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Antinociceptive effects of systemic paeoniflorin on bee venom-induced various 'phenotypes' of nociception and hypersensitivity

Hou-You Yu [a,c], Ming-Gang Liu [b], Dan-Na Liu [a], Gang-Wei Shang [a], Yan Wang [a], Chao Qi [d], Kui-Ping Zhang [d], Zu-Jun Song [c,*], Jun Chen [a,b,*]

[a] *Institute for Biomedical Sciences of Pain and Institute for Functional Brain Disorders, Tangdu Hospital, Fourth Military Medical University, Xi'an 710038, PR China*
[b] *Institute for Biomedical Sciences of Pain, Capital Medical University, Beijing 100069, PR China*
[c] *Department of Emergency, Xijing Hospital, Fourth Military Medical University, Xi'an 710032, PR China*
[d] *Nhwa Brain Bio-Med-Pharma Co., Ltd. (Shaanxi), Xi'an 710015, PR China*

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Abstract

Paeoniflorin (PF), one of the active chemical compounds identified from the root of *Paeonia lactiflora* Pall, has been well-established to exhibit various neuroprotective actions in the central nervous system (CNS) after long-term daily administration. In the present study, by using the bee venom (BV) model of nociception and hypersensitivity, antinociceptive effects of PF were evaluated by intraperitoneal administration in conscious rats. When compared with saline control, systemic pre- and post-treatment with PF resulted in an apparent antinociception against both persistent spontaneous nociception and primary heat hypersensitivity, while for the primary mechanical hypersensitivity only pre-treatment was effective. Moreover, pre- and early post-treatment with PF (5 min after BV injection) could successfully suppress the occurrence and maintenance of the mirror-image heat hypersensitivity, whereas late post-treatment (3 h after BV) did not exert any significant impact. In the Rota-Rod treadmill test, PF administration did not affect the motor coordinating performance of rats. Furthermore, systemic PF application produced no significant influence upon BV-induced paw edema and swelling. Finally, the PF-produced antinociception was likely to be mediated by endogenous opioid receptors because of its naloxone-reversibility. Taken together, these results provide a new line of evidence showing that PF, besides its well-established neuroprotective actions in the CNS, is also able to produce analgesia against various 'phenotypes' of nociception and hypersensitivity via opioid receptor mediation.

Keywords: Paeoniflorin; Antinociception; Persistent spontaneous nociception; Hyperalgesia; Opioid receptor; Bee venom model

1. Introduction

Paeoniflorin (PF) is one of the active chemical compounds identified from white paeony root (*Paeoniae alba radix* or referred to as Baishao in Chinese), the dried peeled root of *Paeonia lactiflora* Pall. (family Paeoniaceae) (for its chemical structure see Fig. 1) (Chen et al., 1999c; Liu et al., 2005). PF has been previously shown to exhibit profound neuroprotective effects in the mammal central nervous system (CNS), including prevention of calcium overloading-induced cell injury in cultured primary cortex neurons (Tsai et al., 2005), amelioration of spatial cognitive impairment caused by scopolamine in rats (Ohta et al., 1993a,b), improvement of learning impairment of aged rats in an operant brightness discrimination task (Ohta et al., 1994), as well as attenuation of chronic cerebral hypoperfusion-produced learning dysfunction and brain damage (Liu et al., 2006). Further study has also demonstrated that neuroprotective effects of PF are caused by its actions on multiple

* Corresponding authors. Reprints request to Dr. Jun Chen, Institute for Biomedical Sciences of Pain, Tangdu Hospital, Fourth Military Medical University, 1# Xinsi Road, Baqiao District, Xi'an 710038, PR China. Tel.: +86 29 84777942; fax: +86 29 84777945 or Institute for Biomedical Sciences of Pain, Department of Neurobiology, Capital Medical University, #10 You'anmenwai Xitoutiao, Fengtai, Beijing 100069, PR China. Tel.: +86 10 83911513; fax: +86 10 83911491.
E-mail addresses: junchen@fmmu.edu.cn, chenjun@ccmu.edu.cn (J. Chen).

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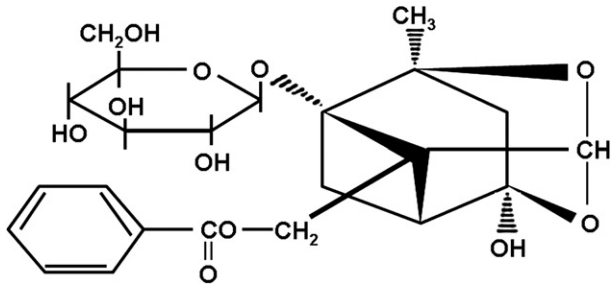

Fig. 1. Chemical structure of PF.

targets in the CNS, including arachidonic acid metabolism pathways, nitric oxide system, mitogen-activated protein kinases, the nuclear factor-κB pathway and several molecules involved in neuronal damage (Chen et al., 2006). However, so far the antinociceptive effects of PF or *Paeoniae alba radix* remain less studied experimentally, with one exception (Tsai et al., 2001).

It has been gradually known that clinical pain has multiple symptoms and signs, associated with tissue or nerve injury in forms of persistent spontaneous pain, thermal and mechanical hyperalgesia or allodynia evoked by different modalities of stimuli (Merskey and Bogduk, 1994). Similar to diverse manifestations of clinical pain, experimental studies have also demonstrated that genetic 'phenotypes' of nociception and hypersensitivity are distinct and can be basically classified into 5 types, including: (1) baseline thermal nociception; (2) spontaneous responses to noxious chemical stimuli; (3) thermal hypersensitivity; (4) mechanical hypersensitivity; and (5) afferent input-dependent hypersensitivity (Lariviere et al., 2002). Based upon genetic 'phenotypes' of pain, regardless of etiology, duration, assay methods or animal models, all of the above five types are considered to be stimulus modality-related in terms of chemical, thermal and mechanical stimuli or state-related in terms of physiological and pathological states (Lariviere et al., 2002; Shang et al., 2006). Thus, to realize better control of clinical pathological pain, it is of particular importance to screen potential analgesics from traditional Chinese medical herbs by evaluating their antinociceptive roles in an animal model of pain with up to three types of nociception and hypersensitivity in an individual subject (Chen, 2007). This could definitely minimize the discrepancy of a drug effect caused by inter-model differences.

