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Brain Research Bulletin 71 (2006) 279–286

BRAIN RESEARCH BULLETIN

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Changes in pain behavior induced by formalin, substance P, glutamate and pro-inflammatory cytokines in immobilization-induced stress mouse model

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Received 23 June 2006; received in revised form 5 September 2006; accepted 12 September 2006
Available online 4 October 2006

Abstract

In the present study, we examined the change of pain behaviors induced by formalin injected subcutaneously (s.c.) into the hind paw, or substance P (SP), glutamate, and pro-inflammatory cytokines (TNF-α, IL-1β, and IFN-γ) injected intrathecally (i.t.) in the mouse immobilization stress model. The mouse was restrained either once for 1 h or five times for 5 days (once/day). In the formalin test, a single immobilization stress attenuated pain behaviors (licking, biting or scratching) in the second phase, while it had no effect on the pain behaviors revealed during the first phase. In addition, repeated immobilization stress attenuated pain behaviors revealed during the second phase but not in the first phase. A single as well as repeated immobilization stress decreased pain behaviors induced by substance P i.t. injection, but there were no significant changes in the glutamate test. In the pro-inflammatory cytokine pain model, a single immobilization stress decreased the pain behaviors induced by TNF-α, IL-1β administered i.t. but not by IFN-γ administered i.t. Moreover, a mouse applied with repeated immobilization stress did not show any changes in pain behaviors elicited by pro-inflammatory cytokines (TNF-α, IL-1β and IFN-γ) compared to the control group. These results suggest that a single and repeated immobilization stress differentially affects such nociceptive processing induced by formalin, SP, glutamate and pro-inflammatory cytokines in different manners.

Keywords: Immobilization stress; Pain behavior; Stress-induced analgesia

1. Introduction

Stress is defined as a state of disharmony or threatened homeostasis and results in various physiological and behavioral changes [15]. It has been characterized that stress influences brain activity and promotes long-term changes in various neural systems. Stress therefore elicits a cluster of neuronal disorders that is implicated in cognitive, endocrinal and psychiatric problems [39,47,52]. In addition, a series of studies has demonstrated that stress generally decreases the nociception referred as a stress-induced analgesia that is considered to be implicated with endogenous opioid systems [2,4,32,50,54].

Immobilization is widely used stress model, which inflict potent physical and psychological stress on experimental animals to make various psychopathology [25]. Immobilization stress produces antinociceptive effects which are supported by the findings that immobilization stress increases the latency of the hot-plate response [2]. Although the exact roles underlying immobilization-induced antinociception are not fully understood, it has been suggested that the endogenous opioid system is attributed to the production of antinociceptive effects induced by immobilization, at least in part [3,6,8,42]. Recent studies have shown that single and repeated immobilization stresses induced antinociception effects differently to tail-flick latency [31]. In addition, it has also been shown that a single immobilization attenuated only the second phase of formalin induced pain behaviors in male and female rats, respectively [1]. Although many previous studies have demonstrated mainly immobilization induced analgesia or antinociception in the

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doi:10.1016/j.brainresbull.2006.09.012

tail-flick [20,33,36,57] and the hot-plate test [26], it has not been well known that antinociceptive profiles of a single- and repeated immobilization stress occur in various pain models.

Subcutaneous (s.c.) injections of 1% formalin in mouse left hind paw induce nociceptive behaviors like licking, biting, scratching forward to injected sites. Generally, nociceptive behaviors induced by formalin s.c. show a biphasic pattern. The early phase of the nociceptive response normally peaks 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 [27].

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 showed 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 [11,16,30]. Although the exact mechanism leading to formalin-induced nociceptive response is not well known yet, several studies have been suggested that spinally located SP may play important roles in the nociceptive processing of both the first and second phase of pain behaviors, which are consisted with direct mechano- or chemo-receptor activation and inflammation, respectively, induced by formalin s.c. injection [19,28,44]. Furthermore, glutamate are mainly involved in the central sensitization which is induced by inflammatory processing in the second phase of formalin responses or neuropathic pain [9,17,37,43,48].

