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The differential effects of acetaminophen on lipopolysaccharide induced hyperalgesia in various mouse pain models

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ABSTRACT

We investigated effects of acetaminophen on LPS-induced hyperalgesia in various pain models. We examined the changes of pain behaviors induced by formalin injected subcutaneously (s.c.) in the hind paw, with substance P (SP) and glutamate injected inthrathecally (i.t.). Hyperalgesia was induced by LPS intraperitoneal injection 1 day prior to the pain test. LPS-induced hyperalgesia was exhibited in nociceptive behaviors induced by formalin s.c. (only in the second phase), SP and glutamate i.t. injection. APAP showed a dose-dependent antinociceptive effect on the saline- and LPS-pretreated group in the formalin and SP pain model. However, the analgesic effect of APAP was not observed in the glutamate pain model. To clarify the action site, APAP was administered i.t. or intracerebroventricularly (i.c.v.) 30 min prior to behavioral tests. The 2nd phase of formalin response was not only increased by LPS, but it also significantly attenuated by i.c.v. injections of APAP. However, the effect of APAP was observed only in the LPS-pretreatment, but not in the control group. These results suggest that LPS-induced hyperalgesia in the formalin 2nd phase may be involved in the SP-sensitive neuronal pathways, in which the hyperalgesic response elicited by LPS attenuated by APAP with supraspinal pain modulatory mechanisms.

1. Introduction

Pain hypersensitivity elicited by inflammation is well understood for an abnormal neuronal activity associated with the inflammatory mediators including excitatory amino acids, nitric oxides, pro-inflammatory cytokines and prostaglandins (Wieseler-Frank et al., 2005). In that, alteration of neuronal activity elicited by glial activation is regarded as a key mechanism of inflammation-induced hyperalgesia, and it was recently the focus of a new treatment target for pain hypersensitivity (Watkins and Maier, 2003). The bacterial endotoxin-lipopolysaccharide (LPS) induced hyperalgesia has been widely used as a model for inflammation-induced hyperalgesia (Kemper et al., 1998; Mason, 1993; Ueno et al., 2001; Watkins et al., 1994b; Wiertelak et al., 1994b) or inflammation-induced anti-analgesia (Johnston and Westbrook, 2005). It has been suggested that LPS induced hyperalgesia may be elicited by activation of the descending pain facilitatory system, which is mainly involved not only with the nucleus solitary tract and the raphe magnus within the rostral ventromedial but also the spinal cord (Watkins et al., 1994b). Moreover, it has also been introduced that substance P, cholecystokinin-2, *N*-methyl-D-aspartate (NMDA) receptor agonist and nitric oxide synthesis in the spinal cord (Watkins et al., 1994a,c; Wiertelak et al., 1994a) as well as prostaglandin production by cyclooxygenase-2 may play an important role in the regulation of hyperalgesia induced by LPS pretreatment (Matsumoto et al., 1998).

Acetaminophen (APAP) has been extensively used as an antipyretic and analgesic drug for over one century, but its mode of action has not been clarified yet. In addition, anti-hyperalgesic effect of APAP in the various pain models was not characterized yet. Contrary to aspirin and other nonsteroidal anti-inflammatory drugs (NSAIDs) which inhibit the synthesis of prostaglandin by effects on both cyclooxygenase (COX)-1 and COX-2 (Mitchell and Warner, 1999; Seibert et al., 1997; Smith et al., 2000; Vane et al., 1998; Wu, 1995), APAP does not inhibit COX in peripheral tissues, which could explain its very weak anti-inflammatory activity (Swierkosz et al., 2002). However, it has been widely assumed that APAP strongly inhibits prostaglandin synthesis in the central nervous system (Flower and Vane, 1972; Tolman et al., 1983). Recently, several authors demonstrated that the analgesic

Abbreviations: APAP, acetaminophen; COX, cyclooxygenase; LPS, lipopolysaccharide; NMDA, *N*-methyl-D-aspartate; NSAID, non-steroidal anti-inflammatory drug; SP, substance P; s.c., subcutaneously; i.t., intrathecal; i.c.v., intracerebroventricular.

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effect of the APAP might be mediated by monoaminergic (Courade et al., 2001a; Libert et al., 2004) or endogenous opioid systems (Raffa et al., 2004), which are affected by prostaglandin action in the central nervous system.

Subcutaneous (s.c.) injections of 1% formalin in the hind paw of mice induces nociceptive behaviors like licking, biting and shaking of the injected site. Generally, nociceptive behaviors induced by formalin s.c. show a biphasic pattern. The early phase of the nociceptive response normally peaks at between 0 to 5 min, and the late phase is manifested between 20 to 40 min after formalin injection, representing the direct effect on nociceptors and inflammatory nociceptive responses, respectively (Hunskaar and Hole, 1987). The exact mechanism leading to formalin-induced nociceptive response is not well known yet. However, there is strong evidence that spinally located SP may play important roles in the nociceptive processing of both the 1st and 2nd phase of pain behaviors, which are consisted with direct mechano- or chemo-receptor activation and inflammation, respectively, induced by formalin s.c. injection (Cridland and Henry, 1986; Hunt and Mantyh, 2001; Ohkubo et al., 1990). Furthermore, glutamate are mainly involved in the central sensitization which is induced by inflammatory processing in the 2nd phase of formalin responses or neuropathic pain (Chen and Lipton, 2006; Coderre and Melzack, 1992; Lucifora et al., 2006; Murray et al., 1991; Skilling et al., 1988). It has also been reported that i.t. injections of substance P (SP) or glutamate in mice induce a behavioral response similar to that caused by noxious stimulation and show a similar response consisting of biting, scratching, and licking the lumbar and caudal parts of the body. For these reasons, i.t. SP or glutamate injection has been widely used for pain models to study the nociceptive/antinociceptive mechanism (Choi et al., 2001; Chung et al., 2000; Hylden and Wilcox, 1981).

