Pharmacology, Biochemistry and Behavior 100 (2011) 199–204

Contents lists available at SciVerse ScienceDirect

Pharmacology, Biochemistry and Behavior

journal homepage: www.elsevier.com/locate/pharmbiochembeh

Antinociceptive effects of intragastric dl-tetrahydropalmatine on visceral and somatic persistent nociception and pain hypersensitivity in rats

Fa-Le Cao [a,1], Gang-Wei Shang [a,1], Yan Wang [a], Fan Yang [a], Chun-Li Li [a], 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

ARTICLE INFO

Article history:
Received 3 June 2011
Received in revised form 11 August 2011
Accepted 20 August 2011
Available online 25 August 2011

Keywords:
dl-Tetrahydropalmatine
Bee venom test
Formalin test
Acetic acid-induced writhing test
Pain
Hyperalgesia
Analgesia

ABSTRACT

Although tetrahydropalmatine (THP), an alkaloid constituent of plants from the genera *Stephania* and *Corydalis*, is known to have analgesic property, the antinociceptive effects of THP have not been well evaluated experimentally and the appropriate indications for treatment of clinical pain remain unclear. In the present study, nociceptive and inflammatory models of both somatic and visceral origins were used to assess the antinociceptive and antihyperalgesic effects of intragastric (i.g.) pretreatment of dl-THP in rats. In the bee venom (BV) test that has been well established experimentally, i.g. pretreatment of three doses of dl-THP (20, 40, 60 mg/kg, body weight) resulted in less stably antinociceptive effect on the BV-induced persistent paw flinches that are known to be processed by spinal nociceptive circuit, however the drug of the two higher doses produced distinct suppression of the BV-induced persistent nociception rated by nociceptive score that reflects both spinal and supraspinal mediation. Similarly, the antinociception of dl-THP (60 mg/kg) was only significant for phase 1 but not for phase 2 of the formalin-induced persistent paw flinches, however, the inhibition was distinct for both phase 1 and phase 2 of the formalin nociceptive score. For the antihyperalgesic effect, in contrast, pretreatment of dl-THP (60 mg/kg) produced significant inhibition of both primary hyperalgesia to either thermal or mechanical stimuli and the mirror-image thermal hyperalgesia identified in the BV test. In the acetic acid writhing test, the number of writhes was completely blocked at the first 5-min interval followed by a sustained suppression in the remaining period of the whole time course comparing to the vehicle control.

These data suggest that i.g. pre-administration of dl-THP could more effectively inhibit visceral nociception as well as thermal and mechanical inflammatory pain hypersensitivity (hyperalgesia) than persistent nociception. Moreover, the drug is likely to produce more effectiveness on supraspinally processed nociceptive behaviors than spinally mediated nociceptive behaviors, implicating an action of THP at the supraspinal level.

1. Introduction

Tetrahydropalmatine (THP), a tetrahydroprotoberberine isoquinoline alkaloid, is a primary active constituent from the genera *Stephania* and *Corydalis*. Studies have shown that THP exhibits potential utility in treating drug abuse (Liu et al., 2009; Mantsch et al., 2007; Xi et al., 2007; Yang et al., 2008) and epileptic attacks (Chang and Lin, 2001; Lin et al., 2002), and has hypothermic (Lin et al., 2001), antihypertensive (Lin et al., 1996), sedative, hypnotic and neuroprotective effects in rats (Chang et al., 1999). Aside from them, however, THP has always been thought to alleviate pain, such as headache, chest pain, hypochondriac pain, epigastric pain, abdominal pain, backache, arthralgia, dysmenorrheal and trauma (Huang, 1994). Although THP was most well known as a traditional analgesic agent, it is surprisingly noted that few pharmacological studies have been carried out to explore the possible antinociceptive action of THP and to evaluate its analgesic effects in animals.

Actually, there are many animal models for studying pain and evaluating the effects of various analgesic drugs. For instance, it has been clearly demonstrated that bee venom (BV) test is a well-established animal model for elucidating peripheral and central mechanisms of pathological pain (Chen, 2003, 2007, 2008; Chen and Lariviere, 2010). In behavioral assays, the BV-inflamed animals show unique expressions of persistent spontaneous nociception (PSN) and hypersensitivity (including primary heat and mechanical hyperalgesia, and secondary or mirror-image heat hyperalgesia) (Chen et al., 1999, 2000, 2006; Chen and Chen, 2000, 2001). The formalin test is another persistent ongoing pain model, which is characterized by a biphasic nociceptive response consisting of immediate

Abbreviations: BV, bee venom; i.g., intragastric; PSN, persistent spontaneous nociception; PWMT, paw withdrawal mechanical threshold; PWTL, paw withdrawal thermal latency; s.c., subcutaneous injection; TAC, total alkaloids of Corydalis yanhusuo W.T. Wang; THP, tetrahydropalmatine.

* Corresponding author at: 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.
E-mail address: junchen@fmmu.edu.cn (J. Chen).

[1] Equal contributors.