The bee venom (BV) test, a well-established experimental animal model mimicking honeybee sting-induced natural tissue injury, is produced by subcutaneous (s.c.) injection of a given dose of honeybee venom into one hind paw (Lariviere and Melzack, 1996; Chen et al., 1999b; Chen and Chen, 2001; Lariviere et al., 2002). Following s.c. BV treatment, the animal shows most types of nociception and hypersensitivity corresponding to the above genetic classification, including: (1) immediate persistent spontaneous nociception (PSN) such as the paw flinching reflex and licking; (2) primary thermal hypersensitivity; (3) primary mechanical hypersensitivity and (4) secondary or mirror-image heat hypersensitivity (Chen et al., 1999b, 2000; Chen and Chen, 2001; Lariviere et al., 2002; Chen, 2003, 2007). Previous electrophysiological studies have shown that BV-elicited PSN and hypersensitivity are mediated by a persistent increase in spontaneous discharges and subsequent enhanced heat/mechanical responsiveness of wide dynamic range (WDR) neurons in the spinal dorsal horn, suggesting a contribution of spinal neuronal plasticity to various pain-related behaviors in the BV test (Chen et al., 1998, 1999a; You and Chen, 1999; You et al., 2002; Zheng et al., 2002). In addition, our behavioral pharmacological survey found that different intracellular messenger-mediated signal transduction pathways were likely to be involved in mediation of different temporal processing of nociception and hypersensitivity (Chen, 2003, 2007). Hence, the purpose of the present study was to employ the BV model to evaluate possible antinociceptive effects of systemic PF administration against multiple aspects of nociception and hypersensitivity.

2. Materials and methods

2.1. Animals

The experiments were performed on Sprague–Dawley male albino rats weighing from 180 to 250 g. The animals were provided by the Laboratory Animal Center of Fourth Military Medical University (FMMU) and use of the animals was reviewed and approved by the FMMU Animal Care and Use Committee. The present work was carried out in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23 revised 1985) or the UK Animals Scientific Procedures Act 1986 and associated guidelines, or the European Communities Council Directive of 24 November 1986 (86/609/EEC). The animals were housed in plastic boxes in groups of 4–6 at 22–26 °C with food and water available *ad libitum* in the colony room. A 12:12 h light dark cycle with lights on at 08:00 was maintained and testing was done between 9:00 and 18:30. The rats were acclimated to the laboratory and habituated to the test boxes for at least 30 min each day for 5 days before testing.

2.2. Drugs

PF was provided by the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China) and dissolved in physiological saline in different concentrations for intraperitoneal (i.p.) injection. Naloxone was purchased from Beijing Four-Rings Pharmaceutical Company (Beijing, China) and dissolved in saline. A volume of 0.05 ml saline containing 0.2 mg lyophilized whole venom of honeybee (*Apis mellifera*, Sigma, St. Louis, MO, U.S.A.) was used during the whole experiment since it has been shown to be the optimal dose to induce both long-lasting spontaneous nociception and hyperalgesia in previous studies (Lariviere and Melzack, 1996).

2.3. Assessment of spontaneous pain-related behaviors

A 30×30×30 cm transparent plexiglas test box with a transparent glass floor was placed on a supporting frame of 30 cm

high above the experimental table to allow the experimenters to observe the paws of the animals without obstruction. The rat was placed in the test box for at least 30 min before administration of any chemical agents. After the acclimation period, s.c. injection of BV was made into the center of the plantar surface of one hind paw with slight restraint. The rat was then returned to the test box, and pain-related spontaneous responses were determined by counting the number of paw flinches occurring during 5 min intervals for 1 h following BV injection.

2.4. Behavioral assays of pain sensitivity

2.4.1. Quantitative measurement of thermal pain sensitivity

Thermal pain sensitivity of rats was determined by testing paw withdrawal thermal latency (PWTL, s) in response to heat stimuli applied in bilateral hind paws. As described previously (Chen et al., 1999b), the rat was placed on the surface of a 2 mm thick glass plate covered with a plastic chamber (20×20×25 cm) to measure the sensitivity to heat stimuli with a TC-1 radiant heat stimulator (new generation of RTY-3 made in Xi'an Bobang Technologies of Chemical Industry Co. Ltd., PR China). The radiant heat source was a high intensity halogen lamp bulb (150 W) positioned under the glass floor directly beneath target area on the hind paw. The distance between the projector lamp bulb and lower surface of the glass floor was adjusted to produce a light spot on the floor surface with 5 mm diameter. Four stimuli were repeated for each site and the latter three values were averaged as mean PWTL. The inter-stimulus interval was more than 10 min at the same site on the same hind paw and 5 min for different site of another hind paw. The thermal latency was determined as the duration from the onset of heat stimulus to the occurrence of hind paw withdrawal reflex. The stimulus was stopped if the latency exceeded 30 s so as to avoid excessive tissue injury and the region was considered to be completely unresponsive. For comparison of PWTL between groups, % inhibitory effect was obtained through equation: % inhibitory rate = (test latency − baseline / cutoff − baseline) × 100%.

2.4.2. Quantitative measurement of mechanical pain sensitivity

Mechanical pain sensitivity of rats was determined by testing paw withdrawal mechanical threshold (PWMT, g) in response to mechanical stimuli applied in ipsilateral hind paw produced by ascending graded individual von Frey monofilaments with bending forces of 2.0, 2.5, 3.0, 4.0, 5.0, 7.0, 10.0, 15.0, 20.0, 25.0, 30.0, 35.0, 40.0, 50.0, 60.0 g. The rat was placed on a metal mesh floor covered with the same plastic chamber and von Frey filaments were applied from underneath the metal mesh floor to the testing site of the target hind paw. A von Frey filament was applied 10 times (several seconds for each stimulus) to each testing area. The bending force of the von Frey filament being able to evoke an approximate 50% occurrence of paw withdrawal reflex was expressed as the PWMT. The stimulus was stopped if the threshold exceeded 60.0 g (cutoff value). For comparison of PWMT between groups, % inhibitory effect was obtained through equation: % inhibitory rate = (test threshold − baseline / cutoff − baseline) × 100%.