So far, it has been reported that traditional NSAIDs or selective COX-2 inhibitors have a modulatory effect in LPS-induced hyperalgesia (Matsumoto et al., 1998; Padi and Kulkarni, 2005; Satyanarayana et al., 2004). However, the direct prediction of APAP's effect on pain facilitation is difficult due to the molecular mechanisms and action sites which are quite different from traditional NSAIDs or selective COX-2 inhibitors. The present study, therefore, designed to characterize anti-hyperalgesic effect of APAP on various pain models for the first time. We firstly investigated the effects of APAP on LPS-induced hyperalgesia in formalin, SP, and glutamate pain models, respectively. Then, we also characterized the action site of APAP on the LPS-induced hyperalgesia.

2. Materials and methods

The experimental protocol was approved by an Institutional Review Committee for the use of Human or Animal Subjects or that procedures are in compliance with at least the Declaration of Helsinki for human subjects, or the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985), the UK Animals Scientific Procedures Act 1986 or the European Communities Council Directive of 24 November 1986 (86/609/EEC). 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 Institute of Health and the ethical guidelines of the International Association for the Study of Pain.

2.1. Animals

Male ICR mice (MJ Ltd., Seoul, Korea) weighing 25–28 g were used for all the experiments. Animals were housed 5 per cage in a room maintained at 22±0.5 °C with an alternating 12 h light–dark cycle for at least 5 days before the experiments were started and 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 used only once. To reduce variation, all experiments were performed during the light phase of the cycle (10:00–17:00).

2.2. Induction of hyperalgesia

For the induction of hyperalgesia, mice were injected with LPS 24 h prior to the pain test. Lipopolysaccharide (*Escherichia coli* O111:B_4) was injected intraperitoneally (i.p.) 1 mg/kg, 20 ml/kg. Control animals received an equivolume vehicle (0.9% physiologic normal saline). The concentration of LPS selected was based on previous studies that used i.p. administration (Matsumoto et al., 1998; Ueno et al., 2001; Watkins et al., 1994b) and our pilot study.

2.3. Intracerebroventricular (i.c.v.) and intrathecal (i.t.) injection of drugs

The i.t. injections were made according to the procedure of Hylden and Wilcox (Hylden and Wilcox, 1981) using a 25 μl Hamilton syringe with a 30-gauge needle. The i.c.v. administration followed the method described by Haley and McCormick (1957). The i.c.v. and i.t. injection volumes were 5 μl and the injection sites were verified by injecting a similar volume of 1% methylene blue solution and determining 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 through the ventricular spaces and reached the ventral surface of the brain and the upper cervical portion of the spinal cord. The dye injected i.t. was distributed both rostrally and caudally but within a short distance (about 0.5 cm) and no dye was found visually in the brain. The success rate for the injections, before the experiments were done, was consistently found to be over 95%.

2.4. Formalin treatment and nociceptive behavioral analysis

Ten microliters of 1.0% formalin solution, made up in physiologic normal saline, was injected subcutaneously (s.c.) under the plantar surface of the left hindpaw. For the behavioral study, animals were pretreated with LPS or saline administration i.p. 24 h prior to the study. Following subcutaneous injection in the mice's left hindpaw, the animals were immediately placed in an acrylic observation chamber (20 cm high, 20 cm diameter), and the time spent licking, shaking, and biting their injected paws was measured with a stopwatch timer and considered as indicative of nociception (Hunskaar et al., 1985).

2.5. Substance P or glutamate-induced nociceptive behavioral test

Mice were injected i.t. with substance P (SP; 0.7 mg) or glutamate (20 mg). For the behavioral study, animals were pretreated with LPS or saline administration i.p. 24 h prior to the study. Immediately after the i.t. injection, the mice were placed in a glass cylinder chamber (20 cm high, 20 cm diameter) and the duration of nociceptive behavioral responses, which were manifested by licking, biting, and scratching directed toward the lumbar and caudal region, was measured for 30 min (Hylden and Wilcox, 1981).

2.6. Drugs

All drugs were purchased from Sigma Chemical Co. (St. Louis, MO). Formalin, SP, glutamate and LPS (*Escherichia coli* 0111:B_4) were prepared with physiologic normal saline (0.9% weight/volume of NaCl). Acetaminophen was dissolved in 20% dimethyl sulfoxide for oral administration or dissolved in 5% ethanol-distilled water for i.t. or i.c.v. administration. Control animals received an equivolume vehicle (0.9% saline, 20% dimethyl sulfoxide and 5% ethanol-distilled water) respectively.