0091-3057/$ – see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2011.08.016

(the first) and tonic (the second) phases, representing another unique characteristic of inflammatory pain state (Tjolsen et al., 1992). Moreover, intraperitoneal injection of diluted solutions of acetic acid is a model of tonic visceral pain in rodents (writhing test) (Mogil et al., 1996). Therefore, in the present study, we assessed the effects of dl-THP on different nociceptive responses to the tissue injury produced by three noxious chemical agents injected into either somatic or visceral site of the body.

2. Material and methods

2.1. Animals

The experiments were performed on male Sprague–Dawley albino rats (180–220 g) purchased from Laboratory Animal Center of Fourth Military Medical University (FMMU), Xi'an, PR China. The animals were kept one per cage at 25–26 °C with 12-hour light-dark cycles (with the lights on at 8:00 a.m. to 8:00 p.m.) and were fed standard laboratory diet and water *ad libitum*. All experiments were approved by Animal Care and Use Committee at FMMU and accord with the Declaration of the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85–23, revised 1985).

2.2. Pain models and drug treatment

Solutions of BV (4 μg/μl, Sigma, St. Louis, MO, USA), acetic acid (0.6%, Sigma, St. Louis, MO, USA) and formalin (2.5%, Sigma, St. Louis, MO, USA) were prepared in 0.9% sterile saline. Subcutaneous injection (s.c.) of BV (50 μl) and formalin (50 μl) was administered into the posterior plantar surface (Chen et al., 1999; Hunskaar and Hole, 1987), while acetic acid (100 μl) was given intraperitoneally. Sterile saline (50 μl or 100 μl) was used as vehicle control of the above treatment. All vehicle or dl-THP (purchased from Shaanxi Huike Botanical Development Co. Ltd., China) was administered through intragastric (i.g.) administration 30 min before BV, formalin or acetic acid injection.

2.3. Measurement of nociception and pain hypersensitivity

2.3.1. Quantitative assessment of persistent spontaneous nociception

For behavioral testing, PSN was estimated by counting the number of paw flinches occurring during every 5-min interval for 1 h following intraplantar BV or formalin injection. A nociceptive score was also used for evaluation of supraspinal origin of nociceptive behaviors according to a previous report (Coderre and Melzack, 1992; Ren et al., 2008). Briefly, when the injected paw bears the rat's weight on the ground, it is scored "0"; when the rat lightly rests its injected paw and bears only some of its weight on the floor, it is scored "1"; when the rat raises the injected paw off the ground, it is scored "2"; when the animal licks, bites, or shakes the injected paw, it is scored "3". A weighted average of nociceptive score (0–3), was calculated by multiplying the time spent in each category by the category weight, and then divided by the total time for each 5-min time block (see Chen et al., 1998).

In the acetic acid model, the number of writhes was cumulatively counted each 5-min period for 1 h, starting 5 min after the administration of the acetic acid solution (Mogil et al., 1996). A writhe was defined as a contraction of the abdominal muscles accompanied by an elongation of the body and extension of the hind limbs.

2.3.2. Quantitative assessment of pain hypersensitivity

As described previously, rats were placed in a plastic chamber on the surface of a 2 mm thick glass plate 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., China, 10 V) at 3 h after s.c. BV injection. The latency was determined as the duration from the beginning of heat stimuli to the occurrence of a marked paw withdrawal reflex. Four stimuli were repeatedly applied to both the injection site and the corresponding area of the contralateral paw, and the latter three values were averaged as mean paw withdrawal thermal latency (PWTL, s) (Chen et al., 1999). The inter-stimulus interval for each heat test was more than 15 min at the same hind paw or at different site of another hind paw.

Mechanical pain sensitivity of rats was determined by testing paw withdrawal mechanical threshold (PWMT, mN) in response to mechanical stimuli by using ascending graded individual von Frey monofilaments with bending forces of 4.9, 9.8, 19.6, 39.2, 58.8, 78.4, 98.0, 117.6, 137.2, 156.8, 176.4, 196.0, 245.0, 343.0, 441.0, and 588.0 mN. A single von Frey filament was applied 10 times (several seconds for each stimulus) to each testing area of bilateral hind paws. The bending force of the von Frey filament being able to evoke a not less than 50% occurrence of paw withdrawal reflex (e.g. 5/10) was expressed as the PWMT (Chen et al., 1999).

2.4. Motor coordination test

Motor coordination was evaluated by a Rota-Rod treadmill (Ugo, Basile, Italy) in rats among three groups, namely, naive, saline-treated or 60 ml/kg dl-THP-treated group. The accelerating speed of the Rota Rod was set to raise from 6 r/min to 30 r/min within 120 s. Rats 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. As shown in Table 1, animals were trained on the Rota-Rod in a protocol containing 8 trials (T1-T8 from 0 min to 360 min). Trials 1–3 were carried out to allow animal accommodation to the testing apparatus. The remaining trials (T4–T8) were subjected to statistical analysis.

2.5. Data analysis and statistics

All results were presented as mean ± S.E.M. One-way ANOVA (Fisher's PLSD test) was used for statistical analyses. A statistical difference was accepted as significant if $P<0.05$.