2.5. Quantitative measurement of local inflammatory response

A plethysmometer (Ugo, Basile, Italy) was used to measure the volume of rat hind paw prior to and 1 h after s.c. administration of BV. The increase in volume of the injected hind paw was an index to the inflammatory intensity. The injected hind paw was immersed into a water cell full of 0.04–0.05% NaCl solution according to the user's manual, and the volume of displacement was the volume of hind paw. The increment in paw volume (IPV) was calculated as: %IPV = test volume − baseline volume / baseline volume.

2.6. Assessment of motor coordinating performance of rats

Motor coordination of rats was tested by a Rota-Rod treadmill (Ugo, Basile, Italy). The accelerating speed of the Rota Rod was set to increase from 6 r/min to 30 r/min within 2 min. The animals were placed on the treadmill and the timers were started with acceleration and automatically stopped when the animal fell off, with a maximal cutoff time of 300 s. Animals were trained on the Rota-Rod at least three times to allow accommodation to the testing apparatus before drug injection (PF, 200 mg/kg, i.p.).

2.7. Experimental design

For evaluating the effects of PF on PSN, the drug was systemically administered 10 min prior to (three doses: 5 mg/kg, 50 mg/kg and 100 mg/kg, i.p) or 5 min after s.c. BV injection (50 mg/kg, i.p.) and the testing of PSN began immediately after BV or PF treatment for pre- and post-administration paradigm, respectively. For examining its effects on thermal and mechanical hypersensitivity, in the pre-treatment paradigm, the same three doses of PF were intraperitoneally applied 10 min prior to s.c. BV injection, while, in the post-treatment paradigm, PF at a dose of 50 mg/kg was administered either 5 min or 3 h after s.c. BV injection so as to see whether there existed any differences in antihyperalgesic actions of PF when given at different time points. To note, in any case, testing of thermal or mechanical hypersensitivity was consistently performed at 3 h after s.c. BV injection. In general, to exclude the side-effects brought about by experimenting orders, we performed PWMT measurements first followed by PWTL testing. It is also important and necessary to mention here that our preliminary experiments found no significant effects of PF administration on basal thermal latency or mechanical threshold under normal conditions (without BV-initiated inflammation), so the single PF application group was not incorporated in the present experimental design.

To clarify whether PF has any impact on motor coordinative performance, after three trials of accommodation, a high dose of PF (200 mg/kg, i.p.) was given and then Rota-rod test was performed for another three trials. Rats without any treatment or with injection of equal volume of vehicle were also included and compared. As for the inflammation assays, following a baseline test, three doses of PF (5 mg/kg, 50 mg/kg and 100 mg/kg, i.p) were administered followed by s.c. BV treatment 10 min later and the hind paw volume was measured at 1 h after BV injection. In those experiments regarding naloxone (2 mg/kg body weight,

i.p.) preconditioning, PF (50 mg/kg, i.p.) was administered 10 min after naloxone injection followed by intraplantar BV administration. Then PSN, PWTL and PWMT were sequentially measured.

2.8. Data analysis

All results were expressed as mean±SEM. Two-way repeated ANOVA was used to analyze group differences in mean time courses of PSN and motor coordinating performance (treadmill test). One-way ANOVA (Fisher's PLSD test) was applied to compare differences in averaged mean numbers of flinching reflex for 1 h in the pre-treatment paradigm, the thermal or mechanical hypersensitivity measurements as well as inflammatory assays. Student's *t*-test was adopted to analyze differences in averaged post-treatment data of PSN. A statistical difference was accepted as significant if P value <0.05.

3. Results

3.1. Effects of i.p. PF administration on PSN induced by s.c. BV injection

In the BV test, s.c. injection of whole BV solution could immediately produce PSN revealed by long-term spontaneous paw flinching or licking for 1–2 h (Chen et al., 1999b; Chen and Chen, 2001; Chen, 2003, 2007), which could also be evidently seen in our control group (Fig. 2). In comparison with vehicle control, i.p. pre-treatment with PF (5 mg/kg, 50 mg/kg and 100 mg/kg) resulted in a dose-dependent suppression of the BV-induced persistent paw flinching reflex (Fig. 2A) over the 1 h time course observation. The significant inhibition of PSN by PF started from the first 5-min time block and lasted for 15 min to 50 min with different doses. The total mean number of paw flinches of 1 h time course following pre-treatment with saline or PF was shown in Fig. 2B. The inhibitory rate of PF at 5 mg/kg, 50 mg/kg and 100 mg/kg was 26.32%, 50.20% and 59.57%, respectively (Fig. 2B).

Systemic post-treatment with PF at 50 mg/kg 5 min after s.c. BV could also produce 19.43% inhibition of the BV-induced PSN in the subsequent 55 min time course when compared with the control group (Fig. 2C and D). In the post-treatment group, the inhibitory action of PF started from 5 min after i.p. application and lasted for nearly 20 min.