2.7. Statistical analysis

Data were presented as the mean±SEM. The statistical significance of differences between groups was assessed with two-way ANOVA with Dunnett's post-hoc test using SAS version 9.1.3 for Windows XP (SAS institute Inc., SAS Campus Drive Cary, NC 27513-2414 USA). The specific tests used are presented in the figure legends. *P* values less than 0.05 were considered to indicate statistical significance.

3. Results

3.1. The effects of acetaminophen administered orally on LPS-induced hyperalgesia in pain behaviors induced by s.c. formalin injection

Although i.p. LPS (1 mg/kg, 20 ml/kg) injection 24 h prior to the behavioral test has no effect on the 1st phase of formalin response (two-way ANOVA: LPS [$F(1,110)=0.22$, $p=0.6433$];), APAP significantly affected the 1st phase of formalin response (two-way ANOVA: APAP [$F(4,110)=8.25$, $p<0.0001$]). Meanwhile, the dose–response comparison did not show any differential response to APAP between the saline and LPS group (two-way ANOVA: Interaction between LPS and APAP [$F(4,110)=0.11$, $p=0.9775$]). The analgesic effect of APAP was verified by two-way ANOVA with a Dunnet's post-hoc test. Oral administration of APAP significantly decreased nociceptive behaviors at the dose of 300 mg/kg. $p<0.05$ was considered to indicate statistical significance (Fig. 1A).

As shown in Fig. 1B, the cumulative behavioral response time in the 2nd phase was significantly affected by LPS pretreatment (two-way ANOVA; LPS [$F(1,110)=15.82$, $p=0.0001$]) and APAP administration (two-way ANOVA; APAP [$F(4,110)=8.64$, $p<0.0001$]), respectively. The differential response to APAP between the saline and LPS group was not observed (two-way ANOVA: Interaction between LPS and APAP [$F(4,110)=2.41$, $p=0.0531$]). The antinociceptive effect of APAP was observed at the dose of 100 to 300 mg/kg (Dunnett's post-hoc test; $p<0.05$ was considered significant).

3.2. The effects of acetaminophen administered orally on LPS-induced hyperalgesia in pain behaviors induced by SP or glutamate i.t. injection

The i.t. injection of SP (0.7 μg/5 μl) or glutamate (20 μg/5 μl) caused an acute, immediate behavioral response, i.e., licking, biting and scratching, which lasted about 30 min. Pretreatment with LPS (1 mg/kg, 20 ml/kg) was performed 24 h prior to the behavioral study. As shown in Fig. 2A, LPS-pretreatment significantly influenced nociceptive behaviors elicited by SP i.t. injection (two-way ANOVA; LPS [$F(1,72)=50.15$, $p<0.0001$]). Moreover, APAP significantly affected the nociceptive behaviors induced by i.t. SP injection (two-way ANOVA; LPS [$F(3,72)=3.03$, $p=0.0346$]). The dose–response comparison did not show any differential response to APAP between the saline and LPS group (two-way ANOVA: Interaction between LPS and APAP [$F(3,72)=1.36$, $p=0.2632$]). The analgesic effect of APAP was verified by two-way ANOVA with a Dunnett's post-hoc test. The antinociception induced by orally administered APAP was observed at the dose of 300 mg/kg ($p<0.05$ was considered significant).

We also examined the dose-dependent effect of APAP on nociceptive behavior elicited by glutamate i.t. injection in the same manner. As shown in Fig. 2B, LPS pretreatment significantly increased pain behaviors induced by glutamate i.t. injection (two-way ANOVA; LPS [$F(1,64)=81.56$, $p<0.0001$]). However, APAP did not show any antinociceptive effect on the nociceptive behavior induced by glutamate i.t. injection overall (two-way ANOVA; APAP [$F(3,64)=1.78$, $p=0.1597$]). Moreover, we did not find any interaction between LPS-pretreatment and the analgesic effect of APAP. We therefore concluded that APAP have same effect at all value of LPS-pretreatment group (two-way ANOVA: Interaction between LPS and APAP [$F(3,64)=1.10$, $p=0.3538$]).