3. Results

3.1. Effects of dl-THP on BV-induced persistent spontaneous nociception and pain hypersensitivity

Fig. 1A and D showed the time courses of the effects of i.g. pretreatment with vehicle or three doses of dl-THP (20, 40, and 60 mg/kg) on the PSN by counting the paw flinches or nociceptive score caused by s.c. injection of BV. Compared with the vehicle group, the three doses of dl-THP only suppressed paw flinches at the first 5 min time block without significant antinociception for the remaining 10–60 min time course. However, in contrast, the same

Table 1
Effect of a single dose of dl-THP (60 mg/kg, i.g.) on motor coordination of rats measured by Rota-Rod treadmill test.

Trials	Baseline	Saline	dl-THP
T1 (0 min)	53.50 ± 4.18	54.33 ± 6.53	56.50 ± 6.89
T2 (30 min)	153.17 ± 9.90	152.67 ± 13.44	149.50 ± 12.19
T3 (60 min)	238.00 ± 16.63	239.33 ± 15.73	236.00 ± 19.30
T4 (120 min)	240.33 ± 17.50	243.67 ± 15.97	233.00 ± 16.97 NS
T5 (180 min)	243.50 ± 16.54	244.83 ± 14.73	231.00 ± 14.42 NS
T6 (240 min)	241.67 ± 16.41	243.33 ± 14.12	234.17 ± 14.23 NS
T7 (300 min)	245.17 ± 15.10	245.33 ± 17.59	236.83 ± 18.33 NS
T8 (360 min)	242.50 ± 18.72	238.50 ± 18.43	237.17 ± 17.02 NS

Notes: Data are expressed as mean ± SEM from 6 animals for each group. NS, no significance, dl-THP-treated group vs. saline control.

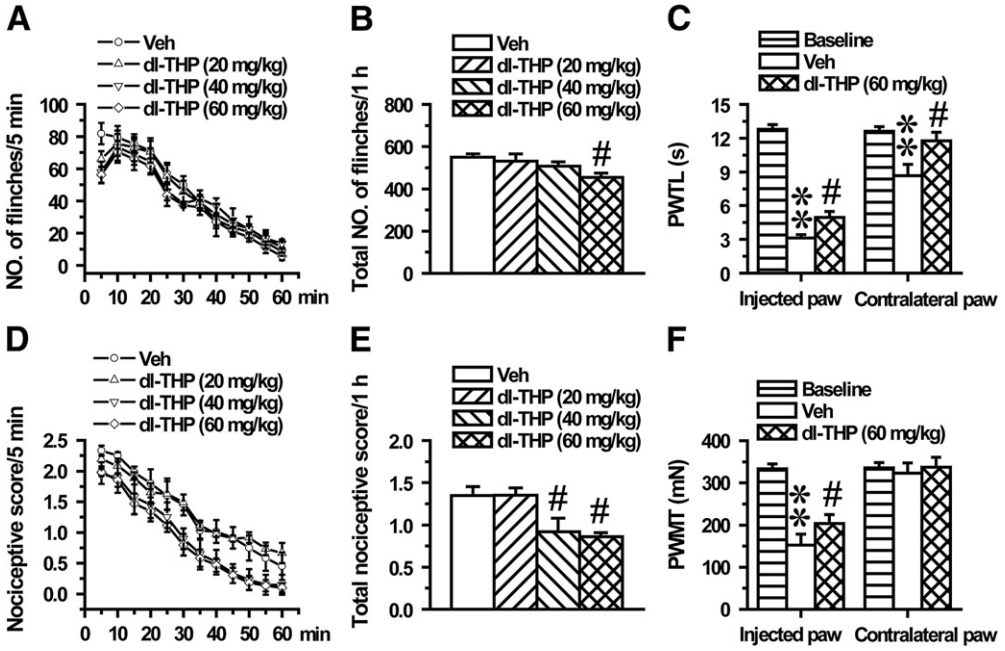

Fig. 1. Effects of intragastric pre-administration of dl-THP (20, 40, 60 mg/kg) on the induction of BV-induced nociception. Persistent spontaneous nociception was evaluated by either spinally-processed paw flinches (A, B) or spinally- and supraspinally-processed nociceptive score (D, E). Curve graphs (A and D) show the time courses of persistent spontaneous nociception, while column graphs (B and E) show the mean total numbers of paw flinches or nociceptive scores in 1 h time course after s.c. BV injection. Thermal and mechanical pain hypersensitivities are evaluated by paw withdrawal thermal latency (PWTL, column C) and paw withdrawal mechanical threshold (PWMT, column F). # $P<0.05$ vs. vehicle-treated groups, ** $P<0.01$ vs. baseline. Error bars: ± S.E.M.

three doses of dl-THP produced less antinociception for the nociceptive score in the initial time block of 0–25 min, however, the antinociceptive effects of the two higher doses became statistically significant in the remaining time course of 30–60 min. Fig. 1B and E showed the total number of paw flinches and nociceptive score in 1 h time course, respectively. The inhibitory rate of dl-THP (20, 40, and 60 mg/kg) for paw flinches was 3.34% ($n=6$, $P>0.05$), 7.52% ($n=6$, $P>0.05$) and 17.35% ($n=7$, $P<0.05$), respectively, while for nociceptive score −0.48% ($n=6$, $P>0.05$), 31.64% ($n=8$, $P<0.05$) and 36.06% ($n=8$, $P<0.05$) were achieved, respectively. There was no distinct dose effect for the three doses used in the present study.

