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European Journal of Pharmacology 552 (2006) 67–77
www.elsevier.com/locate/ejphar

Rat pain-related responses induced by experimental scorpion BmK sting

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Received 22 May 2006; received in revised form 7 September 2006; accepted 8 September 2006
Available online 23 September 2006

Abstract

The developmental and pharmacological characteristics of pain responses induced by the experimental scorpion BmK (*Buthus martensi* Karsch) sting were detailed in this study. Following the unilateral intraplantar injection of BmK venom into rat hind paw, it was found: 1) BmK venom induced an edematogenic response, spontaneous pain and pain hypersensitivity in a dose-dependent manner; 2) the paw edema and flare were induced rapidly and restricted at the injected paw for about 24–48 h; 3) the monophasic tonic spontaneous pain manifested as continuous paw flinching and lifting/licking of the injected paw and lasted for more than 2 h; 4) the detectable thermal hypersensitivity to radiant heat stimuli was just at the injected side for about 72–96 h; 5) the mechanical hypersensitivity to von Frey filaments was evoked surprisingly to be the bilateral and mirror-like for about 2–3 weeks; 6) morphine, indomethacin and bupivacaine could suppress BmK venom-induced pain responses with different intensity and time courses. The results indicated that the experimental BmK sting could evoke the prolonged paw inflammation, tonic spontaneous behaviors, unilateral thermal and bilateral mechanical hypersensitivity. The distinct time development of pain responses induced by experimental BmK sting might be involved in different nervous and/or tissue mechanisms. The experimental BmK sting test thus may be an available tissue injury-induced tonic inflammatory pain model for understanding the mechanisms underlying clinical spontaneous pain, thermal and mirror-imaged bilateral mechanical pain hypersensitivity.

Keywords: Scorpion BmK venom; Paw inflammation; Spontaneous pain; Thermal hypersensitivity; Mechanical hypersensitivity

1. Introduction

Tissue injury often results in chronic inflammation accompanying with ongoing spontaneous pain and hypersensitivity (e.g. hyperalgesia and allodynia). Among the existed inflammatory pain tests, the majority of them such as including carrageenan, yeast (zymason), capsaicin, mustard oil and complete Freund's adjuvant models were employed preferentially to elucidate stimulus-evoked pain hypersensitivity (Gilchrist et al., 1996; Hargreaves et al., 1988; Iadarola et al., 1988; Ma and Woolf, 1996; Neumann et al., 1996). By contrast, the formalin test predominantly exhibited the biphasic tonic spontaneous responses with no significant stimulus-evoked pain (Abbott et al., 1995; Chen et al., 1999; Dubuisson and Dennis, 1977; TjoÈlsen et al., 1992). Thus, animal models combining with the prolonged spontaneous pain and stimulus-evoked pain hypersensitivity may be helpful to understand the dissociation and/or correlation of the pathophysiological mediation and processing for clinical spontaneous pain, thermal and mechanical hypersensitivity (Chen et al., 1999; Lariviere et al., 2002; Meller, 1994).

Scorpion *Buthus martensi* Karsch (BmK), is widely distributed in northwestern China to Mongolia and Korea. Although it is not so dangerously venomous for mammal, the sting by this species could cause severe ongoing pain, inflammation and burning sensation for a long duration (Balozet, 1971). The mechanism underlying this natural pain is far from clear. Our previous study demonstrated that intraplantar (i.pl.) injection of BmK venom evoked the local inflammation concomitant with spontaneous pain and thermal hypersensitivity (Chen et al., 2001, 2002). However, the other kinds of pain responses such as the mechanical hypersensitivity and the time developmental information of these pain responses induced by experimental BmK sting are still unknown.

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doi:10.1016/j.ejphar.2006.09.018

For the aim of evaluating rat pain-related responses induced by experimental BmK sting, in the present study, the kinds of pain responses evoked by i.pl. injection of BmK venom were examined systematically. Then, the whole time windows for the induction, development, maintenance and termination of the inflammation, spontaneous pain and the stimulus-evoked pain hypersensitivity were investigated in detail. Furthermore, the suppression of morphine, indomethacin and bupivacaine on BmK sting-induced pain responses was detected. Finally, the possible contributors and underlying mechanism for BmK sting-induced pain responses were discussed.

2. Materials and methods

2.1. Animals and administration of drugs

The experiments were performed on Sprague–Dawley albino rats weighing from 220 to 250 g. The animals were provided by the Shanghai Experimental Animal Center, Chinese Academy of Sciences. European Community guidelines for the use of experimental animals and the IASP's guidelines for pain research in conscious animals were followed (Zimmermann, 1983). Five rats were kept in individual cages with water and food available *ad libitum*. The animal room was maintained at 21–23 °C with a 12:12 h light:dark cycle. The test was done between 09:00 and 19:00. The rats were acclimatized to the laboratory and habituated to the test boxes for 30 min at least each day during 5 days before testing. The crude BmK venom was purchased from an individual scorpion culture farm in Henan Province, China. The venom was filtered with a Sephadex G-50 column according to the method described by Liu et al. (1996).

