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

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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.

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(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.