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Neurokinin-1 receptor in peripheral nerve terminals mediates thermal hyperalgesia

Wen-Ling Chen, Yu-Qiu Zhang, Zhi-Qi Zhao *

Institute of neurobiology, Fudan University, Shanghai 200433, China

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Abstract

Neurokinin-1 receptor (NK-1) plays an important role in nociception. The present study was to explore whether activation of peripheral NK-1 receptor, especially expressed on primary sensory afferents, could induce hyperalgesia and sensitize C-type sensory afferents. (1) Intraplantar administration of NK-1 agonist [Sar^9, Met(O$_2$)^{11}]SP (Sar-SP, 0.2, 1 nmol, 20 μl) produced significant thermal hyperalgesia and edema, which was blocked by co-injection of NK-1 antagonist WIN51,708 (10 nmol). But in the rats with compound 48/80 treatment for mast cell depletion, the Sar-SP-induced edema, but not hyperalgesia, was attenuated. (2) Close-arterial injection of Sar-SP (1 nmol, 0.1 ml) excited and sensitized sensory C afferents of the sural nerve to heat stimuli. The results suggest involvement of NK-1 receptors expressed on the peripheral afferent terminals in thermal hyperalgesia mediated by directly sensitizing C-type sensory afferents.

Keywords: Neurokinin-1 receptor; Thermal hyperalgesia; Mast cell; Peripheral sensory afferent

Several lines of evidence indicate that substance P (SP) and its highly affinitive receptor, neurokinin-1 receptor (NK-1), play an important role in nociception. SP is produced in a proportion of small primary sensory neurons and released from its central and peripheral terminals by noxious stimulus in the periphery [1,2]. SP delivered from peripheral terminals induces vasodilation and neurogenic inflammation [3]. Peripheral application of SP produces pain favoring response and mechanical hyperalgesia in rats [4,5]. Close-arterial injection of SP excited partial articular polymodal C-fibers and enhanced mechanical sensitivity in rats and cats [6,7]. However, it is not clear whether activation of peripheral NK-1 receptor could produce thermal hyperalgesia and influence the thermal sensitivity of C-type nociceptors. Therefore, in the present study, we used [Sar^9, Met(O$_2$)^{11}]SP (Sar-SP), a highly selective NK-1 receptor agonist, to investigate effects of NK-1 receptor on the paw withdrawal latency (PWL) to heat stimulus and the excitability of afferent C nociceptors.

It is known that mast cells, as immunocompetent cells, are widely distributed along the periphery and close to the nerve endings, in which NK-1 receptors are richly expressed. SP released from peripheral nerve endings binds to mast cells to elicit degranulation [8]. Activated mast cells in turn release several inflammatory mediators to induce vasodilation, plasma extravasation, and excitation of C-type afferent terminals, which result in tissue edema and neurogenic inflammation. A question is whether NK-1 agonist-induced hyperalgesia is mediated directly by activation of NK-1 expressing in the nerve terminals or indirectly by inflammatory mediators released from mast cells, when NK-1 agonist is peripherally administered. To address these possibilities, compound 48/80, an activator of mast cell degranulation, was chronically administered to deplete mast cells.

Materials and methods

Behavioral studies. Male Sprague–Dawley rats (Experimental Animal Center, Shanghai Medical College of Fudan University, China) weighing

* Corresponding author. Fax: +86 21 55522876.
E-mail address: zqzhao@fudan.edu.cn (Z.-Q. Zhao).

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200–250 g were used in the behavioral test. All experiments were conducted in accordance with the guidelines of the International Association for the Study of Pain (IASP) and approved by The Committee for the Ethical Use of Laboratory Animal, Fudan University. Before behavioral test, the rats were acclimated for the experimental environment at least 3 days and housed in groups of 3–4 in plastic cages with soft bedding with a 12:12 h light-dark cycle.