Intragastric administration of the highest single dose of dl-THP (60 mg/kg) into naive animals produced no significant changes in basal pain sensitivity (data not shown). As shown in Fig. 1C, in the BV-inflamed rats, pre-treatment with 60 mg/kg of dl-THP prevented partially the thermal hypersensitivity identified in bilateral hindpaws from occurring, with the inhibitory rates of 58.82% ($n=7$, $P<0.05$) for the primary injury site and 35.77% ($n=7$, $P<0.05$) for the contralateral hindpaw. In Fig. 1F, in comparison with the vehicle group, pre-treatment with the same dose of dl-THP resulted in partially suppressive effects on the primary mechanical hypersensitivity by 33.37% ($n=7$, $P<0.05$).

3.2. Effects of dl-THP on formalin-induced persistent spontaneous nociception

During the 1 h time course of the formalin-induced paw flinches, pre-treatment with single dose (60 mg/kg) of dl-THP resulted in a profound suppression of phase 1 by 53.12% ($n=7$, $P<0.05$), but not phase 2, in comparison with the vehicle control (Fig. 2A and B). However, the same dose of dl-THP produced relatively equivalent antinociception for both phase 1 (by 49.54%, $n=6$, $P<0.05$) and 2 (by 47.26%, $n=6$, $P<0.01$) when nociceptive score was measured (Fig. 2C and D).

3.3. Effects of dl-THP on acetic acid-induced writhing responses

Intraperitoneal injection of acetic acid evoked a stereotypical writhing response in rats. As shown in Fig. 3A, anti-visceral pain effect

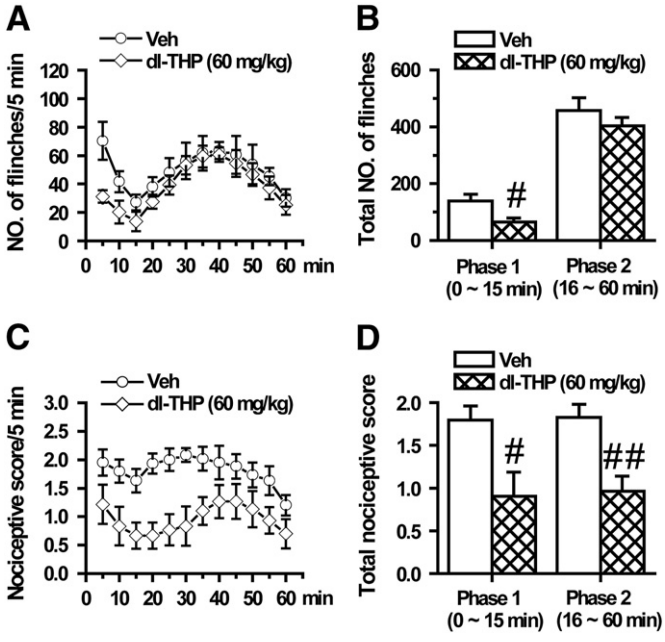

Fig. 2. Effects of intragastric pre-administration of dl-THP (60 mg/kg) on the induction of formalin-induced nociception. Biphasic persistent spontaneous nociception was evaluated by either spinally-processed paw flinches (A, B) or spinally- and supraspinally-processed nociceptive score (C, D). Curve graphs (A and C) show the time courses of persistent spontaneous nociception, while column graphs (B and D) show the mean total numbers of paw flinches or nociceptive scores in 1 h time course after s.c. formalin injection. Phase 1 = 0–10 min; Phase 2 = 11–60 min. # $P<0.05$, ## $P<0.01$ vs. vehicle-treated groups. Error bars: ± S.E.M.

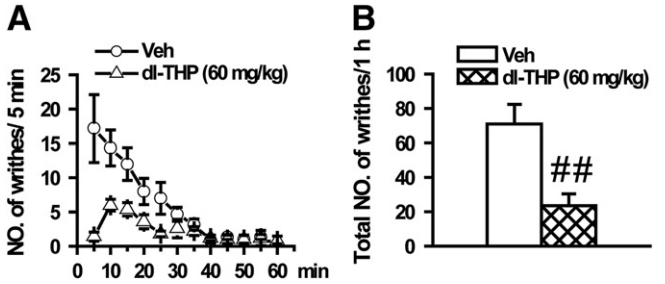

Fig. 3. Effects of intragastric pre-administration of dl-THP (60 mg/kg) on the induction of acetic acid-induced visceral nociception. Curve graph (A) shows the time course and column graph (B) shows the mean total numbers of writhing response averaged from 1 h time course following intraperitoneal acetic acid injection. $^{\#\#}$ $P<0.01$ vs. vehicle-treated groups. Error bars: ± S.E.M.

of i.g. pre-administration of dl-THP (60 mg/kg) was dramatically strong in the first 5 min time block followed by a period of 30 min significant antinociception. Based upon the total number of writhes in 1 h time course, the inhibitory rate of 66.90% ($n=7$, $P<0.01$) was achieved (Fig. 3B).

3.4. Effects of dl-THP on motor coordination ability

In the present study, we used Rota-Rod treadmill test to investigate the possible effect of drug administration on motor coordination ability in rats. Results showed that no significant difference in the time spent on the treadmill among the tested groups (Table 1).

