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Short communication

Resiniferatoxin reversibly blocks adjuvant-induced thermal hyperalgesia in the rat

Yi-Hong Zhang[a,b], Yong Chen[b], Zhi-Qi Zhao[a,*]

[a] Institute of Neurobiology, Fudan University, 220 Handan Road, Shanghai 200433, China
[b] Shanghai Institute of Physiology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, China

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Abstract

The role of capsaicin-sensitive sensory afferents and mast cells in complete Freund's adjuvant (CFA)-induced thermal hyperalgesia and edema was investigated in rats. A single systemic injection of resiniferatoxin produced a reversible prevention of adjuvant-induced thermal hyperalgesia which lasted several days. In addition, resiniferatoxin markedly reduced the early edema. Chronic degranulation of mast cells with compound 48/80 also reduced the thermal hyperalgesia and edema, especially in the early phase of inflammation. Co-pretreatment with resiniferatoxin and compound 48/80 induced effects similar to those of resiniferatoxin alone. The data support the involvement of capsaicin-sensitive fibers in the adjuvant-induced inflammation.

Keywords: Inflammation; Thermal hyperalgesia; Paw edema; Resiniferatoxin; Mast cell

1. Introduction

Adjuvant-induced inflammation, which is well known to be a T cell dysimmunity-mediated disease, is a widely used experimental model. This inflammation has been suggested to be a non-neurogenic response, based on the observation that neonatal capsaicin treatment does not block the thermal hyperalgesia and edema induced by injection of complete Freund's adjuvant into the hind paw (Hylden et al., 1992). However, there was report that capsaicin reduced adjuvant-induced joint swelling and prevented spread of the arthritis (Donaldson et al., 1995). Mice lacking vanilloid receptor-1 (VR1) completely lost their ability to develop the thermal hyperalgesia by carrageenan, mustard oil or adjuvant (Caterina et al., 2000; Davis et al., 2000).

Mast cells, as immunocompetent cells, are located closely to peripheral nerve endings. Antidromic stimulation of peripheral nerves can elicit mast cell degranulation. Plasma extravasation and vasodilatation are generated when mast cells are activated. Mast cells, as well as released peptides from the periphery are all considered to be involved in neurogenic inflammation.

Using resiniferatoxin, an effective tool that selectively desensitizes capsaicin-sensitive afferents and the cationic secretagogue, compound 48/80, which chronically degranulates mast cells, the aim of the present study was to examine the relative roles of these two compounds in mediating the adjuvant-induced thermal hyperalgesia and edema, two robust signs of this autoimmune inflammation.

2. Materials and methods

2.1. Animal use and care

Experiments were performed on 32 adult male rats, weigh 200–300 g, and conducted in accordance with the guidelines of the International Association for the Study of Pain (IASP) and the National regulations, concerning the ethical use of laboratory animals.

2.2. Induction of inflammation

CFA (150 μl, Sigma, St. Louis, MO, USA), suspended in an oil/saline (1:1) emulsion was injected at a concentration

* Corresponding author. Institute of Neurobiology, Fudan University, 220 Handan Road, Shanghai 200433, China. Tel.: +86-21-5552-2877; fax: +86-21-5552-2876.
E-mail address: zqzhao@fudan.edu.cn (Z.-Q. Zhao).

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of 0.5 mg/ml into the plantar surface of the left hindpaw under ether anesthesia.

2.3. Behavioral measurements

Radiant heat, provided by a 50-W projector lamp, was focused on the plantar surface of the left hindpaw. Paw withdrawal latency was measured from the start of heating to the time of withdrawal and was adjusted to around 10 s for the normal rats. A cutoff time of 25 s was applied to avoid tissue damage. Four trials, at least 5 min apart, were conducted with each hindpaw, the last three trials were averaged to give a mean latency.

2.4. Paw size measurements

The rats were fixed within a special tube-shaped container, leaving the hindpaw outside. A calibrated micrometer was used to measure the maximal dorso-ventral thickness of the left hindpaw.