3.2. Effects of i.p. PF administration on thermal hypersensitivity induced by s.c. BV injection

Consistent with our previous results (Chen et al., 1999b, 2000; Chen and Chen, 2000, 2001; Chen, 2003, 2007), s.c. bee

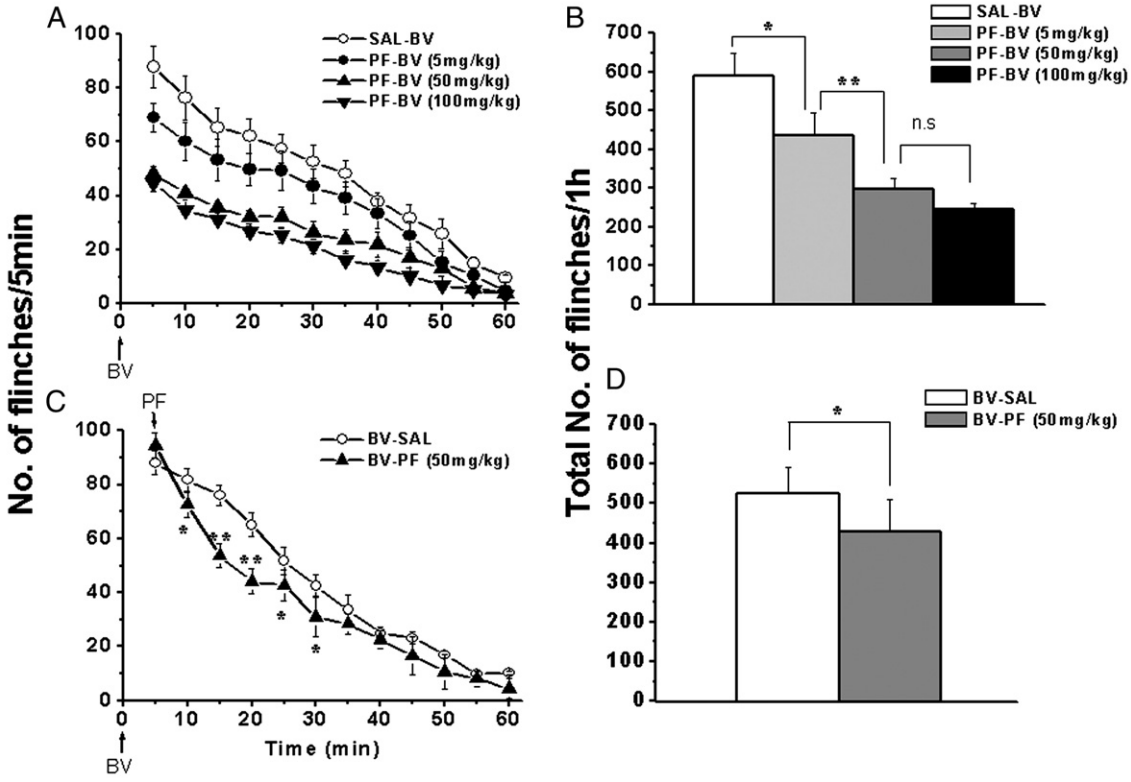

Fig. 2. Effects of i.p. pre- (5 mg/kg, 50 mg/kg, 100 mg/kg) or post-treatment (50 mg/kg) with PF or vehicle (SAL, normal saline) on the induction and maintenance of persistent spontaneous flinching reflex induced by s.c. BV injection. Curve graph (A, C) showing mean time courses and column graph (B, D) showing total mean number of paw flinches for a period of 1 h. For pre-treatment paradigm (A and B), PF was administered 10 min prior to s.c. BV injection (upright arrow) and behavioral measurement was performed immediately after BV treatment. For post-treatment paradigm (C and D), PF was applied (reverse arrow) 5 min after BV injection (upright arrow) and PSN measurement was performed immediately after PF treatment. $n=8$ for each group. *$P<0.05$, **$P<0.01$; n.s., no significance. Vertical bars: ±SEM.

venom injection could result in significant reduction of thermal latency in bilateral hind paws in the vehicle control group, implicating the occurrence of both primary and mirror-image heat hyperalgesia (Fig. 3). Pre-treatment with PF caused a partial prevention of the occurrence of thermal hyperalgesia identified in both primary injury site and non-injected hind paw (Fig. 3A). The inhibitory effects were statistically significant for all three doses, although there was no dose-dependence here. The thermal latency in the injection site was increased by 27.11%, 29.46% and 28.71%, while that in the non-injected hind paw was increased by 22.24%, 22.86% and 24.15% at three doses of PF, respectively (Fig. 3A).

Systemic post-treatment with PF at 50 mg/kg 5 min after s.c. BV injection also exerted marked reversal effects on both primary and mirror-image heat hypersensitivity (Fig. 3B). The inhibitory rate was 24.52% for primary heat hyperalgesia and 19.18% for mirror-image hyperalgesia (Fig. 3B).

Interestingly, when administered 3 h after BV injection (at this time the hyperalgesic state was fully established), the same dose of PF could only reverse the maintenance of primary heat hyperalgesia without affecting the mirror-image heat hyperalgesia (Fig. 3C). For primary thermal hyperalgesia, PF treatment produced a 18.49% increase of thermal latency.

3.3. Effects of i.p. PF administration on mechanical hypersensitivity induced by s.c. BV injection

In saline-pretreated group, s.c. injection of whole BV solution elicited a significant decrease in the mechanical threshold of the injected hind paw, indicating generation of primary mechanical hyperalgesia (Fig. 4), which was also in line with our previous findings (Chen et al., 1999b; Chen and Chen, 2001; Chen, 2003, 2007). Pre-treatment with PF clearly blocked the development of primary mechanical hypersensitivity although

