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Antinociceptive Effect of Nicotine in Various Pain Models in the Mouse

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The antinociceptive effect of nicotine administered intracereboventricularly (i.c.v.) or intrathecally (i.t) in several pain models was examined in the present study. We found that i.t. treatment with nicotine (from 5 to 20 g) dose-dependently blocked pain behavior revealed during the second phase, but not during the first phase in the formalin test. In addition, i.c.v. treatment with nicotine (from 0.1 to 10 μg) dose-dependently attenuated pain behavior revealed during both the first and second phases. In addition to the formalin test, nicotine administered i.c.v. or i.t. attenuated acetic acid-induced writhing response. Furthermore, i.c.v. or i.t. administration of nicotine did not cause licking, scratching and biting responses induced by substance P, glutamate, TNF-α (100 pg), IL-1β (100 pg) and INF-γ (100 pg) injectied i.t. The antinociception induced by supraspinally-administered nicotine appears to be more effective than that resulting from spinally administered nicotine. Our results suggest that nicotine administration induces antinociception by acting on the central nervous system and has differing antinociceptive profiles according to the various pain models.

Key words: Nicotine, Antinociception, Intracerebrobentrivularly injection, Intrathecally injection, Formalin

INTRODUCTION

Nicotine, acting at neuronal nicotinic acetylcholine receptors, is the primary component of tobacco that drives addiction (Benowitz and Jacob, 1990). Nicotine is also used medicinally to treat Alzheimers disease, Parkinsons disease and chronic pain (Bannon *et al.*, 1998).

Several studies have demonstrated that nicotine plays an important role in modulating pain transmission. Specifically, activation of nicotinic receptors elicits an antinociceptive effect in a variety of species and pain tests (Aceto *et al.*, 1986; Mattila *et al.*, 1968; Phan *et al.*, 1973). Furthermore, metanicotine produces significant antinociceptive effects in mice and rats subjected to either acute thermal (tail-flick), mechanical (paw-pressure), chemical (para-phenylquinone), persistent (formalin), or chronic (arthritis) pain stimuli (Damaj *et al.*, 1998). Indeed, acute nicotine (0.5, 1, 3, and 6 mg/kg, sc) administration induces antinociceptive response in the tail-immersion and the hot-plate test (Castane *et al.*, 2002). Although the effects of nicotine may not extend to all types of pain and appear to be dependent on the mode of administration, recent observation suggests that nicotine reduces pain in humans (Perkins *et al.*, 1994; Rau *et al.*, 1993), implying a true analgesic component. Although antinociceptive effects of nicotine have been previously reported, the antinociceptive spectrum of nicotine has been largely revealed only in pain models such as the tail-flick or hot-plate tests. In addition, differences in antinociceptive response between nicotine administered surpraspinally or spinally have not been well characterized.

The dorsal horn of the spinal cord is an important site, for the integration and modulation of synaptic transfers of sensory input from the periphery to the central nervous system. Various neurotransmitters such as substance P and glutamate mediate pain transmission in the spinal cord (Hopkins *et al.*, 1995; Kidd *et al.*, 2001; Riedel *et al.*, 2001). Intrathecally administered substance P- and glutamate-induced pain models have been widely used to study nociceptive/andtinociceptive mechanisms (Choi *et al.*, 2001; Chung *et al.*, 2001b; Hylden *et al.*, 1981).

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Moreover, proinflammatory cytokines have been shown to induce central sensitization, modulating pain behavior during peripheral inflammation. For example, after a variety of nerve injuries, including transection of the L5 spinal nerve, increased TNF in both the dorsal root ganglia (DRG) and the spinal cord is associated with neuropathic pain (DeLeo *et al.*, 1997; Schafers *et al.*, 2002; Wagner *et al.*, 1998). Parallel to these findings, TNF antagonism reduces hyperalgesia associated with two models of neuropathic pain: chronic constriction injury and partial nerve transection (Sommer *et al.*, 2001b, 2001a; Wagner *et al.*, 1998). At the spinal cord level, i.t. administration of IL-1β produces a significant hyperalgesic effect in a hot plate test (Falchi *et al.*, 2001) or mechanical allodynia and hyperalgesia (Reeve *et al.*, 2000). Furthermore, it has also been reported that i.t. injection of recombinant interferon-γ (IFN-γ) facilitates the spinal nociceptive flexor reflex in rats (Xu, 1994) and IFN-γ injected spinally elicits caudally directed biting-scratching behaviours, putative signs of nociception (Gamae and Saria, 1986; Hylden JI, 1981). These data suggest that the proinflammaory cytokines, such as TNF-α, IL-1β or IFN-γ appear to be involved in neuropathic pain.