2.5. Reduction of cell function

2.5.1. Primary capsaicin-sensitive afferents

A single s.c. injection of 0.3 mg/kg resiniferatoxin in a volume of 0.3 ml (Sigma) dissolved in 20% ethanol, 80% physiological saline solution was used. Control animals were treated with an equal volume of vehicle; 24 h later, CFA was injected to induce inflammation.

2.5.2. Mast cells

The rats received an ascending series of doses of compound 48/80 over a 4-day period (Woolf et al., 1996), starting with 25 μg (i.p) 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. After 24 h, inflammation was induced by CFA injection.

2.6. Toluidine blue staining

Mast cell degranulation was evaluated after toluidine blue staining of a stretch preparation of loose connective tissue from the hindpaw hypodermis. The tissue from one pretreated rat (protocols described above) and one control rat was removed under ether anesthesia, stretched carefully, and transferred to fixative containing 90% ethyl alcohol and 10% formalin for 10 min at room temperature, then was stained with 0.5% toluidine blue. The stained tissue was examined under a light microscope.

2.7. Statistical analyses

Data are presented as means ± S.E.M and analyzed using a two-way analysis of variance (ANOVA) with repeated measures, paired or unpaired *t*-test where appropriate. In all cases, $P < 0.05$ was considered significant.

3. Results

3.1. Adjuvant-induced inflammation

Thermal hyperalgesia appeared as early as 1 h after CFA injection and reached its peak at 10 h from the baseline paw withdrawl latency 10.94 ± 0.26 to 4.21 ± 0.34 s ($P < 0.0005$) persisting for several days then slowly returning to baseline by day 11 ($P > 0.05$). Simultaneously, edema became apparent at 2 h, reached its peak at 12 h after CFA injection from the baseline, 4.54 ± 0.04 mm, to 7.84 ± 0.16 mm ($P < 0.001$), then gradually diminished but was still pronounced at 5 days post injection and remained at a relatively high level for several weeks. Local redness lasted throughout the entire observation periods. Elevation of the inflamed hindlimb was observed in one rat, and disappeared at 3 days post-injection.

3.2. Resiniferatoxin blocked the thermal hyperalgesia and edema induced by CFA

Respiratory depression was observed immediately after injection of resiniferatoxin but ceased after about 30 min. Vehicle injection showed no significant effects. In contrast, resiniferatoxin pretreatment produced a complete blockade of the thermal hyperalgesia induced by CFA. During the first 10 h after CFA injection, none of the animals in the resiniferatoxin-pretreated group responded to noxious heat in the inflamed paw. The blockade persisted at a high level for 50 h and began to decrease slowly 3 days after CFA injection. A significant overall difference between the resiniferatoxin and vehicle-treated inflamed rats was observed at most of the time points tested. Paw withdrawl latency was restored to baseline about 11 days after CFA injection in resiniferatoxin-pretreated rats (Fig. 1A). Resiniferatoxin pretreatment also reduced the adjuvant-induced edema. Though its effect on edema was far less than that on thermal hyperalgesia, resiniferatoxin still markedly attenuated the paw edema at the early development periods, with statistical significance during 1, 3 and 6 h after injection of CFA (Fig. 1B). There was no significant change in paw withdrawl latency and paw thickness in the saline-treated rats during the entire observation period.

3.3. Mast cell degranulation and inflammation

Compound 48/80 did not alter the baseline paw withdrawl latency. However, thermal hyperalgesia was attenuated to some extent by compound 48/80 during the early development period as well as at later times (significantly at 6 h, 20 h, 30 h, 9 days and 10 days, $P < 0.05$). Compared to that of the control group, the peak time point of thermal hyperalgesia in compound 48/80-treated rats was delayed until 3 days post-injection of CFA. Compound 48/80 also reduced the early edema to some extent, with statistic significance at 2 and 4 h. None of the rats showed elevation

of the inflamed hindlimb in the compound 48/80-treated group.

Co-pretreatment with resiniferatoxin and compound 48/80 induced effects on thermal hyperalgesia and edema similar to that of resiniferatoxin alone as to both extent and timing.