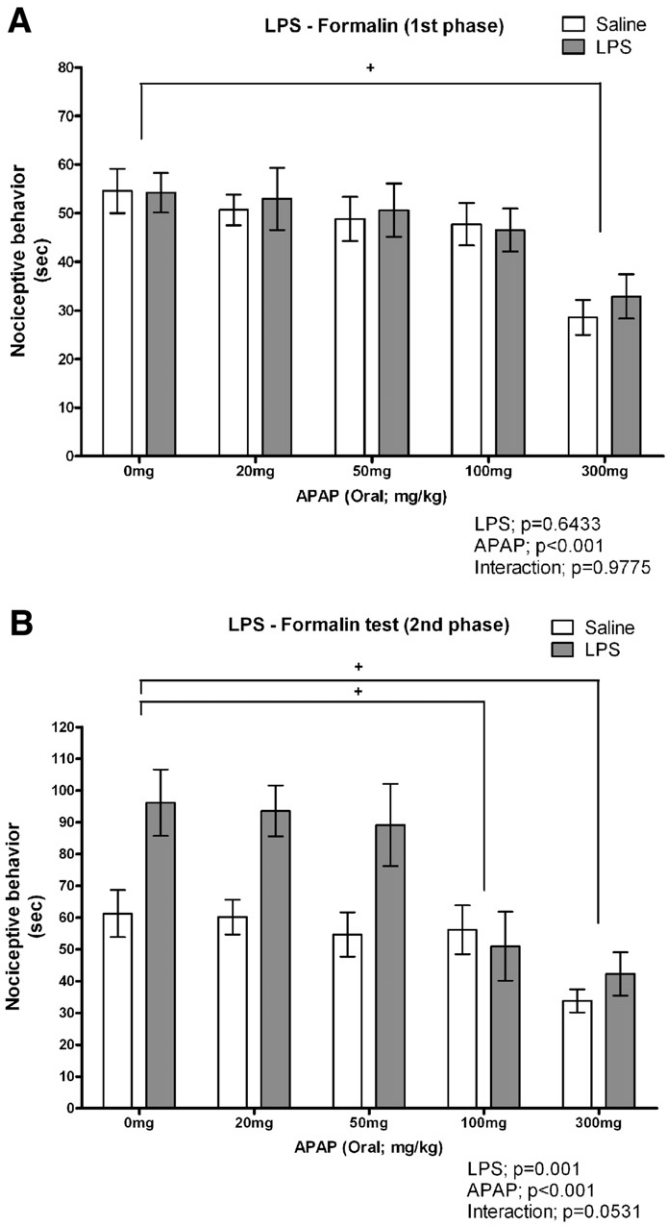

Fig. 1. The effects of orally administered acetaminophen on LPS-induced hyperalgesia in pain behaviors induced by s.c. formalin injection. Animals were injected with vehicle (0.9% normal saline; 20 ml/kg) or LPS (1 mg/kg, 20 ml/kg) intraperitoneally 1 day prior to the pain test. APAP was administered orally at various doses for 30 min prior to the s.c. formalin (1%, 10 μl) injection into the left hindpaw. The cumulative response time of licking, shaking and biting the injected paw was measured during the period of 0–5 min [the 1st phase] and 20–40 min [the 2nd phase]. The vertical bars denote the standard error of the mean. (A) The dose-dependent effect of APAP was examined in the 1st phase of the formalin test. LPS-induced hyperalgesia, the analgesic effect of APAP and the interaction between LPS and APAP were analyzed by two-way ANOVA. The dose-dependent effect of APAP on the 1st phase of formalin test was assessed by two-way ANOVA with Dunnett's post-hoc test. $p<0.05$ was considered significant. (+$p<0.05$; indicated the effect of APAP compared to 0 mg treated group, $n=12$) (B) The dose–response test of APAP was conducted in the 2nd phase of the formalin test. The LPS-induced hyperalgesic effect, antinociceptive response to APAP treatment and interaction between saline- and LPS-pretreatment group were analyzed by two-way ANOVA. The dose–response effect of APAP on the 2nd phase of formalin test was assessed with Dunnett's post-hoc test. $p<0.05$ was considered significant. (+$p<0.05$; indicated the effect of APAP compared to 0 mg treated group, $n=12$).