The present study was designed to characterize the antinociceptive effects of i.c.v. or i.t. administered nicotine on pain behavior induced by spinally administered formalin, acetic acid, substance P, glutamate, or proinflammatory cytokines (TNF-α, IL-1β or IFN-γ).

MATERIALS AND METHODS

Experimental animals

Male ICR mice (M.J. LTD., Seoul, Korea) weighing 23-25 g were used for all the experiments. Animals were housed five per cage in a room maintained at 22 ± 0.5 °C with an alternating 12 h light/dark cycle. Food and water were available *ad libitum*. The animals were allowed to adapt to the laboratory for at least 2 h before testing and were only used once. Experiments were performed during the light phase of the cycle (10:00-17:00 h). These experiments were approved by the University of Hallym Animal Care and Use Committee. All procedures were conducted in accordance with the Guide for Care and Use of Laboratory Animals, published by the National Institutes of Health and the ethical guidelines of the International Association for the Study of Pain.

Intracerebroventricular (i.c.v.) and intrathecal (i.t.) injections

I.c.v. injections were made according to the procedure of Haley and McCormick (Haley TJ, 1957). The i.t. injection was performed free-hand between the L5 and L6 lumbar space in unanesthetized male mice according to the methods described by Hylden and Wilcox (Hylden JI, 1980). The cumulative response time of licking, scratching and biting episodes directed toward the lumbar and caudal regions of the spinal cord were measured. I.c.v. and i.t. injection volume was 5 μL and the injection sites were verified by injecting a similar volume of 1% methylene blue solution and determing the distribution of the injected dye in the ventricular space or in the spinal cord.

The dye injected i.c.v. was found to be distributed throughout the ventricular spaces and reached the ventral surface of the brain and upper cervical portion of the spinal cord. The dye injected i.t. was distributed both rostrally and caudally but did not travel far (about 0.5 cm from the injection site) and no dye was found visually in the brain.

Intraplantar formalin tests and acetic acid-induced writhing

For the formalin test (Hunskaar *et al.*, 1985), 10 μL of 1.0% formalin solution, in a 0.9% saline solution, was injected subcutaneously (s.c.) under the plantar surface of the left hindpaw. Two groups of mice (control and treated) were immediately placed in an acrylic observation chamber (20 cm high, 20 cm diameter), and the time spent licking, shaking and biting the injected paw was measured with a stop-watch timer and considered as indicative of nociception. Control animals received a similar volume of physiologic normal saline. The early phase of the nociceptive response normally peaked 0 to 5 min after formalin injection and the late phase 20 to 40 min after formalin injection, representing the direct effect on nociceptors and inflammatory nociceptive responses, respectively (Hunskaar and K., 1987).

For the writhing test (Koster *et al.*, 1959) 0.25 mL of 1% acetic acid dissolved in saline was administered intraperitoneally in the mice. The number of writhes was counted during a 30 min period following the injection of acetic acid. A writhe was defined as a contraction of the abdominal muscles accompanied by an extension of the forelimbs and elongation of the body.

Substance P, glutamate and cytokine-induced nociceptive behavioral tests

The i.t. substance P, glutamate and cytokine-induced nociceptive behavioral tests were performed according to the following procedures. Each mouse was acclimated in an observation chamber for at least 30 min before the injection of substance P (0.7 μg), glutamate (20 μg) and cytokines (TNF-α, IL-1β, IFN-γ; 100 pg). Immediately after i.t. injection with substance P, glutamate or cytokines, the mice were placed in an observation chamber and the pain behavioral response was recorded for 30 min. The cumulative response time(s) of licking, scratching and biting

episodes directed toward the lumbar and caudal region of spinal cord were measured.