In the first series of experiments, the dose- and time-dependent effects of BmK venom were investigated. The rats were randomly divided into two groups: (1) test group: rats with i.pl. injection of BmK venom at doses of 5, 10, 20 and 50 μg in 50 μl sterile saline ($n=8$, for each dose), respectively; (2) control group: rats with i.pl. injection of the sterile saline (50 μl for each animal, $n=8$). After injection, the inflammation, spontaneous pain and hypersensitivity induced by BmK venom were monitored.

In the second series of experiments, the effects of distinct pharmacological treatments on BmK venom-induced inflammatory and pain responses were assayed. Three experimental groups were designed: (1) morphine (Morphine hydrochloride, Shenyang First Pharmaceutical Factory, China) at 6 mg/kg body weight was injected subcutaneously (s.c.)10 min before i.pl. administration of 50 μg of BmK venom ($n=6$). Rats treated with sterile saline were used as control ($n=6$); (2) indomethacin (Sigma, USA) at 2.5 or 25 mg/kg body weight was intraperitoneally (i.p.) administered 10 min before i.pl. injection of 50 μg BmK venom ($n=6$, for each dose). Animals treated with 60% dimethyl sulfoxide (DMSO, v/v in saline) or saline were used as control; (3) 0.7% bupivacaine (Bupivacaine hydrochloride, Shanghai Harvest Pharmaceutical Co. Ltd., China) was co-administered (i.pl.) with 50 μg BmK venom ($n=6$). Control animals were treated with same volume of saline ($n=6$). Following the injection of BmK venom, the suppression of the above drugs on BmK venom-induced pain responses was evaluated.

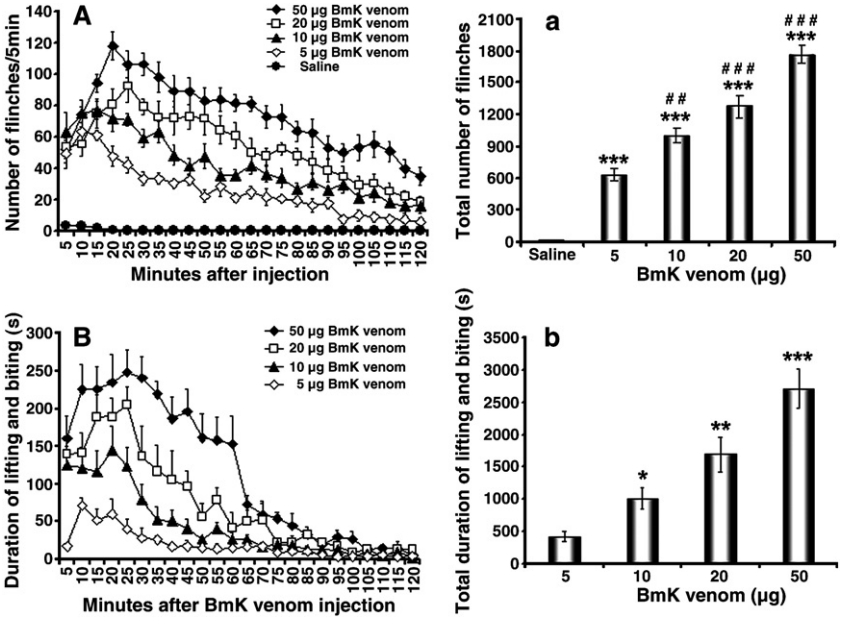

Fig. 1. Spontaneous behaviors induced by the intraplantar injection of BmK venom. (A), time course for the number of paw flinches per 5 min induced by BmK venom (5, 10, 20 and 50 μg in 50 μl saline, i.pl.) or saline (50 μl, i.pl.). There is a significant overall effect of the doses of BmK venom ($P<0.001$, $n=8$). (a), dose-dependent effects of BmK venom on the total number of paw flinches during 2 h observation. ***$P<0.001$, compared with group treated with saline; $^{\#\#}P<0.01$ and $^{\#\#\#}P<0.001$ compared with group treated with 5 μg BmK venom ($n=8$). (B), time courses for the duration of paw lifting/biting per 5 min induced by BmK venom (5, 10, 20 and 50 μg in 50 μl saline, i.pl.). There was a significant overall dose effect of BmK venom ($P<0.001$, $n=8$ for each dose). (b), dose-dependent effects of BmK venom on the total duration of the paw lifting/biting during the 2 h observation after the administration of the venom. *$P<0.05$, **$P<0.01$ and ***$P<0.001$ compared with group treated with 5 μg BmK venom ($n=8$, for each dose). All the data were presented as mean±S.E.M.