Drug injection. Intraplantar injections of drugs were performed using a 28 gauge needle to a 25 μl Hamilton syringe. The needle was pushed into the subcutaneous space of the plantar skin, and 0.2 or 1 nmol Sar-SP (RBI, USA) was injected in 20 μl volume. In some rats, 10 nmol W51,708 (RBI,USA), an antagonist of NK-1 receptor, was co-injected with 1 nmol Sar-SP. The control rats were injected the same volume of saline.

Thermal hyperalgesia. Thermal hyperalgesia was determined by measuring the paw withdrawal latency (PWL) of the hindpaw in response to radiant heat stimulus (model 336 combination unit, IITC/life Science Instruments, Woodland Hill, CA, USA). Rats were placed within individual transparent observation chamber and allowed to acclimate for 30 min before test. The intensity of the thermal stimulus was adjusted to obtain baseline latencies of ~10 s and a cut-off time of 20 s to prevent tissue damage. Before injection of drugs, the hindpaw was tested for three trials at each time period with 5 min intervals between each trial and the average of the three trials was then determined as the baseline. After drugs' administration, the hindpaw was tested only one trial at different time-point during 1 h.

Edema measurement. The thickness of hindpaw was measured at 30 min after injection to evaluate the extent of edema. The rats were fixed in a plastic cylinder with the hindpaw outside. A calibrated micrometer was used to measure the maximal dorsal–ventral thickness of left hindpaw. Three trials were conducted and averaged to give a mean thickness.

Chronic mast cell degranulation. Some rats received an ascending series of doses of compound 48/80 (Sigama, USA) in a 4-day period to induce chronic mast cell depletion [9]. Briefly, compound 48/80 was administered intraperitoneal (i.p.) with 25 μg on the first day, 60 μg on the second day, 125 μg on the third day, and two injections of 200 μg on the fourth day interval 6 h. The control rats received saline as the same process.

Electrophysiological studies. Single fiber recording from the sural nerve was performed in rats weighing 220–300 g. Animal was anesthetized with i.p. injection of urethane (1.5 g/kg, supplemented about 0.5 g/kg as necessary during experiment). The trachea, right carotid artery were cannulated. Core temperature, respiration, heart rate, electrocardiogram, and arterial blood pressure were continuously monitored and maintained under the physiological criteria. An additional cannula was inserted into the saphenous or epigastric arteries for close-arterial injection of drugs.

The left sciatic nerve was exposed and a mineral oil pool was made by the cut edge of skin. The sural nerve was detached from the tibial and peroneal nerves. Microfilament containing one or two unit activity was teased apart using sharpened forceps and cut centrally, and then placed on a single platinum recording electrode. A reference electrode was inserted in the surrounding tissues. The action potential was amplified with an AC-coupled amplifier, filtered, and input into an oscilloscope, then recorded and stored on computer. An A/D converter card (SMUP-PC, Shanghai Medical College, Fudan University, China) was used to digitize and store data.

The conduction velocity (CV) of each unit was determined by electrical stimulation (duration 0.1 ms for Aβ- and Aδ-fibers and 0.5 ms for C fibers, 10–100 V, 1 Hz) using two fine needle electrodes inserted into the skin just proximal to the receptive field (RF). Units with CV of less than 2 m/s were considered as C fibers.

Thermal, mechanical, and chemical stimulation. To measure the thermal threshold of the recorded units, a feedback-controlled lamp was placed on the surface of the skin, and the beam was focused on the receptive field. The radiant heat stimulation was allowed to rapidly rise to the set temperature less than 1 s. The minimal temperature required to activate the unit was considered as the thermal threshold.

The mechanical threshold was determined using calibrated von Frey filaments (Stoelting, IL, USA) ranging from 0.04 to 26 g. An ascending order of filaments was applied to the receptive field until an action potential could be consistently evoked by a given filament. This was considered as the mechanical threshold.

Close-arterial injection of drugs. All drugs were injected through close-arterial cannula in a volume of 0.1 ml, followed by washing with 0.2 ml saline. The stock solutions of [Sar^9, $Met(O_2)^{11}$]SP and WIN51,708 were diluted in saline for injection.