Drugs

Formalin, acetic acid, substance P, nicotine and glutamate were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). TNF-α, IL-1β and IFN-γ were purchased from Calbiochem Novabiochem Co. (La Jolla, CA).

Statistical analysis

Statistical analysis was carried out by one-way analysis of variance (ANOVA). The Bonferroni test was used for *post-hoc* comparisons. *P* values less than 0.05 were considered to indicate statistical significance.

RESULTS

The effect of nicotine injected i.c.v. or i.t. on the pain behavioral response elicited by intraplantar injection of formalin

The control and treated groups of mice were treated i.c.v. with 5 μL of saline or various doses of nicotine 5 min, respectively, before intraplantar administration of formalin. As shown in Fig. 1, nicotine treated i.c.v. at doses from 0.1 to 10 mg reduced pain behavior induced by intraplantar formalin injection during the first (0-5 min) and second (20-40 min) phases, in a dose dependent manner. The cumulative response time of the formalin-induced pain behavior was significantly attenuated both first and

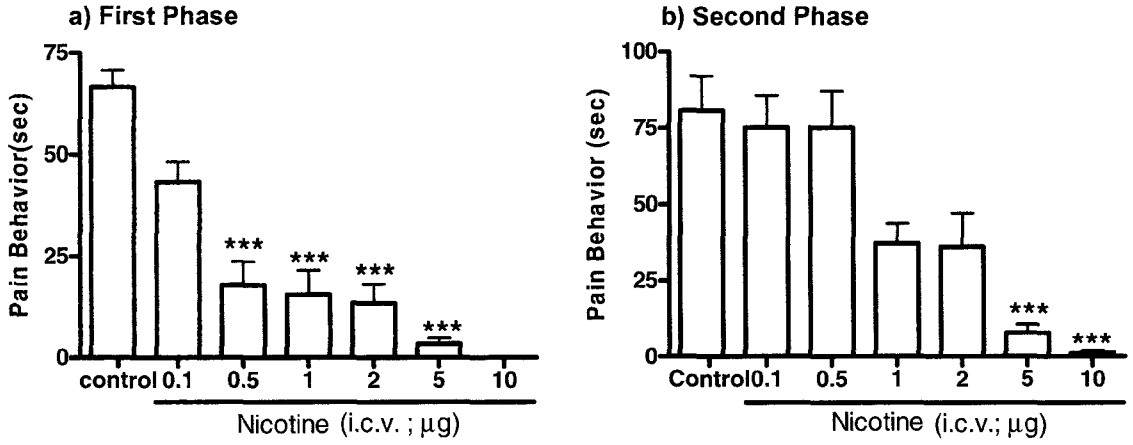

Fig. 1. The effects of pretreatment with nicotine administered i.c.v. on formalin-induced pain behavior manifested during the first phase (a) and second phase (b). Mice were treated with nicotine (0.1 to 10 μg) i.c.v. 5 min prior to formalin (1%, 10 μL) injection into the plantar aspect of the left side hindpaw subcutaneously. The cumulative pain response time of licking, shaking and biting the injected paw was measured during the period of 0-5 min (first phase) and 20-40 min (second phase). The vertical bars denote the standard error of the mean. The number of animals used for each group was 8-10. *** P< 0.001 vs. control.