4. Discussion

In the present study, we compared the effects of i.g. pre-administration of dl-THP in three animal models of pain representing both somatic and visceral origins. Moreover, the behavioral phenotypes of pain symptoms and signs identified from the three animal models can well represent complex state of inflammatory pain and tissue injury. As consequences, first, we found that pre-treatment of dl-THP could more effectively inhibit visceral persistent spontaneous nociception induced by intraperitoneal injection of acetic acid than somatic persistent spontaneous nociception induced by either BV or formalin. Second, the drug is likely to produce more effectiveness on supraspinally processed nociceptive behaviors than spinally mediated nociceptive behaviors approved in both BV and formalin models, implicating an action of THP at the supraspinal level. Third, for the somatic origin of nociception and evoked pain hypersensitivity (hyperalgesia and allodynia), pre-treatment of dl-THP produced more anti-hyperalgesic and anti-allodynic effect (thermal and mechanical) than persistent nociception. Finally, the antinociceptive and anti-hyperalgesic dose of the drug did not produce any side effect on the motor coordination performance tested by Rota Rod treadmill.

The unique behavioral 'phenotypes' of nociception and hypersensitivity identified in the rodent BV model are believed to reflect a complex pathological state of inflammatory pain that is appropriate to the study of phenotype-based mechanisms of pain and hyperalgesia (Chen, 2003, 2007, 2008; Chen and Lariviere, 2010). In the BV-inflamed rats, five symptom-based behavioral phenotypes can be identified: (1) immediate and long-term lasting tonic nociceptive behaviors displaying as spontaneous flinching reflex of injured paw that has been known to be mediated by spinally organized nociceptive reflex circuitry (You et al., 2003a,b,c; You and Chen, 1999) or licking or lifting behaviors assessed by nociceptive score that are mediated by supraspinal origins of facilitation (Chen et al., 2003; Ren et al., 2008); (2) primary thermal and mechanical hyperalgesia occurred in the BV injection site (Chen et al., 1999; Chen and Chen, 2000, 2001); (3) secondary thermal hyperalgesia occurred in the remote area surrounding the BV injection site (Chen and Chen, 2000); and (4) the mirror-image heat hyperalgesia occurred in the symmetric area of the contralateral hind paw to the BV injection site (Chen et al., 1999; Chen and Chen, 2000, 2001). As for the neural mechanisms underlying different phenotypes of pain-related behaviors and hyperalgesia or allodynia induced in the BV model, different spinal and peripheral mechanisms have been proposed based upon a series of experimental data collected in our and other labs (Chen, 2003, 2007, 2008; for more details see review Chen and Lariviere, 2010). Briefly, melittin, a major ingredient peptide of BV (>50% in dry weight) has been demonstrated to be able to activate and sensitize peripheral nociceptors by activation of transient receptor potential vanilloid receptor 1 (TRPV1) by metabolites produced via phospholipase A 2 (PLA2) – arachidonic acid (AA) – cyclooxygenases (COX1 and COX2)/lipoxygenase (LOX) pathways (Chen and Lariviere, 2010; Du et al., 2011). The activated nociceptors by melittin and other substances give rise to persistent firing of action potentials that are conveyed by capsaicin-sensitive primary afferent fibers to the dorsal horn of the spinal cord (Chen and Chen, 2001), leading to activation and sensitization of central pain-related neurons (Li and Chen, 2004) that mediate persistent spontaneous nociception and hyperalgesia to both thermal and mechanical stimuli in the primary injection site (Chen et al., 2006). As for the mechanisms of the mirror-image heat hyperalgesia, spinal segmental sensitization and descending facilitation originated from the brain stem have been shown to be involved (Chen et al., 2003; You et al., 2005). Collectively, the BV-induced pain and hyperalgesia involve both peripheral and central sensitization (Chen and Lariviere, 2010). Similar to one feature of the BV model, s.c. formalin can also produce a biphasic persistent nociception in rodents but with less hyperalgesia or allodynia in the injection site or surrounding area (Chen et al., 1998, 1999; You and Chen, 1999). It has been well established that formalin-induced persistent nociception is mediated by initial and long-lasting activation of primary C- and A fibers (Chen and Koyama, 1996; Puig and Sorkin, 1996) via activation of cold nociceptor TRPA1 (McNamara et al., 2007; Macpherson et al., 2007) and thermal nociceptor TRPV1 (Tian et al., 2009) that lead to enhanced activation or sensitization of spinal pain-related neurons (Chen et al., 1996; Chen and Koyama, 1998; You and Chen, 1999). Thus, both BV and formalin are likely to share at least in part the same peripheral mechanisms mediated by TRPV1 and sensitization of spinal dorsal horn pain-related neurons. Thus, dl-THP may produce antinociception through actions at both peripheral and central sites. However, direct peripheral action of dl-THP is not likely because in the current survey it produced more effective antinociception on the supraspinally-mediated pain-related behaviors but not spinally-mediated paw flinches induced in either BV or formalin test. As supporting evidence, some previous reports suggested that THP produced analgesia through blocking dopamine D2 receptors in the striatum and the arcuate nucleus of the hypothalamus, leading to activation of descending antinociceptive system from the midbrain periaqueductal gray (PAG) to the rostral ventromedial medulla, and to the spinal dorsal horn where nociceptive activities are suppressed (Hu and Jin, 1999a,b, 2000). Inhibition of the mirror-image thermal hyperalgesia by dl-THP is also likely to be produced through activation of supraspinal central site of the descending antinociceptive system because the BV-induced descending pain facilitation might be compromised by dl-THP-produced pre-empted analgesia.