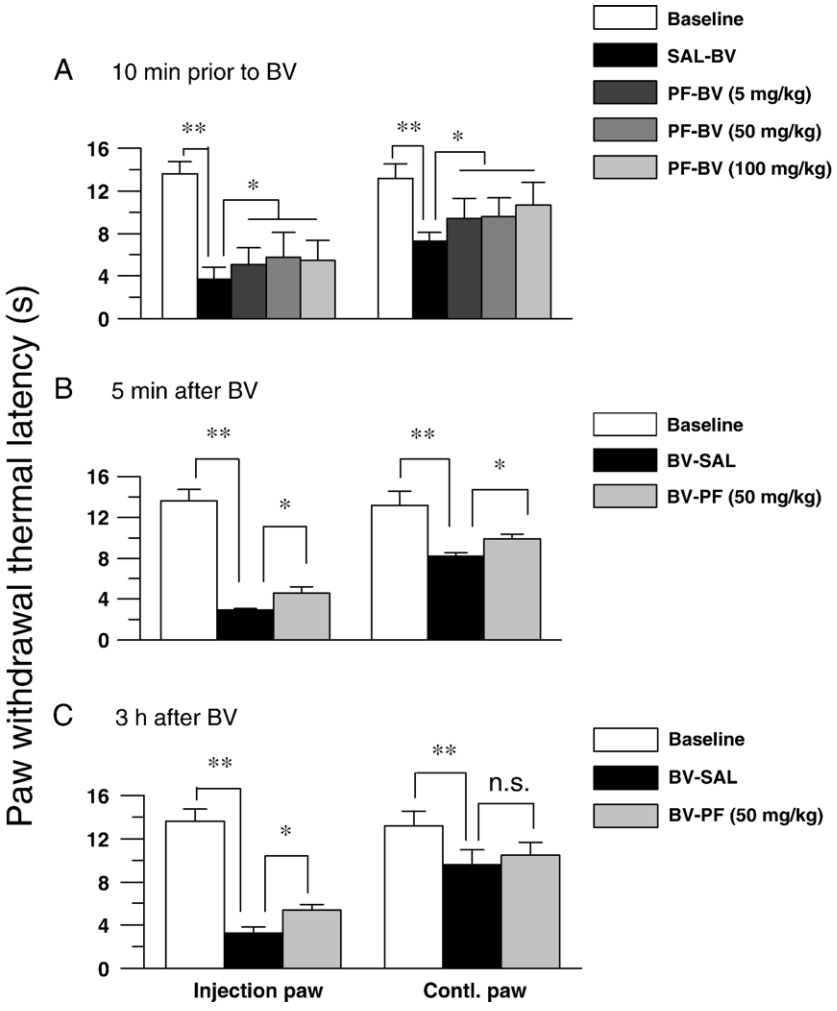

Fig. 3. Effects of i.p. pre-treatment (5 mg/kg, 50 mg/kg, 100 mg/kg), post-treatment at 5 min (50 mg/kg) and 3 h (50 mg/kg) after s.c. BV injection with PF or vehicle (SAL, normal saline) on the induction and maintenance of primary (Injected paw) and mirror-image (Contl. paw) heat hyperalgesia. For pre-treatment paradigm (A), performance assays were begun 10 min after PF treatment (at the time point of BV injection). For post-treatment paradigm (B and C), PF was administered at either 5 min (B) or 3 h (C) after BV injection and behavioral testing was performed uniformly at 3 h time point. $n=8$ for each group. *$P<0.05$, **$P<0.01$; n.s., no significance. Vertical bars: ±SEM.

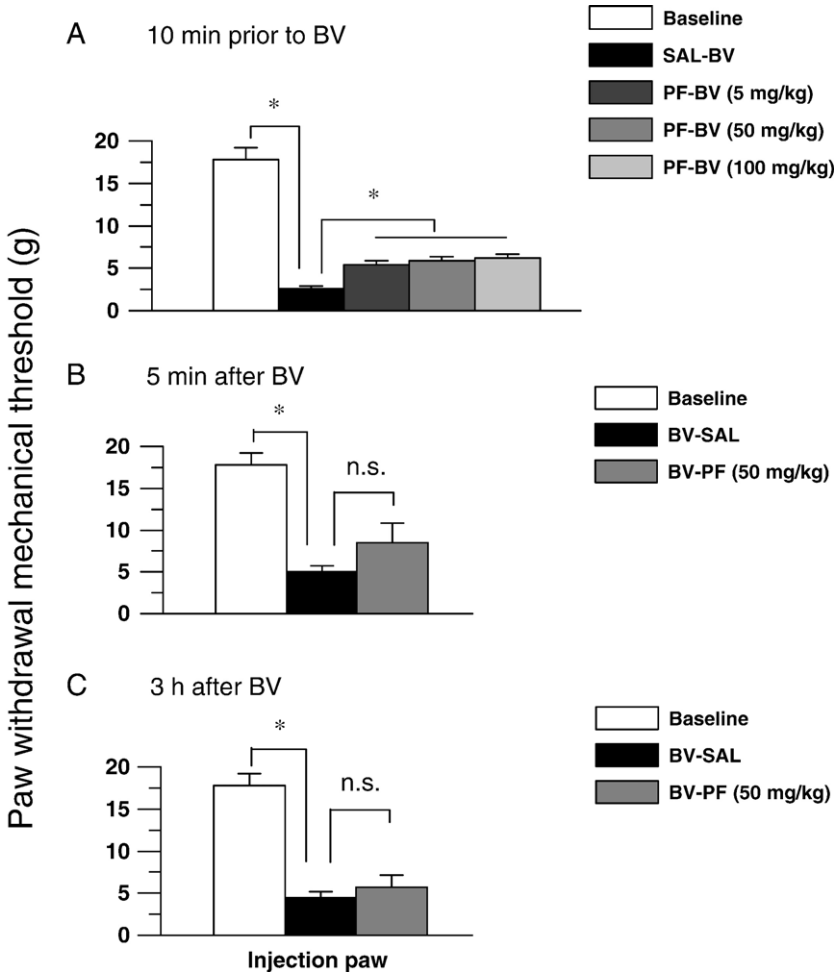

Fig. 4. Effects of i.p. pre-treatment (5 mg/kg, 50 mg/kg, 100 mg/kg), post-treatment at 5 min (50 mg/kg) and 3 h (50 mg/kg) after s.c. BV injection with PF or vehicle (SAL, normal saline) on the induction and maintenance of primary (Injected paw) mechanical hyperalgesia. For pre-treatment paradigm (A), performance assays were begun 10 min after PF treatment (at the time point of BV injection). For post-treatment paradigm (B and C), PF was administered at either 5 min (B) or 3 h (C) after BV injection and behavioral testing was performed uniformly at 3 h time point. $n=8$ for each group. *$P<0.05$, **$P<0.01$; n.s., no significance. Vertical bars: ±SEM.

no dose-dependence was observed either (Fig. 4A). The mechanical threshold in the injection site was increased by 52.77%, 53.24% and 53.82% at three doses of PF, respectively (Fig. 4A).