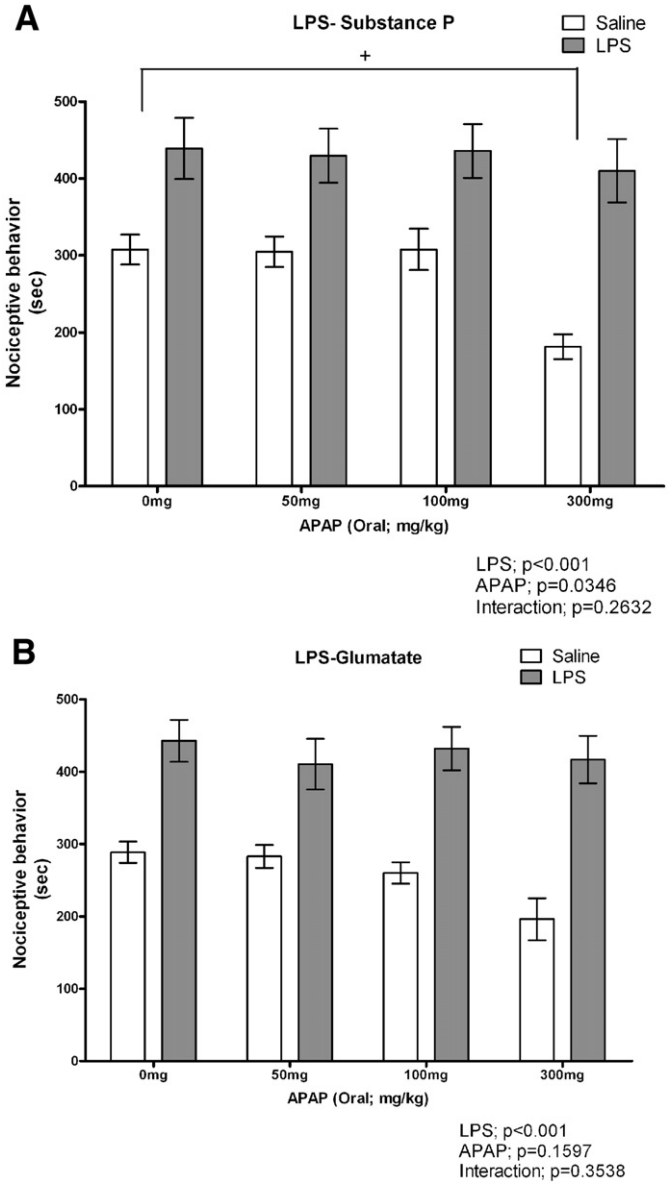

Fig. 2. The effects of acetaminophen administered orally on LPS-induced hyperalgesia in pain behaviors induced by i.t. SP or glutamate injection. Animals were injected with LPS (1 mg/kg, 20 ml/kg) intraperitoneally 1 day prior to the pain test. APAP was administered orally at various doses for 30 min prior to the substance P (0.7 μg/5 μl) or glutamate (20 μg/5 μl) i.t. injection. The cumulative response time of licking, biting and scratching episodes was measured for 30 min. The vertical bars denote the standard error of the mean. (A) The cumulative nociceptive response comparison reveals a hyperalgesic effect of LPS which was assessed by two-way ANOVA. The analgesic effect of APAP was analyzed by two-way ANOVA with a Dunnett's post-hoc test. Each comparisons were determined as significant at the $p<0.05$ level. The differential response to APAP between saline- and LPS-pretreatment was not observed. (+$p<0.05$; indicated the dose-dependent effect of APAP compared to 0 mg treated group, $n=10$). (B) The effect of APAP was assessed in the nociceptive behavior induced by glutamate i.t. injection. The hyperalgesic effect of LPS, the antinociceptive effect of APAP and the differential response to APAP between saline- and LPS-pretreated groups were analyzed by two-ANOVA. ($n=9$). The effect of APAP as well as interaction between LPS and APAP was not apparent.

3.3. The effects of acetaminophen administered intracerebroventricularly on LPS-induced hyperalgesia in pain behaviors induced by s.c. formalin injection

The dose–response effect was determined by supraspinal (i.c.v.) administration of APAP. The 1st phase of formalin response was not changed by LPS-pretreatment 24 h prior to the behavioral test (two-way ANOVA; LPS [$F(1,72)=0.43$, $p=0.5147$]. Furthermore, i.c.v. administration of APAP did not show an antinociceptive effect on the 1st phase of formalin response (two-way ANOVA; APAP [$F(3,72)=0.12$, $p=0.9472$]). Since, interaction between LPS-pretreatment and the