In the current study, we also demonstrated that dl-THP could partially suppress the BV-induced primary thermal and mechanical hyperalgesia. In one of our previous reports, it was demonstrated that bilateral chemical lesions of anterior cingulate cortex (ACC) resulted in a relief of both primary thermal and mechanical hyperalgesia, suggesting existence of a descending facilitation from the ACC that exacerbates hyperalgesia in the injured area of the body. The dl-THP-produced antihyperalgesic and antiallodynic effects might be associated with blockade of the ACC-mediated descending pain facilitation system that remains unclear and requires to be further studied.

In the current study, the most striking finding was that pre-treatment of dl-THP resulted in a more profound analgesia in the acetic acid writhing test. It has been suggested that intraperitoneal injection of acetic acid should produce inflammation of the wall of the abdominal cavity and evoke a sustained writhing behavior caused by visceral stimuli (Nakagawa et al., 2003). Because there has been rare experimental data showing central sensitization at the spinal cord dorsal horn when rats receiving intraperitoneal injection of acetic acid, it is still unknown where dl-THP can act to produce anti-visceral pain. Since it is well known that primary visceral nociceptive afferent may project to the spinal dorsal horn and share the same ascending pain pathways with the somatic system, inhibition of the acetic acid-induced writhing responses by dl-THP is also likely to be produced through activation of supraspinal central site of the descending antinociceptive system as proposed above.

THP is a bioactive ingredient of Corydalis yanhusuo W.T. Wang (TAC) which was shown to be able to suppress both phases of the formalin-induced responses when administered through intragingival route prior to establishment of inflammatory pain state (Wang et al., 2010). Because TAC contains more than 100 compounds, the inhibitory effect of the total alkaloids of TAC on the formalin response was probably caused by integrated actions of THP and some other ingredients. In the tail-flick test, l-THP was shown to increase tail-flick latency in rats in a dose-dependent manner (Xu et al., 1982). However, THP was not shown to be able to change the level of prostaglandins (Zhang et al., 1986). The opioid receptors are not the molecular targets of THP either because it does not have any affinity for any subtypes of opioid receptors (Frussa-Filho et al., 1996). As proposed above, the central dopaminergic systems may play an important role in regulating nociception (Huang and Jin, 1992; Magnusson and Fisher, 2000; Roane et al., 1998; Taylor et al., 2003). It has been shown that l-THP is a non-selective antagonist of different dopamine receptors (Guo et al., 1997; Hu and Jin, 1999a; Mantsch et al., 2007) and that the D2 dopamine receptor is likely to be involved in antinociceptive effects of THP through activation of descending antinociceptive system aforementioned (Hu and Jin, 1999a,b, 2000; Xu et al., 1989).

5. Conclusions

The current study evaluated the overall analgesic effect of dl-THP in rats and provided scientific basis for its use in clinical therapies. It is implicated that pharmacological pretreatment with dl-THP is able to produce stronger analgesic effect on visceral nociception than somatic nociception. Pre-treatment of dl-THP is more beneficial to supraspinally-processed behaviors of nociception and pain hypersensitivity, but with less benefit to spinally-processed behaviors of nociception.

Acknowledgments

This work was supported by the NSFC grant (81070899), 973 program (2006CB500808, 2011CB504100) and National New Drug R&D Project (2009ZX09301-009-RC07) to J.C.