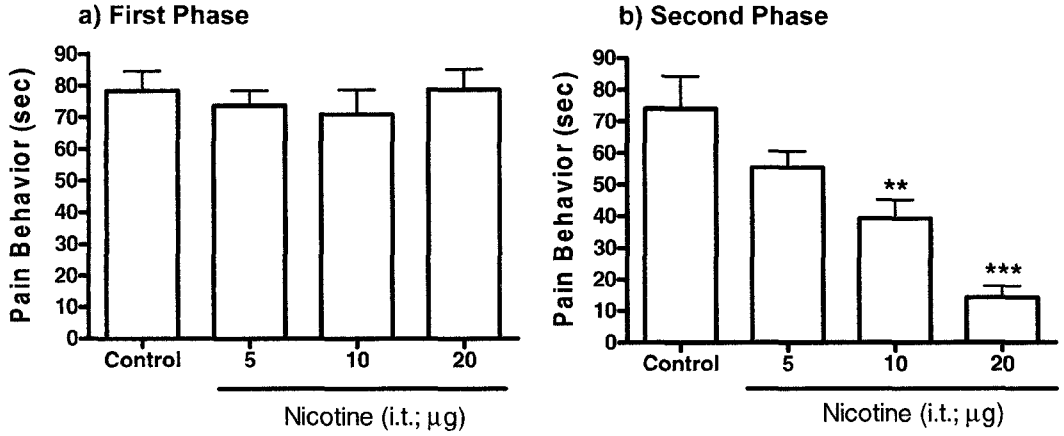

Fig. 2. The effects of pretreatment with nicotine on formalin-induced pain behavior manifested during the first phase (a) and second phase (b). Mice were treated i.t. 5 min prior to formalin (1%, 10 μL) injection into the plantar aspect of the left side hindpaw subcutaneously. The cumulative pain response time of licking, shaking and biting the injected paw was measured during the period of 0-5 min (first phase) and 20-40 min (second phase). The vertical bars denote the standard error of the mean. The number of animals used for each group was 8-10. ** P< 0.01, *** P<0.001 vs. control.

second phases, showing a dose dependent pattern (Fig. 1a, b). Pain behavior in control and treated mice was also documented. Nicotine treated i.t. mice behaved differently compared to i.c.v. nicotine treated mice. Because as shown in Fig. 2, nicotine treated i.t. at doses of 5 to 20 μg could not block nociception induced by intraplantar formalin injection in the first (0-5 min) phase (Fig. 2a) but effectively attenuated the pain behavior does-dependantly during the second (20-40 min) phase. (Fig. 2b).

The effect of nicotine administered i.c.v. or i.t. on acetic acid-induced writhing response

Writhing response was observed after noxious visceral stimulation induced by 1% acetic acid intraperitoneally. Group of mice were pretreated i.c.v. or i.t. with 5 μL of saline (control 0 or various doses of nicotine) 5 min (treated) before intraperitoneal administration of 1% acetic acid. As shown in Fig. 3, nicotine treated i.c.v. (at doses 1 to 10 μg) or i.t. (from 5 to 20 μg) mice, reduced the number of writhing responses induced by acetic acid compared to control group.

The effect of nicotine injected i.c.v. or i.t. on pain behavioral response induced by substance P and glutamate administered i.t.

I.t. injection of substance P (0.5 μg) and glutamate (20 μg) caused an acute, immediate behavioral response, i.e., licking, scratching and biting, which lasted approximately 30 min. As shown in Fig. 4a, mouse given substance P i.t. showed a pain behavioral response. Nicotine, given i.c.v. (5 μg) or i.t. (20 μg) 5 min before the injection of substance P, attenuated significantly substance P-induced