Data statistic. The data were presented as means ± SEM. In the behavioral studies, the repeated measure ANOVA followed by Tukey post hoc test was used to compare the different groups. The differences between before and after injection were determined with Student's paired t test. In the electrophysiological studies, Student's t test was used and chi-square test was used for incidence data. A value of $p < 0.05$ was considered as statistically significant.

Results

Behavioral test

Thermal hyperalgesia and edema induced by Sar-SP

The intraplantar injection of Sar-SP significantly decreased the paw withdrawal latency, which disappeared 1 h after injection (Fig. 1A). As shown in Fig. 1A, the effect of Sar-SP at 1 nmol was more potent than that at 0.2 nmol. Moreover, both of 0.2 and 1 nmol Sar-SP induced an obvious edema in the injected hindpaw,

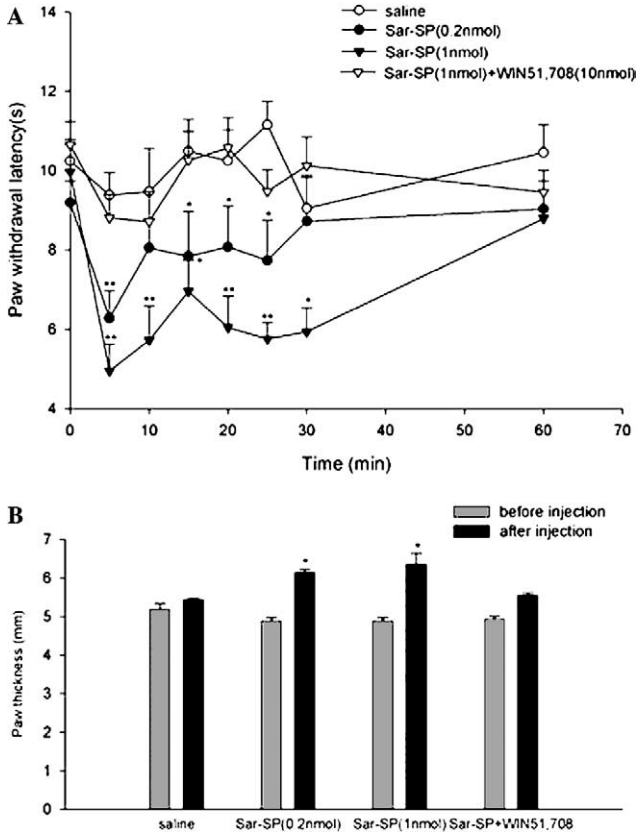

Fig. 1. Thermal hyperalgesia and edema induced by intraplantar injection of Sar-SP. Time course of the paw withdrawal latency to radiant heat stimuli in the hindpaw (A) and the hindpaw thickness (B) following administration of Sar-SP (0.2 and 1 nmol, 20 μl) or co-administration of Sar-SP(1nmol) and WIN51,708 (10 nmol). Data indicate means ± SEM from 7 to 8 rats. *$p < 0.05$, **<0.01 vs. saline.

expressing as an increase in the hindpaw thickness (Fig. 1B). When 10 nmol WIN51,708, a selective antagonist of NK-1 receptor, was co-injected with 1 nmol Sar-SP, the thermal hyperalgesia was completely blocked and the edema was significantly alleviated compared with the control rats (Figs. 1A and B).

The effect of chronic mast cell degranulation

Activation of peripheral NK-1 receptor may elicit mast cell degranulation, which releases several inflammatory mediators such as histamine and serotonin to generate or enhance neurogenic inflammation [10]. To determine the influence of mast cell degranulation on the Sar-SP-induced behavioral response, we tested those responses in the rats treated with chronic mast cell depletion by repeated treatment of compound 48/80. After repeated treatment with compound 48/80, mast cells were significantly depleted (not shown) and intraplantar injection of 1 nmol Sar-SP induced significant thermal hyperalgesia similar with those in the rats repeatedly treated with saline (Fig. 2A). However, the edema induced by 1 nmol Sar-SP largely lightened in the rats treated with compound48/80 (Fig. 2B).