References

Chang CK, Lin MT. DL-Tetrahydropalmatine may act through inhibition of amygdaloid release of dopamine to inhibit an epileptic attack in rats. Neurosci Lett 2001;307:163–6.
Chang CK, Chueh FY, Hsieh MT, Lin MT. The neuroprotective effect of DL-tetrahydropalmatine in rat heatstroke. Neurosci Lett 1999;267:109–12.
Chen J. The bee venom test: a novel useful animal model for study of spinal coding and processing of pathological pain information. In: Chen J, Chen ACN, Han JS, Willis WD, editors. Experimental pathological pain: from molecules to brain functions. Beijing: Science Press; 2003. p. 77-110.
Chen J. Processing of different 'phenotypes' of pain by different spinal signaling pathways. In: Kumamoto K, editor. Cellular and molecular mechanisms for the modulation of nociceptive transmission in the peripheral and central nervous systems, recent research development series. Kerala: Research Sign Post; 2007. p. 147–65.
Chen J. Spinal processing of bee venom-induced pain and hyperalgesia. Sheng Li Xue Bao 2008;60:645–52.
Chen HS, Chen J. Secondary heat, but not mechanical, hyperalgesia induced by subcutaneous injection of bee venom in the conscious rat: effect of systemic MK-801, a non-competitive NMDA receptor antagonist. Eur J Pain 2000;4:389–401.
Chen J, Chen HS. Pivotal role of capsaicin-sensitive primary afferents in development of both heat and mechanical hyperalgesia induced by intraplantar bee venom injection. Pain 2001;91:367–76.
Chen J, Koyama N. Effects of subcutaneous formalin on responses of identified primary afferent units in the cat: an electrophysiological study. Chin J Neuroanat 1996;12:381–93.
Chen J, Koyama N. Differential activation of spinal dorsal horn units by subcutaneous formalin injection in the cat: an electrophysiological study. Exp Brain Res 1998;118:14–8.
Chen J, Lariviere WR. The nociceptive and anti-nociceptive effects of bee venom injection and therapy: a double-edged sword. Prog Neurobiol 2010;92:151–83.
Chen J, Koyama N, Yokota T. Effects of subcutaneous formalin on responses of dorsal hornwide dynamic range neurons and primary afferent neurons in the cat. Pain Res 1996;11:71–83.
Chen J, Luo C, Li HL. The contribution of spinal neuronal changes to development of prolonged, tonic nociceptive responses of the cat induced by subcutaneous bee venom injection. Eur J Pain 1998;2:359–76.
Chen J, Luo C, Li H, Chen H. Primary hyperalgesia to mechanical and heat stimuli following subcutaneous bee venom injection into the plantar surface of hindpaw in the conscious rat: a comparative study with the formalin test. Pain 1999;83:67–76.
Chen HS, Chen J, Sun YY. Contralateral heat hyperalgesia induced by unilaterally intraplantar bee venom injection is produced by central changes: a behavioral study in the conscious rat. Neurosci Lett 2000;284:45–8.
Chen HS, Li MM, Shi J, Chen J. Supraspinal contribution to development of both tonic nociception and referred mirror hyperalgesia: A comparative study between formalin test and bee venom test in the rat. Anesthesiology 2003;98:1231–6.
Chen YN, Li KC, Li Z, Shang GW, Liu DN, Lu ZM, et al. Effects of bee venom peptidergic components on rat pain-related behaviors and inflammation. Neuroscience 2006;138:631–40.
Coderre TJ, Melzack R. The contribution of excitatory amino acids to central sensitization and persistent nociception after formalin-induced tissue injury. J Neurosci 1992;12:3665–70.
Du YR, Xiao Y, Lu ZM, Ding J, Xie F, Fu H, et al. Melittin activates TRPV1 receptors in primary nociceptive sensory neurons via the phospholipase A2 cascade pathways. Biochem Biophys Res Commun 2011;408:32–7.
Frussa-Filho R, Rocha JB, Conceicao IM, Mello CF, Pereira ME. Effects of dopaminergic agents on visceral pain measured by the mouse writhing test. Arch Int Pharmacodyn Ther 1996;331:74–93.
Guo X, Wang LM, Liu J, Jin GZ. Characteristics of tetrahydroprotoberberines on dopamine D1 and D2 receptors in calf striatum. Zhongguo Yao Li Xue Bao 1997;18:225–30.
Hu JY, Jin GZ. Supraspinal D2 receptor involved in antinociception induced by l-tetrahydropalmatine. Zhongguo Yao Li Xue Bao 1999a;20:715–9.
Hu JY, Jin GZ. Effect of tetrahydropalmatine analogs on Fos expression induced by formalin-pain. Zhongguo Yao Li Xue Bao 1999b;20:193–200.
Hu JY, Jin GZ. Arcuate nucleus of hypothalamus involved in analgesic action of l-THP. Acta Pharmacol Sin 2000;21:439–44.
Huang TK. Handbook of the composition and pharmacology of common Chinese drugs. Beijing: Chung-kuo i yao ko chi chu pan she; 1994.
Huang KX, Jin GZ. The antagonistic effects of tetrahydroprotoberberines on dopamine receptors: electrophysiological studies. Sci China B 1992;35:688–96.
Hunskaar S, Hole K. The formalin test in mice: dissociation between inflammatory and non-inflammatory pain. Pain 1987;30:103–14.
Li KC, Chen J. Altered pain-related behaviors and spinal neuronal responses produced by s.c. injection of melittin in rats. Neuroscience 2004;126:753–62.
Lin MT, Chueh FY, Hsieh MT, Chen CF. Antihypertensive effects of DL-tetrahydropalmatine: an active principle isolated from Corydalis. Clin Exp Pharmacol Physiol 1996;23:738–42.
Lin MT, Chueh FY, Hsieh MT. The hypothermic effects of dl-tetrahydropalmatine in rats. Neurosci Lett 2001;315:53–6.
Lin MT, Wang JJ, Young MS. The protective effect of dl-tetrahydropalmatine against the development of amygdala kindling seizures in rats. Neurosci Lett 2002;320:113–6.
Liu YL, Yan LD, Zhou PL, Wu CF, Gong ZH. Levo-tetrahydropalmatine attenuates oxycodone-induced conditioned place preference in rats. Eur J Pharmacol 2009;602:321–7.
Macpherson LJ, Xiao B, Kwan KY, Petrus MJ, Dubin AE, Hwang S, et al. An ion channel essential for sensing chemical damage. J Neurosci 2007;27:11412–5.
Magnusson JE, Fisher K. The involvement of dopamine in nociception: the role of D(1) and D(2) receptors in the dorsolateral striatum. Brain Res 2000;855:260–6.
Mantsch JR, Li SJ, Risinger R, Awad S, Katz E, Baker DA, et al. Levo-tetrahydropalmatine attenuates cocaine self-administration and cocaine-induced reinstatement in rats. Psychopharmacology (Berl) 2007;192:581–91.
McNamara CR, Mandel-Brehm J, Bautista DM, Siemens J, Deranian KL, Zhao M, et al. TRPA1 mediates formalin-induced pain. Proc Natl Acad Sci U S A 2007;104:13525–30.
Mogil JS, Kest B, Sadowski B, Belknap JK. Differential genetic mediation of sensitivity to morphine in genetic models of opiate antinociception: influence of nociceptive assay. J Pharmacol Exp Ther 1996;276:532–44.
Nakagawa T, Katsuya A, Tanimoto S, Yamamoto J, Yamauchi Y, Minami M, et al. Differential patterns of c-fos mRNA expression in the amygdaloid nuclei induced by chemical somatic and visceral noxious stimuli in rats. Neurosci Lett 2003;344:197–200.