Recent study has shown that i.t. injections of mouse pro-inflammatory cytokines are a useful pain model which also evoked nociceptive behaviors [10]. Pro-inflammatory cytokines (TNF-α, IL-1β and IFN-γ) are well known to be involved in the pathophysiology of pathological pain states that are related in hyperalgesia or allodynia [45,51,56].

As we mentioned above, the effects of a single and repeated immobilization stress on nociceptive behaviors elicited by various pain models have not been well characterized yet. In the present study, we therefore examined the effect of a single and repeated immobilization stress on nociceptive behaviors induced by formalin, SP, glutamate and pro-inflammatory cytokines.

2. Materials and methods

These experiments were approved by the Hallym University 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.

2.1. Experimental animals

Male ICR mice (MJ 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 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 experimental condition in the laboratory for at least 2 h before immobilization stress or pain testing. To reduce variation, all experiments were performed during the light phase of the cycle (10:00–17:00).

2.2. Immobilization stress procedure

The mice were subjected to restraint stress as described in a previous study [50]. In brief, restraint was carried out by placing the mouse in a 50 ml corning tube, and adjusting it with an iron nail on the outside, which crossed in the caudal part of the animal. Adequate ventilation was provided by means of holes at the sides of the tubes. The mice were stressed by restraint for 1 h for daily, and for 5 days in the repeated model. In the single model there was a single exposure. The control group was submitted to the same handling at the same time except for the immobilization procedure.

2.3. Intrathecal (i.t.) injection of drugs

The i.t. administration was performed in conscious mice following the previously established method [29,30] using a 30-gauge needle connected to a 25 μl Hamilton syringe with polyethylene tubing. The i.t. injection volume was 5 ml and the injection site was verified by injecting a similar volume of 1% methylene blue solution and determining the distribution of the injected dye in the spinal cord. The dye injected i.t. was distributed both rostrally and caudally but with in a short distance (about 0.5 cm) and no dye was found in the brain. The success rate for the injections was consistently found to be over 95%, before the experiments were done.

2.4. Formalin treatment and nociceptive behavioral analysis

A 10 μl of 1.0% formalin solution, made up in physiologic saline (0.9% NaCl), was injected subcutaneously (s.c.) under the plantar surface of the left hind paw. For the behavioral study, animals were restrained once for 1 h or daily for 5 days prior to the behavioral study although the control group was not submitted to restraint and was injected with formalin without delay. Following the intraplantar injection of formalin, the mouse was 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 [27].

2.5. Substance P or glutamate-induced nociceptive behavioral test

For the behavioral study, mice were restrained once for 1 h or daily for 5 days prior to the behavioral study although the control group was not submitted to restraint. The mouse was injected i.t. with the Substance P (SP; 0.7 μg/5 μl) or glutamate (20 μg/5 μl) after immobilization stress without delay. Following the intrathecal injection of SP or glutamate, the animals were immediately 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 a nd scratching directed toward the lumbar and caudal regions of the spinal cord, was measured for 30 min [30]. Characteristic behaviors (biting, licking and scratching at the abdomen and hind portions of the body) induced by pharmacological effects of SP or glutamate were not observed in the vehicle-treated control group [16,30,45].

2.6. Pro-inflammatory cytokines-induced nociceptive behavioral test

The intrathecally injected TNF-α, IL-1β and IFN-γ-induced nociceptive behavioral tests were performed by the following procedures. Mice were restrained once for 1 h once or daily for 5 days prior to the behavioral study although the control group was not submitted to restraint. Mice were injected i.t. with the TNF-α (100 pg/5 μl), IL-1β (100 pg/5 μl) or IFN-γ (100pg/5 μl) after immobilization stress without delay. Immediately after the i.t. injection, each mouse was placed in an observation chamber (20 cm high, 20 cm diameter) and their behavioral responses such as licking, biting and scratching directed toward the lumbar and caudal regions of the spinal cord were recorded for 30 min. The cumulative response time(s) of scratching and biting episodes were measured with a stop-watch timer. Characteristic behaviors (biting, licking and scratching at the abdomen and hind portions of the body) induced by pharmacological effects pro-inflammatory cytokines (TNF-α, IL-1β and IFN-γ) were not observed in the vehicle-treated control group [16,30,45].