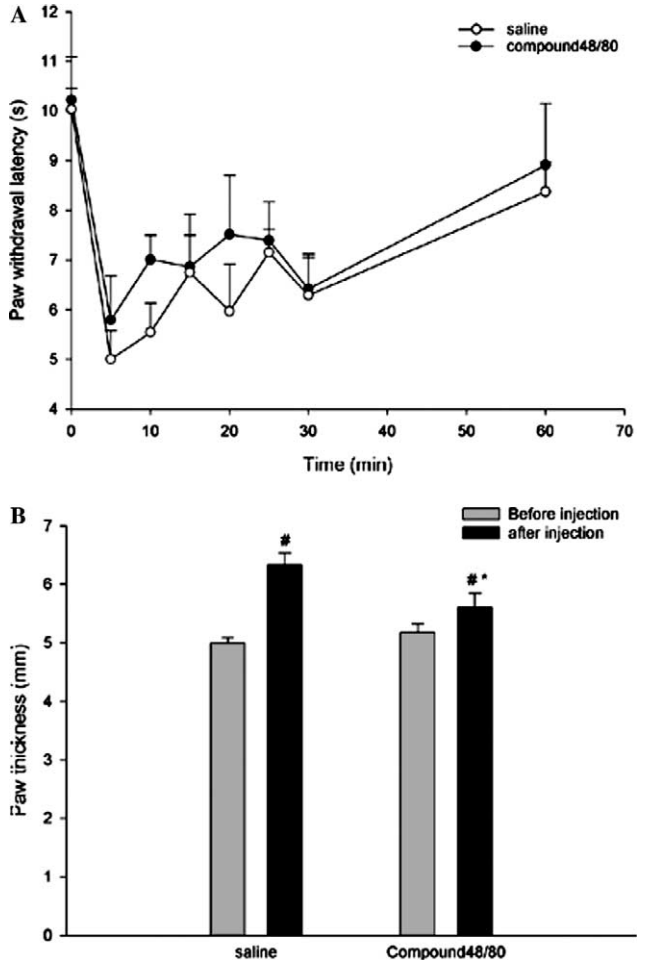

Fig. 2. In the mast cell depletion rats, the paw withdrawal latency and the hindpaw thickness showed in (A,B). Data indicate means ± SEM from 7 to 8 rats. $^{*}p < 0.05$ vs. saline; $^{\#}p < 0.05$ vs. before injection.

Single fiber recording in vivo

In 24 normal rats, a total of 53 mechanosensitive C afferents in the sural nerve were recorded. The mean conduction velocity was 0.87 ± 0.34 m/s and the von Frey threshold ranged from 0.04 to 15 g. Forty-eight of C-type fibers ($n = 53$) were sensitive to thermal stimulation and were thus classified as CMH-fiber, while the other were CM fibers not responding to thermal stimulation. All fibers had no spontaneous discharge during recording.

Excitatory effect of Sar-SP on C afferents

After close-arterial application of 1 nmol Sar-SP, 26 of 53 C-type fibers (49.06%) were excited with a significant increase in firing frequency (Fig. 3A). The latencies varied from 5.20 to 24.30 s (mean 12.43 ± 3.04 s). The temporal pattern of firings induced by Sar-SP was usually an un-regular pattern, with duration ranging from 1.32 to 5 min (mean 3.65 ± 1.45 min). The same volume of saline injection did not show any effect. In 8 CMH fibers, application of 10 nmol WIN51,708 at 2 min prior to the second Sar-SP injection significantly attenuated the second Sar-SP response compared with the first Sar-SP response