Puig S, Sorkin LS. Formalin-evoked activity in identified primary afferent fibers: systemic lidocaine suppresses phase-2 activity. Pain 1996;64:345–55.
Ren LY, Lu ZM, Liu MG, Yu YQ, Li Z, Shang GW, et al. Distinct roles of the anterior cingulate cortex in spinal and supraspinal bee venom-induced pain behaviors. Neuroscience 2008;153:268–78.
Roane DS, Bounds JK, Ang CY, Adloo AA. Quinpirole-induced alterations of tail temperature appear as hyperalgesia in the radiant heat tail-flick test. Pharmacol Biochem Behav 1998;59:77–82.
Taylor BK, Joshi C, Uppal H. Stimulation of dopamine D2 receptors in the nucleus accumbens inhibits inflammatory pain. Brain Res 2003;987:135–43.
Tian LJ, Du YR, Xiao Y, Lu ZM, Yu YQ, Cui XY, et al. Mediating roles of the vanilloid receptor TRPV1 in activation of rat primary afferent nociceptive neurons by formaldehyde. Acta Physiol Sin 2009;61:404–16.
Tjolsen A, Berge OG, Hunskaar S, Rosland JH, Hole K. The formalin test: an evaluation of the method. Pain 1992;51:5-17.
Wang C, Wang SW, Fan GR, Zou HF. Screening of antinociceptive components in Corydalis yanhusuo W.T. Wang by comprehensive two-dimensional liquid chromatography/tandem mass spectrometry. Anal Bioanal Chem 2010;396:1731–40.
Xi ZX, Yang Z, Li SJ, Li X, Dillon C, Peng XQ, et al. Levo-tetrahydropalmatine inhibits cocaine's rewarding effects: experiments with self-administration and brain-stimulation reward in rats. Neuropharmacology 2007;53:771–82.
Xu J, Zheng LZ, Jin GZ. Relevance of brain prostaglandins to central effects of l-tetrahydropalmatine. Zhongguo Yao Li Xue Bao 1982;3:217–20.
Xu SX, Yu LP, Han YR, Chen Y, Jin GZ. Effects of tetrahydroprotoberberines on dopamine receptor subtypes in brain. Zhongguo Yao Li Xue Bao 1989;10:104–10.
Yang Z, Shao YC, Li SJ, Qi JL, Zhang MJ, Hao W, et al. Medication of l-tetrahydropalmatine significantly ameliorates opiate craving and increases the abstinence rate in heroin users: a pilot study. Acta Pharmacol Sin 2008;29:781–8.
You HJ, Chen J. Differential effects of subcutaneous injection of formalin and bee venom on responses of wide-dynamic-range neurons in spinal dorsal horn of the rat. Eur J Pain 1999;3:177–80.
You HJ, Morch CD, Chen J, Arendt-Nielsen L. Simultaneous recordings of wind-up of paired spinal dorsal horn nociceptive neuron and nociceptive flexion reflex in the rat. Brain Res 2003a;960:228–38.
You HJ, Morch CD, Chen J, Arendt-Nielsen L. Role of central NMDA versus non-NMDA receptor in spinal withdrawal reflex in spinal anesthetized rats under normal and hyperexcitable conditions. Brain Res 2003b;981:12–22.
You HJ, Morch CD, Chen J, Arendt-Nielsen L. Differential antinociceptive effects induced by highly selective COX-2 inhibitor SC-236 on simultaneous dorsal horn neuron responses and spinal cord reflex in anesthetized spinal rats. Neuroscience 2003c;121:459–72.
You HJ, Colpaert FC, Arendt-Nielsen L. The novel analgesic and high-efficacy 5-HT1A receptor agonist F13640 inhibits nociceptive responses, wind-up, and after-discharges in spinal neurons and withdrawal reflexes. Exp Neurol 2005;191:174–83.
Zhang ZD, Jin GZ, Xu SX, Yu LP, Chen Y, Jiang FY, et al. Effects of l-stepholidine on the central nervous and cardiovascular systems. Zhongguo Yao Li Xue Bao 1986;7:522–6.