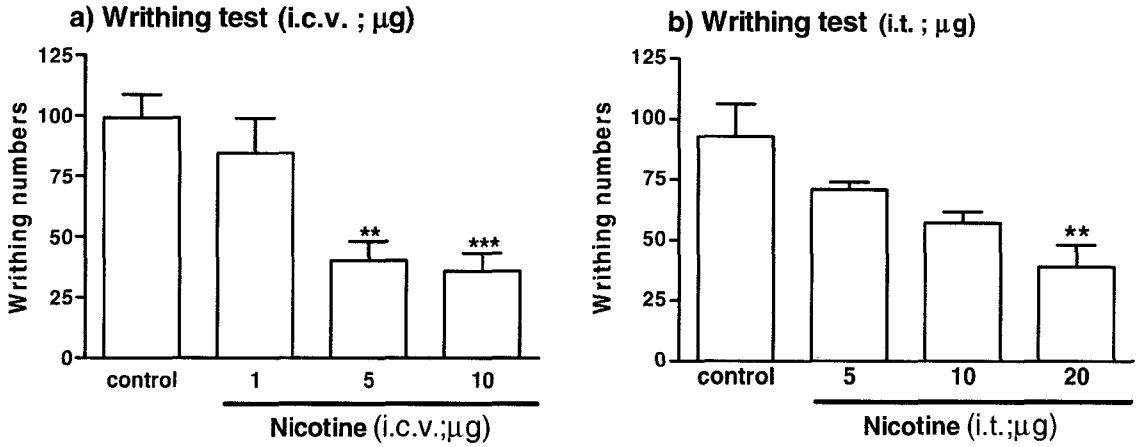

Fig. 3. The effects of nicotine injected i.c.v. or i.t. on the 1% acetic acid-induced pain behavior manifested for 30 min. Mice were treated i.c.v. (a) or i.t. (b) 5 min prior to the 1% acetic acid intraperitoneal injection. The cumulative number of pain response indicated by writhing due to i.p. injection was measured for 30 min. The vertical bars denote the standard error of the mean. The number of animals used for each group was 8-10. * P< 0.05, ** P<0.01 vs. control.

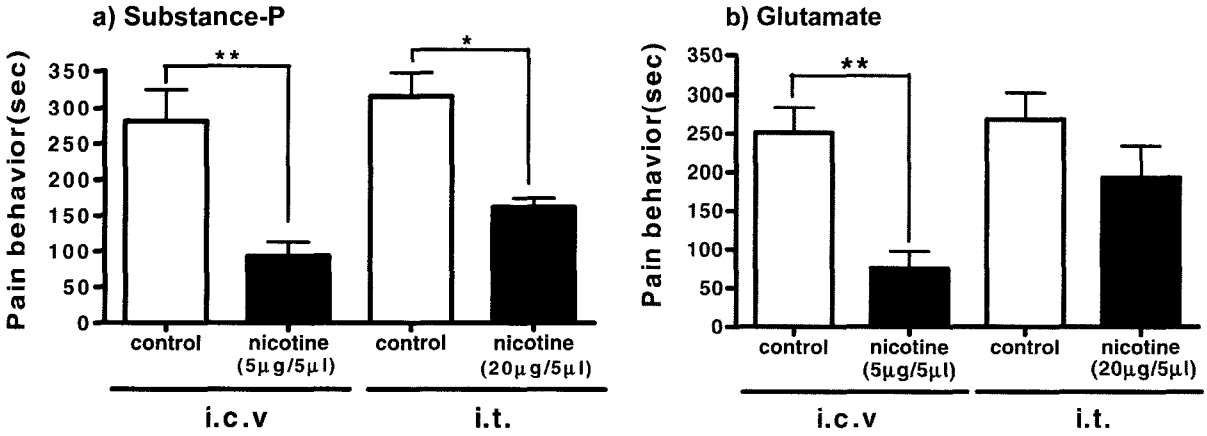

Fig. 4. The effects of nicotine injected i.c.v. or i.t. on the pain behavioral response in sustance P (a)- and glutamate (b)-induced pain models. Mice were treated i.c.v. or i.t. with nicotine 5 min prior to substance P (0.5 μg) or glutamate (20 μg) administered i.t. The pain response induced by substance-P or glutamate was observed for 30 min. The vertical bars indicate the standard error of the mean. The number of animal used for each group was 8-10. *P<0.05, **P<0.01 vs. control.

behavioral responses (Fig. 4a).

As shown in Fig. 4b, the pain behavioral response was also present in mice given glutamate i.t. I.c.v. administration of nicotine 5 min before injection of glutamate, significantly attenuated glutamate-induced pain behavioral responses (Fig. 4b). However, i.t. adminstered nicotine did not affect pain behavior induced by glutamate injected i.t (Fig. 4).