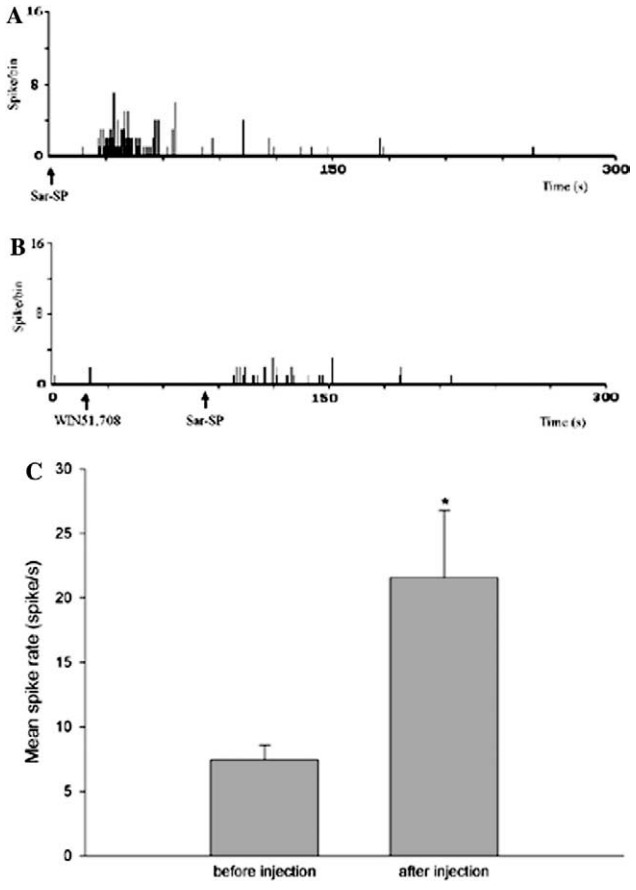

Fig. 3. WIN51,708 blocked Sar-SP- induced excitation of C afferent fibers. (A,B) The excitation of a CMH fiber (CV = 1.24 m/s) induced by 1 nmol Sar-SP before and after administration of WIN51,708. Bin = 0.6 s. (C) Mean spike rate of second Sar-SP with WIN51,708 significantly lower than the first Sar-SP injection. $^{*}p < 0.05$ vs. the first Sar-SP injection.

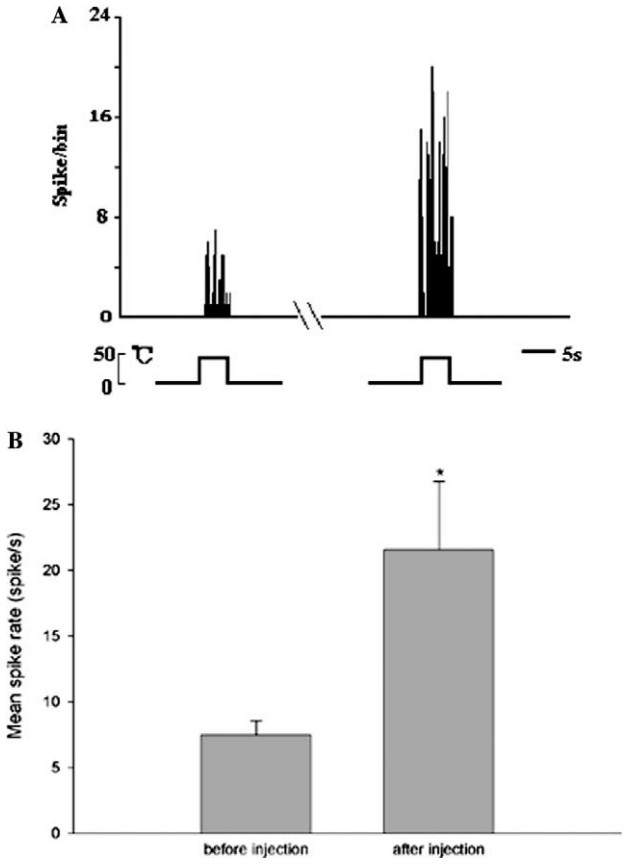

Fig. 4. Sar-SP promoted the response magnitude activated by 50 °C heat noxious stimulus. (A) The spike histograms of a CMH fiber (CV = 0.43 m/s) evoked by 5 s 50 °C heat stimulus before and after Sar-SP injection. Bin = 0.24 s. (B) Mean spike rate after Sar-SP injection evoked by 50 °C heat stimulus was significantly increased ($n = 6$, $^*p < 0.05$ vs. before injection).