However, i.p. post-treatment with the same dose of PF failed to produce any significant influence upon the established primary mechanical hyperalgesia, with application at either 5 min or 3 h after BV injection (as shown in Fig. 4B and C).

3.4. Effects of i.p. PF administration on local inflammation induced by s.c. BV injection

It is well known that s.c. injection of BV could evoke long-term (lasting for 48–96 h) inflammatory responses such as increase in skin temperature, paw edema and local plasma extravasation (Lariviere and Melzack, 1996; Chen et al., 1999b; Calixto et al., 2003). IPV (see Materials and methods for detailed computation) tested 1 h after s.c. BV treatment was not significantly affected by i.p. pre-treatment with PF at any dose used in the present study when compared with the value of saline control group (PF versus saline: 5 mg/kg, 62.17±4.30%; 50 mg/kg, 62.15±1.65%; 100 mg/kg, 61.15±4.43% versus 55.43±4.89%, $n=8$, $P>0.05$).

3.5. Effects of i.p. PF administration on motor coordinating performance of rats

In the present study, using Rota-Rod treadmill test, we examined the possible influence of drug administration on motor coordination ability of experimental animals. As shown in Table 1, there was a trial-dependent increase in the time spent on the treadmill for all three groups of rats. However, that value became stable since the fourth trial, suggesting the fulfillment of an adaptation to the testing apparatus. Thus we took the last three trials for evaluation of the drug effects. Our results revealed no significant difference in the time spent on the

Table 1
Effects of systemic pre-treatment with PF (200 mg/kg, i.p.) on motor coordinating performance of rats measured by Rota-Rod treadmill test

	Baseline (s)	Saline (s)	PF (s)
T1 (0 min)	42.47±3.41	52.35±4.43	55.21±7.40
T2 (30 min)	152.07±13.34	147.46±15.23	139.42±16.39
T3 (60 min)	229.85±12.76	246.37±15.25	203.05±17.30
T4 (120 min)	235.30±16.86	244.00±27.23	237.62±9.65^{NS}
T5 (150 min)	247.38±18.65	238.84±8.10	230.64±23.93^{NS}
T6 (300 min)	224.58±23.83	251.08±24.68	216.55±12.36^{NS}

Data are expressed as mean±SEM from 5 animals for each group. NS, no significance between PF-treated group and saline control.

treadmill among the three tested groups ($n=5$ for each group, $P>0.05$, see Table 1).

3.6. Effects of naloxone preconditioning on antinociception induced by i.p. PF administration

Systemic naloxone preconditioning (2 mg/kg, i.p. $n=6$), a non-selective opioid receptor antagonist, 10 min prior to PF (50 mg/kg, i.p) administration, almost completely eliminated the antinociceptive effects of PF against the BV-induced PSN, primary heat and mechanical hypersensitivity as well as mirror-image heat hypersensitivity (Fig. 5A–D). Systemic pre-treatment of rats with the equal volume of saline ($n=6$) did not significantly affect the PF-produced analgesia.

4. Discussion

Introduction of the BV model, characterized by co-existence of both PSN and hyperalgesia to thermal and mechanical stimuli applied in the injection site or non-injected hind paw, made it possible to screen new endogenous and exogenous analgesics on the basis of experimental evidence (Chen, 2003, 2007). In the present study, using the BV tonic pain model, we systemically investigated the potential antinociceptive effects of PF, a major bioactive component of Paeony roots, against diverse 'phenotypes' of nociception and hypersensitivity relevant to clinical pathological pain. First, our results showed that either i.p. pre- or post-treatment with PF could dose-dependently suppress both induction and maintenance of the persistent flinching reflex evoked by s.c. BV injection (Fig. 2). The development of spontaneous nociception has already been demonstrated to be peripherally-dependent upon ongoing primary afferent impulses originating from the injury site, because peripheral nerve blockade could almost completely abolish BV-elicited persistent increase in spontaneous discharges of spinal dorsal horn WDR neurons and typical behavioral responses (Chen et al., 1996, 1998; Chen and Chen, 2001; Chen, 2003, 2007). Thus, based on