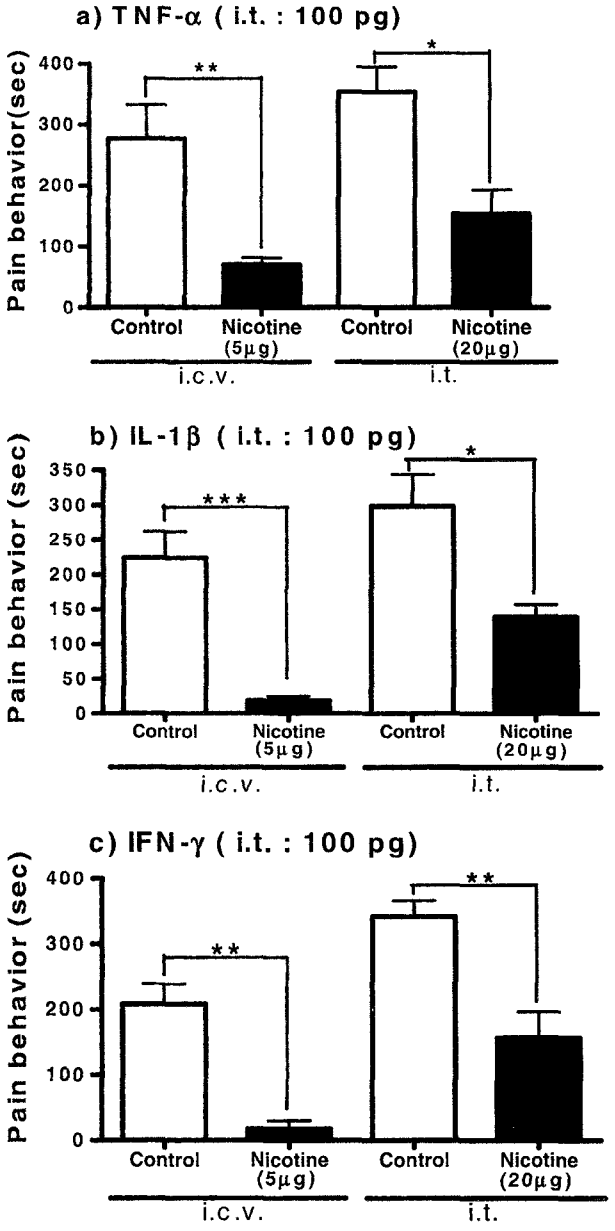

Fig. 5. The effects of nicotine injected i.c.v. or i.t. on the pain behavioral response in TNF-α- (a), IL-1β- (b), or IFN-γ (c)- induced pain models. Mice were treated i.c.v. or i.t. with nicotine 5 min prior to the 100 pg i.t. injection of TNF-α, IL-1β, or IFN-γ. The pain response induced by TNF-α, IL-1β, or IFN-γ was observed for 30 min. The vertical bars indicate the standard error of the mean. The number of animal used for each group was 8-10. **P<0.01, *** P<0.001 vs. control.

The effect of nicotine injected i.c.v. or i.t. on the nociceptive behavioral response in cytokines-induced pain model

In saline-treated control mice, i.t. injection of TNF-α (100 pg), IL-1β (100 pg) or IFN-γ (100 pg) caused an acute, immediate behavioral response, i.e., licking, scratching and biting, which lasted about 30 min. As shown in Fig. 5, nicotine treated i.c.v. or i.t. significantly attenuated TNF-α-, IL-1β- or IFN-γ- induced pain behavioral responses. (Figs. 5a, 5b, and 5c).

DISCUSSION

In the present study, we examined the antinociceptive properties of nicotine in various pain models, specifically formalin, acetic acid, substance P, glutamate and proinflammatory cytokines. These models for nocioception have been regarded as more satisfactory for demonstrating clinical pain than those which elicit only tonic thermal pain, such as the hot-plate or tail-flick test (Abbott *et al.*, 1981; Clavelou *et al.*, 1995).

