal levels [Group(5,52)=5.57, $P<0.01$. Fisher 's LSD *post hoc* test indicated morphine and PF11 4 mg/kg+morphine groups differed from the control group ($P<0.001$); PF11 8 mg/kg+morphine group differed from morphine group ($P<0.001$)] (Fig. 5).

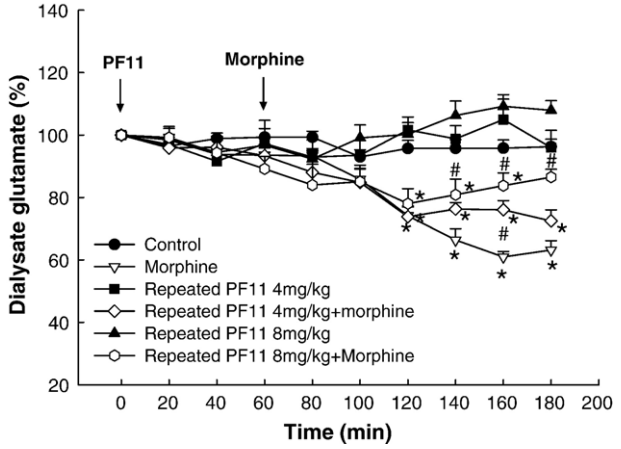

Fig. 4. Effects of repeated pseudoginsenoside-F11 on acute morphine-induced decrease of glutamate in the medial prefrontal cortex in mice. Pseudoginsenoside-F11 (4 or 8 mg/kg, i.g.) was given daily for 6 days, on the test day PF11 was given 60 min before morphine (10 mg/kg, i.p.) injection. Results are expressed as percentage changes (mean±SEM) from the basal value. $n=5$; *$P<0.05$ compared to saline group on each time point; $^{\#}P<0.05$ compared to morphine group on each time point; Repeated measures ANOVA followed by a LSD *post hoc* test vs. control group.

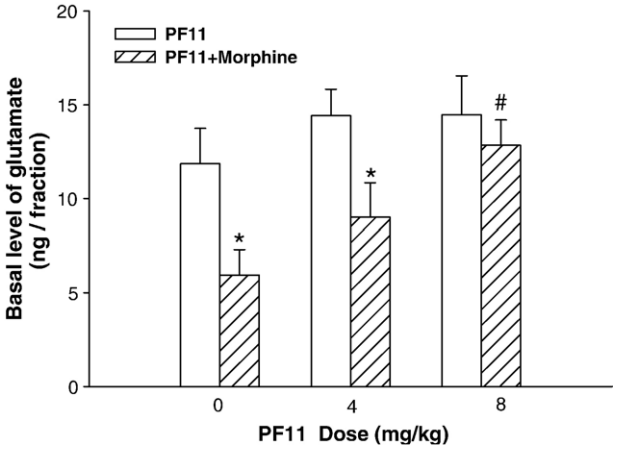

Fig. 5. Effects of repeated pseudoginsenoside-F11 on the basal levels of glutamate in the medial prefrontal cortex in chronic morphine-treated mice. Morphine (10 mg/kg, i.p.) was administered daily for 7 days. Pseudoginsenoside-F11 (4 or 8 mg/kg, i.g.) was given 1 h before each morphine treatment. Results are expressed as ng/sample. $n=5$–6; *$P<0.05$ compared to the control group; $^{\#}P<0.05$ compared to morphine group. One-way ANOVA followed by a LSD *post hoc* test.

4. Discussion

Our previous studies demonstrated PF11, an active ingredient in *Panax quinquefolium* (American ginseng), could antagonize various actions of morphine and MA-induced neurotoxicity (Li et al., 2000; Wu et al., 2003), which implied PF11 might be a potential candidate for treatment of drug abuse. The present results that PF11 prevented the development of morphine-induced behavioral sensitization without influencing locomotor activity per se provided further support on these observations.