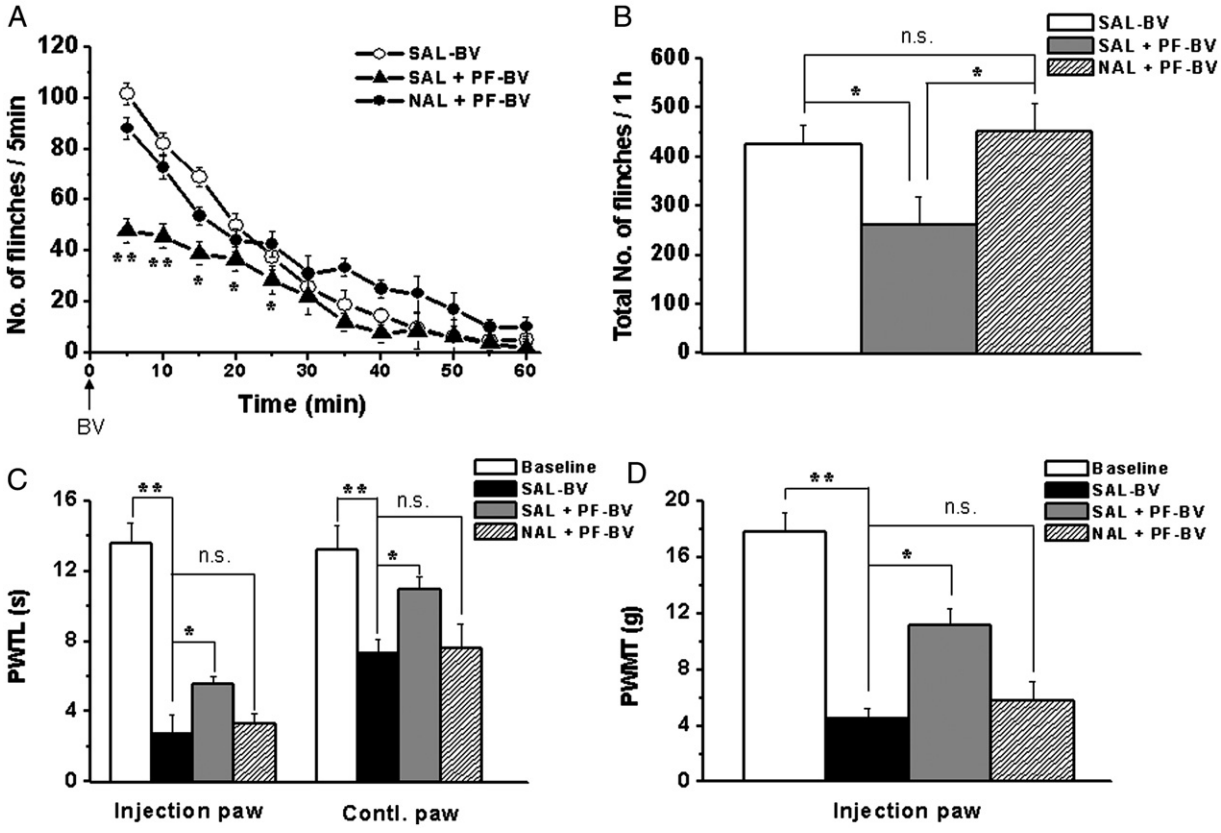

Fig. 5. Influence of pre-treatment with naloxone (NAL, 2 mg/kg, i.p. 10 min before PF administration) or vehicle (SAL, normal saline) on PF antinociception. PF (50 mg/kg, i.p.) was administered 10 min prior to s.c. BV injection. Curve graph (A) showing mean time courses and column graph (B) showing total mean number of paw flinches for a period of 1 h. (C) and (D) showing the influence of naloxone preconditioning on antinociceptive effects of PF against thermal (C) and mechanical (D) hyperalgesia. $n=6$ for each group. *$P<0.05$, **$P<0.01$; n.s., no significance. Vertical bars: ±SEM.

previous findings and present observations, it is reasonable to speculate that PF, when systemically applied, might exhibit antinociceptive effects against BV-produced PSN partially through its probable actions on peripheral nociceptors. In addition, our neuropharmacological survey has disclosed a list of membrane receptors, ion channels and intracellular signaling molecules that may be involved in the generation and maintenance of PSN. It would be interesting, therefore, to elucidate the possible relationship between antinociception of PF and these pharmacological targets.

Our behavioral data regarding PF-induced depression of PSN in the BV test are in accord with one previous study in the formalin test where both early and late phase of the formalin response were dose-dependently suppressed by intracerebroventricular (i.c.v.) pre-treatment with PF (Tsai et al., 2001). There are large numbers of differences between these two reports, such as ways of drug application (i.c.v. versus i.p.), animal species (mice versus rats), drug doses (48, 96, 240, 480 μg versus 5, 50 100 mg/kg) and applied methods used to measure spontaneous nociception (licking time versus number of flinches). Moreover, the neural mechanisms and intracellular signal transduction molecules underlying the BV and the formalin model are also quite different (Chen et al., 1996, 1998, 1999b; You and Chen, 1999). Despite so many disparities, our and other results point unanimously to the fact that pre-administration of PF, i.p. or i.c.v., can immediately inhibit the development of persistent pain and this effect can last for at least 40–50 min. In addition, our current results extend those previous findings by showing that post-treatment with PF could also suppress the maintenance of BV-elicited PSN. This dual blockade of both induction and maintenance of PSN by PF might have important clinical implications in that the compound from Paeony root may be potentially used to both prevent and cure persistent spontaneous pain frequently experienced by chronic pain patients.

The present results also provided a new line of evidence for the antihyperalgesic or antiallodynic actions of PF. It is very important to point out that our pilot experiments failed to detect any significant influence exerted by PF on basal mechanical thresholds or thermal latencies in naive rats (data not shown), suggesting that the PF-dependent signaling pathways associated with antinociception or analgesia are not potently activated under physiological conditions, which precisely meet clinical requirements for pain-relief drugs. The current results demonstrated that either pre- or post-treatment with PF could effectively prevent or reverse BV-induced primary and mirror-image heat hyperalgesia (Fig. 3). Nevertheless, as shown in results of statistical analysis, PF exhibited its antihyperalgesic effects against primary thermal hyperalgesia regardless of the time for administration (prior to or 5 min after or 3 h after BV injection), whereas it displayed its inhibitory actions on mirror-image thermal hyperalgesia only when administered prior to or 5 min after BV treatment. The lack of effectiveness of PF on maintenance of mirror-image heat hyperalgesia when given 3 h after s.c. BV injection is not likely due to the dose used in the current study, because the same dose of PF produced significant repression of PSN as well as primary heat hyperalgesia. The distinction in antihyperalgesic actions of PF against primary and mirror-image heat hypersensitivity indicates that the underlying mechanisms of them are probably mediated by different neuronal chemical components, which has been described previously (Chen and Chen, 2000; Li and Chen, 2003; Chen, 2007). Since the mirror-image heat hypersensitivity has been proved to be maintained by a kind of NMDA and non-NMDA-dependent central sensitization after a time window of spinal summation of ongoing primary afferent barrages as well as descending nociceptive facilitation (Chen et al., 2000, 2001, 2003), it could be logically expected that later treatment of PF after full establishment of these processes might be less operative due to coming of more complicated neural processing. In contrast to heat hypersensitivity, PF was shown to be only effective in suppressing primary mechanical hyperalgesia when applied prior to peripheral noxious insult (Fig. 4). The exact reasons for pharmacological disparation between thermal and mechanical hypersensitivity in the present study are not fully understood, but likely attributable to different central and peripheral neurochemical pathways underlying them (Zheng and Chen, 2001; Li and Chen, 2003). As delineated in our previous pharmacological survey, mGluRs/NKs/VDCC-DAG-PKC pathway is predominantly responsible for the development of BV-induced primary heat hyperalgesia, while the mGluRI-cAMP-PKA pathway is essentially responsible for the development of BV-induced primary mechanical hypersensitivity (Li and Chen, 2003; Chen, 2003, 2007). More importantly, it should be noted that the antinociceptive effects of PF against heat or mechanical hypersensitivity are relatively mild when compared with its impact on PSN (see Figs. 2, 3 and 4 for a comparison). Combined our results showing suppression of BV-evoked PSN by PF with the fact that the half life of PF is less than 2 h in rats (Chen et al., 1999c; Liu et al., 2005), it might be reasonably hypothesized that those observed antihyperalgesic actions of PF should be secondary to its initial inhibition of PSN and associated afferent activity, since we have clearly demonstrated the direct dependence of central hyperexcitable state on the amount of peripheral afferent input (Chen et al., 2001; Chen and Chen, 2001). Taken together, it is suggested that the PF antinociception is closely associated with its time of administration for different ‘phenotypes’ of nociception and hypersensitivity revealed by the BV model, reflecting the possible involvement of different neural signaling systems in the temporal processing of various ‘phenotypes’ of pain and hyperalgesia in terms of thermal, mechanical and chemical features (Chen, 2003, 2007).