We observed that nicotine attenuated pain behavior induced by intraplantar formalin in a dose-dependent manner. The acute first phase of the nociceptive response in the mouse formalin test lasts for about 5 min after formalin injection and is followed by a tonic second phase which persists for 20-40 min after formalin injection (Choi *et al.*, 2001; Chung *et al.*, 2001a; Hunskaar *et al.*, 1985). It is widely agreed that the first and second phases result from the direct activation of primary afferent fibers by nociceptors and the tonic inflammatory nociceptive responses, respectively (Ahmadiani *et al.*, 2000; Hunskaar and K., 1987; Maleki *et al.*, 2001; Puig and Sorkin, 1996). Notably, in the present study, the effect of nicotine treated i.t. was prominent only during the second tonic inflammatory phase. Shibata *et al.* (1989) have reported that peripherally active drugs such as aspirin and glucocorticoid only inhibited the second phase in the formalin test.

The results of our present study clearly demonstrate that nicotine treated supraspinally decreased pain behavior induced by formalin injection during both the first and second phases in a dose-dependent manner. However, nicotine administered spinally decreased formalin-induced pain behavior only during the second tonic phase, but not during the first phase, in a dose-dependent manner. These observations suggest that the effect of nicotine administered intrathecally on formalin-induced pain behavior may be more effective on tonic inflammatory pain than acute noxious pain response in the first phase. However, nicotine administered supraspinally reduces both formalin-induced pain behaviors, i.e. acute noxious pain as well as tonic inflammatory pain. A previous study has demonstrated that systemic injection of nicotine attenuates pain

behavior during the both first and second phases in the formalin test (Zarrindast, 2003). Based on these observations, when nicotine is administered intraperitoneally, it is speculated that a nicotine-induced decrease of pain behavior revealed during the first phase may be due to the action of nicotine at the supraspinal level.

To investigate the antinociceptive effects of nicotine in a visceral pain model, we performed the acetic acid-induced writhing test after i.c.v. or i.t. administration of nicotine. Intraperitioneal injection of acetic acid produced peritoneal inflammation (acute peritonitis), which caused a response characterized by the contraction of abdominal muscles accompanying an extension of the forelimbs and elongation of the body. Writhing is considered to be a visceral inflammatory pain response (Koster *et al.*, 1959; Vyklicky *et al.*, 1979). The i.c.v. or i.t. treatment with nicotine diminished the number of writhing responses in a dose-dependent manner in acetic acid-induced visceral nociception, suggesting nicotine is effective for producing antinocieption in a visceral pain model.

We observed that spinally or supraspinally administered nicotine exerted antinociceptive action on spinally administered TNF-α, IL-1β or IFN-γ. Our results clearly demonstrate, for the first time, that nicotine plays an important role in the regulation of pain behavior induced by pro-inflammatory cytokines such as TNF-α, IL-1β or IFN-γ at the central nervous system level. Furthermore, nicotine is expected to be effective for relieving neuropathic pain. Additionally, Rashid and Ueda (Rashid and Ueda, 2002) have reported that nicotine administered i.t. reduced hyperalgesia in a neuropathic pain midel.

It has been reported that i.t. injection of substance P or excitatory amino acids in mice can elicit nociceptive responses, characterized by biting, scratching and licking the caudal parts of the body (Hunskaar *et al.*, 1986; Hylden JI, 1980). Furthermore, several lines of evidence have demonstrated that i.t. injection of excitatory amino acids cause hyperalgesic response in the hot-plate test (Ferreira *et al.*, 1999). Iontophoretic application of excitatory amino acids shows that the responses to noxious heat, pinch and innocuous tap stimuli are enhanced (Cumberbatch *et al.*, 1994). In the present study, we demonstrated that the cumulative nociceptive response time for i.t. administration of substance P was significantly diminished when nicotine was administered spinally or supraspinally. However, in the glutamate pain model, only supraspinally injected nicotine was effective in attenuating pain behavior. These findings suggest that substance P- or glutamate-induced nociceptive transmission may be mediated by different nociceptive pathways.

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