(0.84 ± 0.27 vs. 2.11 ± 0.45 spike/s, Figs. 3B and C). In 5 rats treated with compound 48/80 to deplete mast cells, we recorded 18 CMH afferent fibers and Sar-SP still induced 44.44% CMH fibers excitatory ($p > 0.05$, χ^2 test).

Sar-SP enhance heat response of CMH afferents

In 16 CMH afferent fibers, thermal threshold was tested using radiant heat stimulus before and after Sar-SP application. Ten of CMH-fibers ($n = 16$) responded to 1nmol Sar-SP and their thermal thresholds were significantly decreased after injection (41.0 ± 0.6 vs. 37.2 ± 0.3 °C, $p < 0.001$). In 6 of these 10 CMH-fibers, the sensitivity to 50 °C 5 s heat stimulus also was measured before and after Sar-SP activation. After Sar-SP application, the mean spike rate was significantly increased compared with the first heat stimulus (21.55 ± 5.21 spike/s vs. 7.46 ± 1.11 spike/s, $p < 0.05$, Figs. 4A and B).

Discussion

Many studies have testified that NK-1 receptor is densely expressed in the superficial spinal dorsal horn and plays an important role in processing nociception in the spinal cord [11,12]. Also, pharmacological and morphological studies have showed that NK-1 receptors are distributed to the peripheral sensory neurons and their peripheral terminals [4,13]. Increasing evidence indicated that the extragenous administration of NK-1 receptor agonists in the peripheral tissue elicited significant pain-like behaviors responsive to mechanical stimuli in normal and pathological conditions [4,5,14,15]. Moreover, in NK-1 receptor knockout mice, behavioral responses to high intensity thermal, mechanical, and chemical stimuli are attenuated [14]. Collectively, the present finding that NK-1 antagonist WIN51,708 blocks Sar-SP-induced thermal hyperalgesia further supports the view that NK-1 receptors expressed on C-type fibers are implicated in processing of peripheral nociceptive information. Paralleling with the behavior results, in the electrophysiological test we also found that Sar-SP could enhance the thermal sensitivity of CMH fibers, suggesting that the activation of peripheral NK-1 receptor could contribute to the thermal hyperalgesia through regulating the thermal sensitivity of afferent C fibers. Consistent with these results, peripheral administration of SP produced increase in firing of testicular nociceptors of dogs and sensitized articular afferents of rats to noxious movements [6,16]. Perfusion of SP evoked inward current in small DRG neurons in vivo and in vitro [13,17].

Peripheral SP elicits mast cell degranulation releasing histamine and other inflammatory mediators to induce vasodilation and plasma extravasation, which result in tissue edema and neurogenic inflammation [8,10]. Therefore, the role of mast cells in Sar-SP-induced thermal hyperalgesia and edema was examined. The previous study has indicated that chronic administration of compound 48/80 could deplete mast cells and attenuates the thermal hyperalgesia after CFA treatment [9]. Our morphological observation showed that most of the mast cells exhibited deterioration following chronic treatment of compound 48/80 (not shown). However, in our experiment, the depletion of mast cells did not significantly influence the thermal hyperalgesia induced by Sar-SP but attenuated the degree of edema. In electrophysiological experiment, we also found that Sar-SP still excited 44.4% C sensory afferents in rats with compound 48/80 treatment. Taken together, these findings suggested that mast cells might be involved in Sar-SP-induced edema, but not in Sar-SP-induced thermal hyperalgesia and sensitization of sensory afferents. The mast cell degranulation path way seems to be not of importance in Sar-SP-induced nociception.

In summary, the present study demonstrated that peripheral application of NK-1 receptor agoinist Sar-SP produced significant thermal hyperalgesia and enhanced the thermal sensitivity of CMH fibers. Given that chronic depletion of mast cells failed to eliminate the thermal hyperalgesia and excited discharges in C-type fibers, it is strongly suggested that NK-1 receptors in C-type fibers preferentially mediate Sar-SP-induced thermal hyperalgesia.

Acknowledgments

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