The current Rota-rod test showed that i.p. PF administration at a much higher dose (200 mg/kg, i.p.) did not affect the motor coordinating performance of rats when compared with saline-treated or naïve animals, suggesting that the antinociceptive effects of PF against pain-related behaviors are mainly caused by its selective actions in somatosensory system without any contribution from the somatomotor system.

To explore the potential working machinery employed by PF to exert antinociception, we, at first, evaluated its anti-inflammatory effects by using the IPV as an index. The present experiments did not detect any significant alterations in IPV

following pre-treatment with PF. It is well known that BV-initiated inflammatory response is mediated by both pro-inflammatory/inflammatory mediators of local tissue origin and neurogenic components of inflammation consisted mainly of capsaicin-sensitive primary afferent-based dorsal root/axonal reflex (CSPA-DRR/AxR) (Willis, 1999; Chen, 2003; Li and Chen, 2004). Due to lack of effects on increased paw volume, it is reasonable to assume that PF may not perform its antinociceptive actions through interfering with formation of local inflammation surrounding the injury site. However, our available data cannot exclude the possibility that PF may somewhat disrupt the functions of spinal glial cells possibly activated by peripheral inflammatory insult (remaining testified), hence blocking the subsequent release and activities of inflammatory mediators or bioactive cytokines from microglia or astroglia and ultimately leading to antinociception via alleviating central inflammatory responses. Also, according to our current findings, we are still unable to distinguish neurogenic inflammatory components from local tissue origin-derived inflammatory components and, of course, it is still hard to say whether both or only one of them is resistant to PF administration. Future more detailed studies are thus required to further address these problems.

Next, we designed another series of experiments to determine the contribution of endogenous opioid receptor system to the PF antinociception by means of pharmacological blocking tools. As illustrated, pre-treatment with naloxone (2 mg/kg, i.p., 10 min prior to PF application), a non-selective opioid receptor antagonist, could apparently abolish the antinociceptive effects of PF against all 'phenotypes' of nociception and hypersensitivity induced by s.c. BV injection (Fig. 5). This phenomenon, with respect to PSN, is partially consistent with one previous study, in which naloxone preconditioning (1 mg/kg, i.p., 10 min before administration of PF) could potently reverse the antinociception of PF in the formalin test (Tsai et al., 2001). However, the reversal of PF actions by naloxone was only evident in the first phase, but not the second phase, while, in the present work, naloxone-reversible effects were sustained for the whole experimental period. The discrepancy is likely ascribed to the basal differences between these two animal models (Dubuisson and Dennis, 1977; Chen et al., 1996, 1998, 1999b; Lariviere and Melzack, 1996; You and Chen, 1999; You and Arendt-Nielsen, 2005). As reported previously, s.c. injection of dilute formalin produced different spontaneous pain-related behaviors in different animal species (Dubuisson and Dennis, 1977; Tjolsen et al., 1992). Unlike the formalin test, it was found that s.c. injection of bee venom resulted in a prolonged, monophasic nociceptive response regardless of animal species (Lariviere and Melzack, 1996; Chen et al., 1998, 1999b; Chen, 2003, 2007). Moreover, following s.c. bee venom administration, dramatical primary mechanical and heat hyperalgesia developed, whereas, following s.c. formalin injection into the same region, dramatic hypoalgesia or analgesia to mechanical and heat stimuli was repeatedly observed (Chen et al., 1999b). BV treatment also elicited enhanced thermal hypersensitivity in the contralateral, non-injected hind paw, which was never seen in the formalin test (Chen and Chen, 2000; Chen et al., 2000, 2001, 2003). These behavioral findings were further confirmed in electrophysiological studies (Dickenson and Sullivan, 1987; Chen et al., 1996, 1998; You and Chen, 1999; You and Arendt-Nielsen, 2005). Taken together, it is believed that the neural mechanisms and intracellular signal transduction molecules underlying BV and formalin model are quite different and the BV model may become more appropriate in elucidation of peripheral and central mechanisms underlying clinical pathological pain as well as screening of endogenous and exogenous effective analgesics. Because of the resultant abatement of PF antinociception by naloxone preconditioning, combined with the widespread distribution of opioid receptors from periphery to CNS (Cox et al., 1980; North 1986; Loh and Smith, 1990), we have reason to suppose that PF might, at least in part, produce analgesia by interacting with opioid receptors along the pain-signaling pathways.

In summary, the present study provided initial evidence showing that systemic application of PF could bring about naloxone-reversible antinociceptive effects against all 'phenotypes' of nociception and hypersensitivity revealed by the BV model. Interestingly, the time of PF administration needed to produce analgesia varied according to the specific pain 'phenotype' to be targeted. This new finding about the antinociceptive or antihyperalgesic actions of PF may open new avenues for developing novel controlling strategies of clinical pathological pain from traditional herbal therapy.

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