Behavioral sensitization is a useful animal model to test plasticity and neuroadaptation related to repeated treatment of opioids (Wolf, 1998; Ikemoto et al., 2000). The mesoaccumbens dopamine system plays an important role in behavioral sensitization. It is reported that morphine has the behavioral stimulating effect through an agonistic action on μ-opioid receptors and resultant acceleration of dopaminergic neurotransmission (Kuschinsky and Hornykiewicz, 1974; Iwamoto, 1981). Evidence also exists that dopamine neurons projecting to the mPFC provide direct and indirect inhibitions of excitatory output to subcortical regions thought to be involved in the initiation of motor activity (Law-Tho et al., 1994; Pierce and Kalivas, 1997; Grobin and Deutch, 1998). Our preliminary studies showed that PF11 antagonized morphine or methamphetamine-induced decrease in DA levels in the limbic area of rat (Wu et al., 2003). Thus, it is reasonable to assume that PF11 might have effects on dopaminergic neurotransmission to affect the development of morphine behavioral sensitization.

Consistent with our previous studies in rats (Hao et al., 2005), the present studies showed that acute morphine significantly decreased the extracellular levels of glutamate in

the mPFC in mice. Single administration of PF11 had no significant effect on the extracellular levels of glutamate, but at dose of 8 mg/kg PF11 could drastically reduce morphine-induced decrease of glutamate. After repeated administrations for 7 days, similar results were obtained. Although PF11 at the lower dose (4 mg/kg) could not antagonize morphine-induced decrease of glutamate, such a kind of tendency was observed, which suggested PF11 maybe act in a dose-dependent manner. Taking together with the results that PF11 antagonized morphine-induced decreases in DA levels, it is suggested PF11 might have effect on the glutamatergic system in mPFC to modulate DA release, and in turn block the development of behavioral sensitization.

The effects of PF11 on the extracellular glutamate may be mediated through different mechanisms. Numerous observations have shown that morphine inhibits the release of glutamate via the activation of opioid receptors. For example, opioid receptors activation decreases glutamate release in the CNS (Nicol et al., 1996; Ostermeier et al., 2000). Morphine decreases capsaicin-evoked release of glutamate from rat spinal dorsal horn slices (Ueda et al., 1995) and veratrine- or K^+-stimulated glutamate release in cerebral cortical slices (Nicol et al., 1996). Experiments in vitro have revealed that PF11 reduces the binding potency of morphine in Chinese hamster ovary (CHO)-mu cells. It significantly attenuates morphine-stimulated [35S] GTPγS binding in a dose-dependent manner and strongly decreases the efficacy of morphine to inhibit intracellular cAMP production (Li et al., 2001). These data suggest, at the cellular level, that PF11 may act as a weak opioid receptor antagonist. Therefore, it is reasonable that PF11 could antagonize morphine-induced glutamate decrease in the mPFC.

After repeated treatment of morphine, the lower basal levels of glutamate were observed, which was similar to the findings in our previously study in rats (Hao et al., 2005). This effect may be due to morphine-induced adaptive changes in the processes of glutamatergic synaptic transmission (Manzoni and Williams, 1999; Xu et al., 2003). Repeated morphine-induced neural damage and maybe exhausted the presynapic glutamate. Evidence also suggests that morphine exerts its inhibitory effects presynaptically, likely through the reduction of Ca^{2+} in nerve terminals, and thereby inhibits the release of glutamate (Yang et al., 2004). Pretreatments of PF11 prevented the decrease of glutamate basal level in the present study. Evidences have showed that ginsenosides, isolated from *Panax ginsing*, prevent the neural damage-induced decrease of glutamate synaptic vesicle numbers in the culture neurons (Wu et al., 2000). Showing a similar property with ginsenosides, PF11 maybe also have neuroprotection effects to prevent the decrease of glutamate levels and improve the material metabolism to increase the production and release of glutamate.

In conclusion, the present study first provided the evidence that PF11 antagonized morphine-induced development of behavioral sensitization and decrease of glutamate in the mPFC in mice. Together with our previous studies, it is suggested that PF11 has effect on glutamatergic system in the mPFC, which may in turn blocks the development of behavioral sensitization. These results imply PF11 might be a potential candidate in the treatment of morphine dependence and addiction.

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