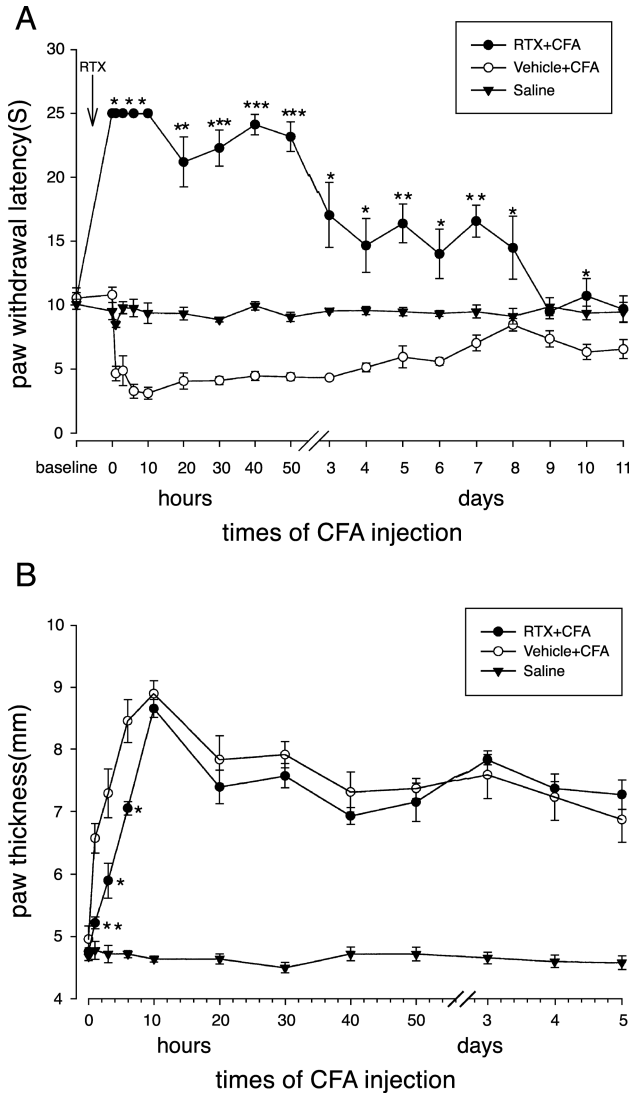

Fig. 1. The time course of effects of resiniferatoxin (0.3 mg/kg, s.c.) or vehicle on paw withdrawal latencies in response to noxious radiant heat on the plantar surface of the hindpaw (A) and paw thickness (B) in rats with adjuvant-induced inflammation. In A, ANOVA with repeated measures reveals a significant effect of different treatment [$F(2,12)=214.18$, $P<0.001$] and time [$F(18,216)=12.82$, $P<0.001$] as well as a significant drug treatment × time interaction [$F(36,216)=23.47$, $P<0.001$]. In B, ANOVA with repeated measures also reveals significant effects of different treatments [$F(2,12)=96.16$, $P<0.001$] and time [$F(11,132)=47.37$, $P<0.001$] as well as a significant drug treatment × time interaction [$F(22,132)=16.57$, $P<0.001$]. In A and B, individual comparisons between resiniferatoxin and vehicle-treated rats with inflammation at each time point are shown (Student's t-test). ***, **, *, representing $P<0.0005$, $P<0.005$, $P<0.05$, respectively. Resiniferatoxin + CFA group (filled circles) vs. vehicle + CFA group (open circles). Saline group served as a control (triangle). $n=5$ for each group.