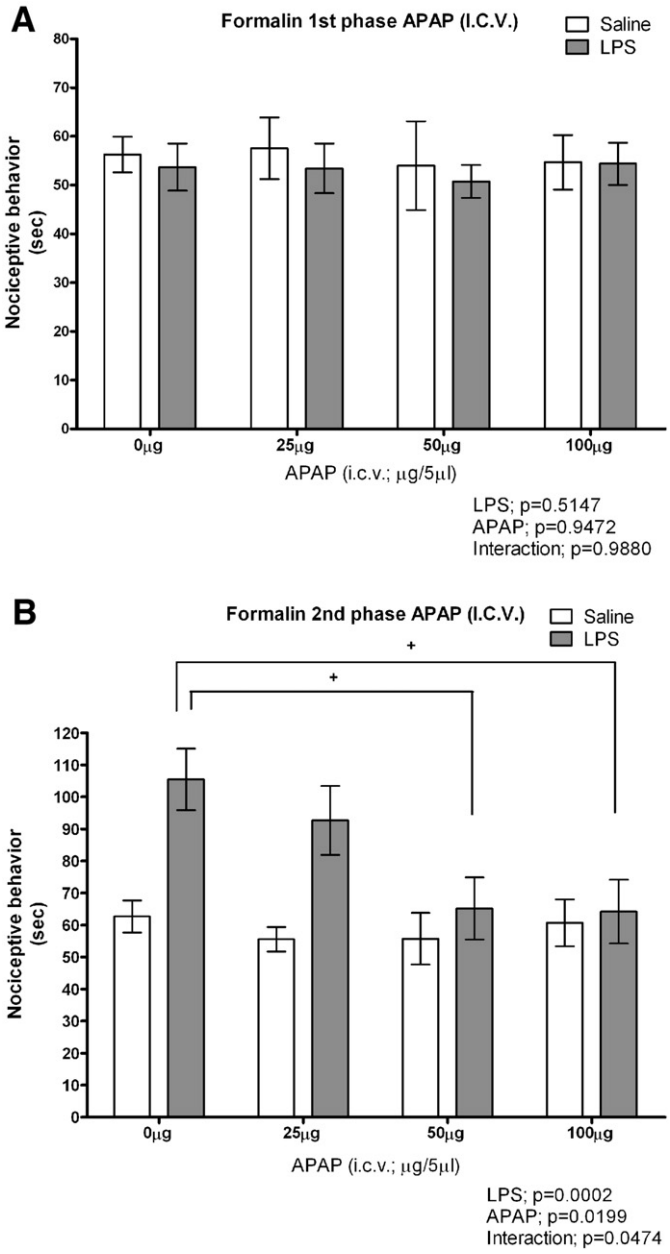

Fig. 3. Supraspinal effects of acetaminophen on LPS-induced hyperalgesia in pain behaviors induced by s.c. formalin injection. Animals were injected with vehicle (0.9% normal saline; 20 ml/kg) or LPS (1 mg/kg, 20 ml/kg) intraperitoneally 1 day prior to the pain test. APAP was administered intracerebroventricularly at various doses for 30 min prior to the s.c. formalin (1%, 10 μl) injection into the left hindpaw subcutaneously. The cumulative response time of licking, biting and shaking the injected paw was measured during a period of 0–5 min [1st phase] and 20–40 min [2nd phase]. The vertical bars denote the standard error of the mean. (A) The dose–response effect of supraspinally administered APAP was examined in the 1st phase of the formalin test. In the two-way ANOVA result, the hyperalgesic effect of LPS, the antinociceptive effect of APAP and the differential response to APAP between saline- and LPS-pretreated groups were not statistically significant. (B) The dose–response test of APAP was conducted in the 2nd phase of the formalin test. The LPS-induced hyperalgesia, antinociceptive response to APAP treatment and interaction between saline- and LPS-pretreatment groups were analyzed by two-way ANOVA. LPS-pretreatment not only significantly induced hyperalgesic response ($n=12$), but i.c.v. administered APAP also robustly affected the 2nd phase of formalin response. Since, dose–response effect of APAP was significantly different between saline and LPS-pretreatment group ($p<0.05$), the analgesic effect was analyzed by one-way ANOVA with Dunnett's post-hoc test in each group (+$p<0.05$; compared with 0 mg treated group, $n=12$). The analgesic effect of APAP was not observed in saline-pretreatment group.

analgesic effect of APAP was not statistically significant, APAP have same effect at all value of LPS-pretreatment group (two-way ANOVA; [$F(3,72)=0.04$, $p=0.9880$]) (Fig. 3A).

As shown in Fig. 3B, pretreatment of LPS significantly increased the 2nd phase of formalin response (two-way ANOVA; LPS [$F(1,88)=15.41$, $p=0.0002$]). Although, supraspinally administered APAP significantly attenuated the 2nd phase of formalin response (two-way ANOVA; APAP [$F(3,88)=3.45$, $p=0.0199$]), the effect of the APAP treatment was completely different between saline- and LPS-pretreatment group (two-way ANOVA: Interaction between LPS and APAP [$F(3,88)=2.75$, $p=0.0474$]). The analgesic effect of APAP was verified by one-way ANOVA with a Dunnet's post-hoc test in each group. In the LPS-pretreatment group, the antinociceptive effect of i.c.v. administered APAP was observed at the dose of 50 and 100 μg/5 μl ($p<0.05$ was considered significant). However, we did not find any effect of APAP in the saline-pretreatment group (Fig. 3B).