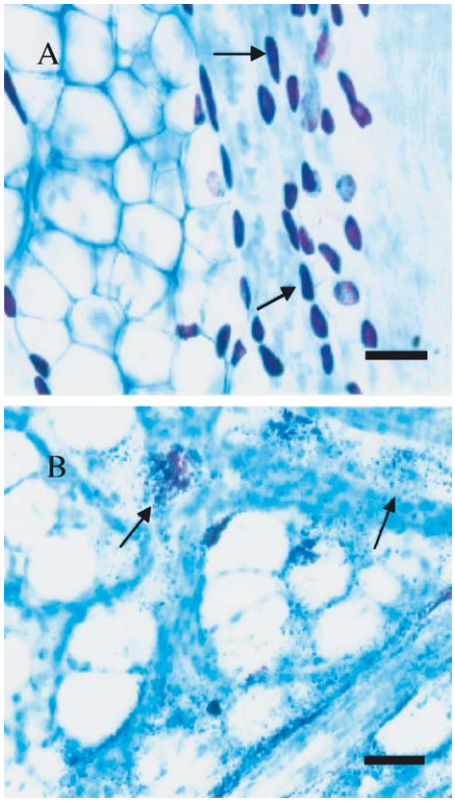

Fig. 2. Light microscopic confirmation of mast cell degranulation. (A) In control rats, mast cells were characterized by many purple metachromatic granules (arrow) following toluidine blue staining. (B) After treatment with compound 48/80, many dispersed amorphous granules were seen outside the cells (arrow).Scale bar, 50 μm.

Fig. 2 shows the effectiveness of degranulation of mast cells by compound 48/80.

4. Discussion

4.1. Capsaicin-sensitive fibers are required for the development of adjuvant-induced thermal heperalgesia

Resiniferatoxin, as an ultrapotent analog of capsaicin, has been demonstrated to desensitize the response of C-fibers to noxious thermal stimuli in normal adult rats, in rats with nerve-injury pain and with spinal cord ischemia (Ossipov et al., 1999; Xu et al., 1997). We now showed that resiniferatoxin also produced a long-lasting, reversible blockade of adjuvant-induced thermal hyperalgesia in the adult rat. The results were consistent with the findings in mice lacking VR1 in which little thermal hypersensitivity was detected for adjuvant-induced inflammation (Caterina et al., 2000). It was also consistent that local application of capsaicin into the draining lymph nodes attenuated the expression of adjuvant-induced arthritis (Lorton et al., 2000). However, the neurotoxic effect of neonatal capsaicin treatment had only a minimal effect on adjuvant-induced thermal hyperalgesia (Hylden et al., 1992). The discrepancy may be due to

differences in treatments. Injection of a large dose of resiniferatoxin produced an initial depolarization of primary sensory neurons followed by a long-lasting desensitization. In our studies, the desensitization was reversible, indicating that the function of C-fibers could recover from the desensitization after several days. The neonatal capsaicin treatment, however, destroyed a large subpopulation of unmyelinated C-afferents. The compensatory changes in dorsal root ganglion neurons and spinal neurons may account for thermal hyperalgesia induced by CFA in neonatal capsaicin treated-rats. There is a recent report that the vanilloid receptor VR1 antagonist, capsazepine, produced significant reversal of carageenan-induced thermal hyperalgesia in the guinea pig, but was ineffective in the rat (Walker et al., 2003). In contrast, our results mentioned above indicated that the capcaicin-sensitive fibers are required for the development of adjuvant-induced thermal hyperalgesia in the rat.

Compared to resiniferatoxin, compound 48/80 showed only limited effects on adjuvant-induced thermal hyperalgesia, and may act by reducing amines and other inflammatory mediators or cytokines in mast cells.

4.2. A neurogenic mechanism plays some role in the early phase of adjuvant-induced inflammation

It has been suggested that the adjuvant-induced inflammatory response does not appear to require capsaicin-sensitive neurogenic mechanisms(Hylden et al., 1992). In our present studies, however, pretreatment with resiniferatoxin or compound 48/80 alone or both significantly reduced the early edema to a similar extent, which indicated that both may act through the same pathway. Activity in unmyelinated afferents as part of a local axon reflex results in peripheral release of substance P and calcitonin gene-related peptide (CGRP) from the nerve terminals. Besides their direct action on the blood vessels, substance P and CGRP induced activation and degranulation of mast cells, which result in rapid and marked histamine release, then induce vasodilatation, increased vascular permeability and contraction of smooth muscles. The whole process is a so-called neurogenic mechanism which may have played a part role in the early phase of adjuvant-induced inflammation in the present study.

In conclusion, we have now presented behavioral evidence that capsaicin-sensitive afferents are necessary for adjuvant-induced thermal hyperalgesia, and neurogenic mechanisms may be partly involved in the early phase of this inflammation.

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