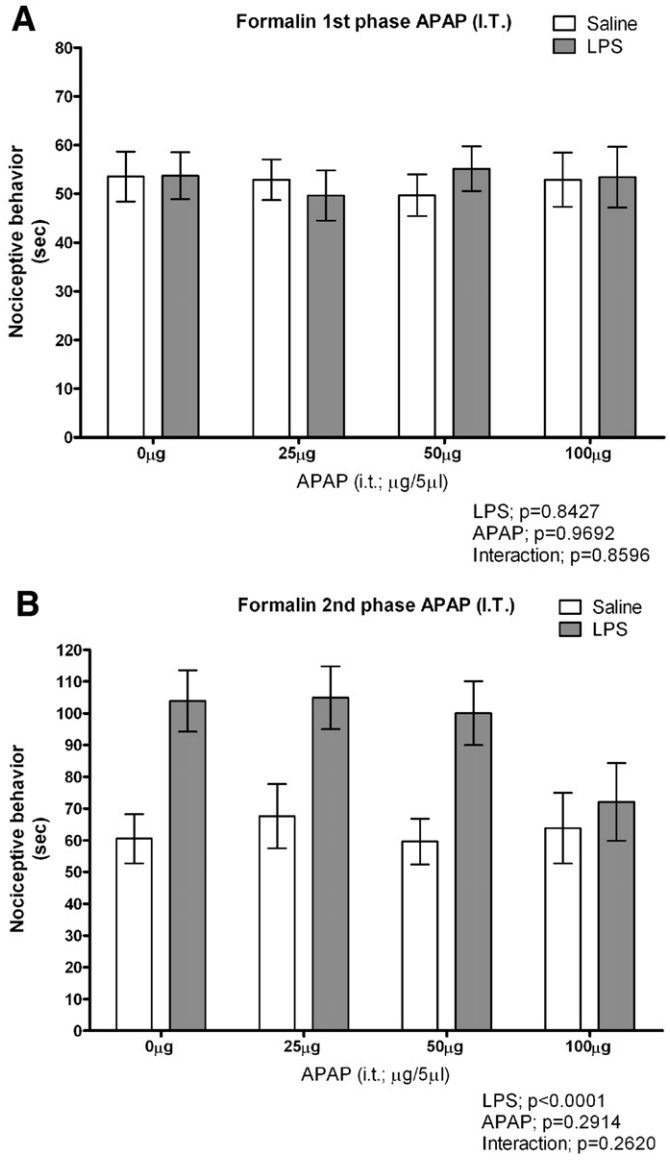

Fig. 4. Spinal effects of acetaminophen on LPS-induced hyperalgesia in pain behaviors induced by s.c. formalin injection. Animals were injected with vehicle (0.9% normal saline; 20 ml/kg) or LPS (1 mg/kg, 20 ml/kg) intraperitoneally 1 day prior to the pain test. APAP was administered intrathecally at various doses for 30 min prior to the s.c. formalin (1%, 10 μl) injection into the left hindpaw subcutaneously. The cumulative response time of licking, biting and shaking the injected paw was measured during a period of 0–5 min [1st phase] and 20–40 min [2nd phase]. The vertical bars denote the standard error of the mean. (A) The dose–response effect of spinally administered APAP was examined in the 1st phase of the formalin test. In the two-way ANOVA result, the hyperalgesic effect of LPS, the antinociceptive effect of APAP and the differential response to APAP between saline- and LPS-pretreated groups were not statistically significant. (B) The dose–response test of APAP was also conducted in the 2nd phase of the formalin test. The LPS-induced hyperalgesia, antinociceptive response of APAP treatment and interaction between LPS and APAP were analyzed by two-way ANOVA. Although, the hyperalgesia induced by LPS pretreatment was apparent in the 2nd phase of formalin response ($n=12$), neither the APAP-induced antinociception nor interaction between saline- and LPS-pretreatment groups has statistical significance.

3.4. The effects of acetaminophen administered intrathecally on LPS-induced hyperalgesia in pain behaviors induced by s.c. formalin injection

We examined the dose-dependent effect of i.t. administered APAP in the same manner. The 1st phase of formalin response was not changed by LPS-pretreatment 24 h prior to the behavioral test (two-way ANOVA; LPS [$F(1,72)=0.04$, $p=0.8427$]). Furthermore, i.t. administration of APAP did not show an antinociceptive effect on the 1st phase of formalin response (two-way ANOVA; APAP [$F(3,72)=0.08$, $p=0.9692$]). In addition, interaction between LPS-pretreatment and the analgesic effect of APAP was not statistically significant (two-way ANOVA; [$F(3,72)=0.25$, $p=0.8596$]) (Fig. 4A).

As shown in Fig. 4B, LPS pretreatment significantly increased the 2nd phase of formalin response (two-way ANOVA; LPS [$F(1,88)=21.43$, $p<0.0001$]). However, there is no significant effect of APAP, which administered spinally, on the nociceptive behavior induced by formalin s.c. injection (two-way ANOVA; APAP [$F(3,88)=1.26$, $p=0.2914$]. A significant interaction about analgesic effect of APAP was not also observed between LPS-pretreated group and saline-pretreated group (two-way ANOVA; Interaction [$F(3,88)=1,35$, $p=0.2620$]).

4. Discussion

In this study, we clearly demonstrated the differential analgesic effects of APAP on LPS-induced hyperalgesia in various pain models which are elicited by s.c. formalin or i.t. SP or i.t. glutamate injection. The hyperalgesic effects elicited by i.p. LPS pretreatment was observed in every pain responses that used in our experiments except the 1st phase of formalin response (Figs. 1B, 2A and B). Oral administration of APAP substantially reduced nociceptive behaviors induced by s.c. formalin (both in the 1st and 2nd phase) as well as i.t. SP injection (Figs. 1A, B and 2A). The antinociceptive and anti-hyperalgesic effect of APAP were also observed in the LPS-induced hyperalgesic state in the 2nd phase of formalin response and in the behavioral test using i.t. SP injection, respectively. However, the effects of APAP were not found in the pain behaviors induced by i.t. glutamate injection (Fig. 2B). Although, these results may not be concordant with our previous report on the differential analgesic mechanisms of aspirin and acetaminophen (Choi et al., 2001), the discrepancy seem to be due to the differential sample size and experimental design. We characterized the supraspinal action of APAP that was responsible for the effects of APAP on LPS-induced hyperalgesia in the 2nd phase of formalin response (Fig. 3B). These pain modulations may occur by a complex interaction of APAP and the descending pain modulatory system.

We could interpret our results from two points of view: the first is the differential mechanisms of nociceptive processing according to pain modality and the second is an interrelationship of hyperalgesic action of LPS and the anti-hyperalgesic action of APAP.

Previous research revealed that nociceptive transmissions may be processed by various neurochemically defined parallel pathways (Hunt and Mantyh, 2001). Particularly, it has been known that inflammatory pain and neuropathic pain transmission were quite different. Inflammatory pain processing was mainly transmitted by SP, CGRP, and TrkA receptor express C-fiber in the spinoparabrachial pathway, but neuropathic pain processing was relayed by the P2X3 purine receptor and the receptor for glial-cell-derived neutotrophic factor express C-fiber (Hunt and Mantyh, 2001; Lucifora et al., 2006;

Nagy and Hunt, 1982). As alluded to earlier, the formalin response consisted with two nociceptive phases; the 1st phase for the acute phasic nociceptive processing and the 2nd phase for the tonic inflammatory nociceptive processing, in which several neurotransmitters are differently involved in these two behavioral phases. It has been suggested that SP is involved with both the 1st phase and the 2nd phase of formalin responses. Moreover, glutamate that is known to be involved in the central sensitization elicited by neuropathic pain and is also involved in tonic nociceptive processing during the 2nd phase of pain behaviors, (Choi et al., 2001; Miczek et al., 1986; Skilling et al., 1988; Vaccarino et al., 1992). However, in this study, anti-hyperalgesic response for APAP between the 2nd phase of the formalin test and other nociceptive behaviors induced by SP or glutamate i.t. injection did not correlate with the preceding studies. The reasons for the differential anti-hyperalgesic effect of APAP on the formalin, SP, or glutamate pain tests are not apparent, but these results imply that APAP may have differential anti-hyperalgesic modalities in various nociceptive pathways. However, the exact regulatory mechanisms of APAP on different nociceptive parallel pathways need to be elucidated in further studies.

A series of studies has reported that illness-inducing agents produce hyperalgesia (Maier et al., 1993; Watkins et al., 1994b; Wiertelak et al., 1994b). Moreover, LPS-induced hyperalgesia has been widely adapted to investigate the neurocircuitry of illness-induced hyperalgesia and the mechanisms of analgesic drugs (Watkins et al., 1994b). According to a previous study, we can classify the effect of LPS by their sites of action, which are supraspinal and spinal action. It has been suggested that LPS enhances both supraspinally-mediated nociceptive processing (formalin response and writhing response) and spinally-mediated pain reflexes (tail-flick response). It has generally been assumed that although the exact roles of LPS on hyperalgesia in the supraspinal and spinal region is not fully understood, the activation of the vicera-to-spinal cord pain pathway as well as glial activation were key mechanisms. In these mechanisms, nucleus raphe magnus (NRM) may be regarded as an important region in which to activate the pain facilitatory system, especially for an important source of substance P involved in pain facilitatory processes (Watkins et al., 1994b). Moreover, it has been reported that substance P is involved in the generation of NMDA-mediated pain facilitatory processes in the spinal cord dorsal horn (Coderre et al., 1993; Meller and Gebhart, 1993). One important consideration is that the effect of APAP was mediated by serotonergic systems in NRM (Courade et al., 2001b; Libert et al., 2004; Pickering et al., 2006), which is strongly involved with LPS-induced hyperalgesia. It would seem therefore that NRM may be an important site for the development of hyperalgesia as well as APAP-induced anti-hyperalgesic action. Although the direct evidence about the substance P-involved NRM neurons was not clarified in this study, our result imply that at least the anti-hyperalgesic effect of APAP may be partially mediated by an inhibition of substance P-involved pain facilitatory system in NRM. Until now, the exact mechanisms of APAP action on substance P, cholecystokinin-2, *N*-methyl-D-aspartate (NMDA) receptor agonist, and nitric oxide synthesis in the spinal and the supraspinal regions were not clarified yet. Therefore, we expect that the exact roles of APAP on differential regulatory sites in nociceptive processing will need to be clarified in further study.

Previous studies have demonstrated that APAP acts by inhibition of central COX activity (Vane, 1994; Vane and Botting, 1998), which is involved with the modulation of microglial activity (Fiebich et al., 2000). Although COX-3, which is a splice variant of COX-1 and related to the specific target for the APAP, was newly suggested (Chandrasekharan et al., 2002), it remains controversial for its inappropriate clinical relevance. More recently, it was suggested that the central mechanism of APAP action may be due to the form of the bioactive fatty acid amide *N*-arachidonoyl phenolamine (AM404) in the central nervous system by conjugation of *p*-aminophenol and arachidonic acid (Hogestatt et al., 2005). Although, we did not find any antinociceptive effect of APAP which administered spinally as well as supraspinally, the anti-hyperalgesic action of APAP on LPS-induced hyperalgesia was apparent only in the supraspinally injected group (Figs. 3B and 4B). This result strongly suggests that APAP as central COX inhibitor may exert their sensitization-relieving effects by modulating nociception at supraspinal sites rather than spinal site.

In the present study, we clearly demonstrated the differential effect of APAP on various pain models. The APAP, which was administered systemically or supraspinally, robustly attenuated LPS-induced hyperalgesia in the 2nd phase of formalin response and nociceptive behaviors induced by SP i.t. injection, but not in the 1st phase of formalin response and nociceptive behaviors induced by glutamate i.t. injection. These results imply that glutamate-sensitive nociceptive pathways may not be implicated in the anti-hyperalgesic action of APAP on LPS-induced hyperalgesia. Furthermore, the anti-hyperalgesic action of APAP on LPS-induced hyperalgesia in inflammatory nociceptive processing may be mediated by supraspinal action.

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