B1-kinin receptors modulate Mesobuthus tamulus venom-induced vasosensory reflex responses in anesthetized rats

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Abstract:
Objective: Intra-arterial injection of Mesobuthus tamulus (BT) venom produces reflex vasosensory responses modulating cardiorespiratory parameters in albino rats. The present study was conducted to understand the role of kinin receptors in modulating vasosensory reflexes evoked by BT venom.

Materials and Methods: In urethane-anesthetized rats, tracheostomy was performed to keep the airway patent. The femoral artery was cannulated proximally, as well as distally, to record the blood pressure (BP) and to inject the chemicals, respectively. Electrocardiographic and respiratory excursions were recorded to compute the heart rate (HR) and respiratory rate (RR). A group of animals was pretreated with saline/kinin receptor antagonists intra-arterially (B1/B2 receptor antagonists) before the injection of venom.

Results: After intra-arterial injection of BT venom (1 mg/kg), there was an immediate increase in RR, which reached to 40% within 30 s, followed by a decrease of 40%. Further, there was sustained increase in RR (50%) up to 60 min. The BP started to increase at 40 s, peaking at 5 min (50%), and remained above the initial level up to 60 min. The bradycardiac response started after 5 min which peaked (50% of initial) at 25 min and remained at that level up to 60 min. In B1 receptor antagonist (des-Arg) pretreated animals, venom-induced cardiovascular responses were attenuated (by 20–25% in mean arterial pressure and HR) significantly but not in B2 receptor antagonist (Hoe-140) pretreated animals. Either of the antagonists failed to alter the RR responses.

Conclusions: BT venom-induced vasosensory reflex responses modulating cardiovascular parameters are mediated via B1-kinin receptors in anesthetized rats.

Key words: B1-Kinin receptor, des-Arg, Hoe-140, scorpion venom, vasosensory reflexes

Indian red scorpion (Mesobuthus tamulus [BT]) envenomation causes systemic toxicity leading to cardiorespiratory changes causing death in severe situation. Several mechanisms of action of BT venom have been proposed for the toxicity of venom, vasosensory reflex mediated response is one of them. In our previous study, BT venom-induced vasosensory reflex responses altering the cardiorespiratory parameters have been demonstrated. It has also been shown that the venom-induced cardiorespiratory changes were prolonged and occurred immediately, intermittently, and lately. Further, it is shown that most of the afferents were running through the ipsilateral somatic nerves and the efferents are located in the sympathetic and vagal parasympathetics. It was further shown that nociceptive vascular reflexes evoked by scorpion venom modulate cardiorespiratory parameters involving transient receptor potential vanilloid 1 (TRPV1).

While comparing the venom-induced vasosensory reflex response pattern with that of other nociceptive agonists (capsaicin/anandamide/αβMe-adenosine 5′-triphosphate)-induced responses, the responses evoked by venom are prolonged. In a study elsewhere, capsaicin or its agonists produced instantaneous hypotensive and hyperventilatory responses which were blocked after ipsilateral somatic denervation, as well as by TRPV1 antagonist pretreatment. It is known that the TRPV1 receptors form one of the mechanisms that can excite the nociceptor terminals. These receptors can also be excited by bradykinin (BK), H1, 5-hydroxytryptamine (5-HT), prostaglandins (PGs), etc. Since BT venom mediates its actions via kinin-dependent...
mechanisms, it becomes necessary to identify the particular type of kinin receptor involved in producing the nociceptive response. Therefore, it was planned to investigate the role of kinin receptor antagonists (B1 receptor antagonist-des-Arg and B2 receptor antagonist-Hoe 140) in mediating the vasosensory reflexes induced by venom.

Materials and Methods

Animals and Anesthesia
All the experiments were performed after getting the approval from Ethical Committee of Institute (Dean/13-14/CAEC/189 dated 09-05-2013). Experiments were performed on healthy male albino rats (Charles-Foster strain), weighing between 200 and 300 g. The animals were kept in the 12:12 h light/dark cycle and were provided with ad libitum animal food (Hindustan Lever Ltd.) and water. Animals were anesthetized with urethane (Merck, Germany), with an initial dose of 1.5 g/kg body weight intraperitoneally and was maintained by injecting the urethane as required.

Dissection and Recordings
Tracheal cannulation was performed to keep the respiratory way patent. The trachea was dissected and exposed after a mid-line incision over the neck. A transverse cut was made between the tracheal rings, and a polyethylene tube of appropriate diameter was inserted and secured firmly. Tracheal secretions were aspirated from time to time by gentle suction through a fine polyethylene tube.

An incision was made on the femoral triangle along the course of the femoral artery. The femoral artery was dissected and skeletonized by clearing the tissues and fascial attachments by blunt dissection. The femoral vein and nerve were separated out from the artery. The cannula was attached with a syringe, containing freshly prepared heparinized saline (20 IU/ml). A small nick was made in the artery near the proximal end then the cannula was inserted and secured firmly with thread. The cannula was in turn connected through a three-way stopcock to a statham strain gauge pressure transducer (Biodevices, Ambala). After the cannulation of the proximal part of the femoral artery, the cannulation was done in the distal part of the same artery to inject the venom/drugs/saline in a local segment of the vessel. The placement of cannulation was ascertained by injecting 0.10 ml saline.

The pressure transducer was filled with the heparinized saline and the cannula was connected to it. The thread clamp was released and free pulsatile flow of blood was ascertained. The pressure was recorded by connecting the transducer to a bridge amplifier via galvanometer. The galvanometer deflections were recorded on a chart paper with the help of a pen. The mean arterial pressure (MAP) was taken as a parameter throughout the study. The instrument was calibrated from time to time during the experiment.

The skin over the xiphisternum was secured with the thread and was attached to a force-displacement transducer. The respiratory movements were recorded on a chart recorder via a bridge amplifier. Respiratory rate (RR) was calculated from these recordings.

The needle electrodes were connected according to the standard limb II configuration. The electrocardiographic (ECG) potentials were recorded on a chart recorder. Heart rate (HR) was calculated manually from R-R intervals of the ECG.

After the surgical procedures, animals were allowed to stabilize for 30 min followed by the initial recording of blood pressure (BP), ECG, and RR. A volume of 0.10 ml of normal saline was injected in the peripheral end of the femoral artery and the cardiorespiratory parameters were recorded at the interval of 5 min up to 15 min as initial recording. This was followed by the injection of venom (1.0 mg/kg) in the peripheral end of the same femoral artery, and the cardiorespiratory parameters were recorded at the interval of 5 min up to 60 min. This response was considered as “venom only” response.

Drugs and Solutions
Crude BT venom was obtained from Haffkine Institute, Mumbai, India. Stock solution (2 mg/ml) of BT venom was prepared in distilled water and refrigerated. BT venom (1 mg/kg) was used to stimulate the perivascular nociceptors as it produces optimal responses. Heparin was obtained from Biological Evans Ltd., Hyderabad, India. des-Arg (B1 receptor antagonist) and Hoe-140 (B2 receptor antagonist) were obtained from Sigma Chemical company, St. Louis, Mo, USA.

Experimental Protocol
In this study, 18 animals were used in three different groups (n = 6). All the chemicals/venom/saline was injected in the peripheral end of the femoral artery. Venom-induced responses were considered as the “venom only” response. In the subsequent two groups, venom was injected after pretreatment with des-Arg (B1 receptor antagonist) and Hoe-140 (B2 receptor antagonist). These antagonists were injected 45 min before the treatment of agonist. BP, ECG, and RR were recorded at the interval of 5 min up to 60 min. The volume of all the injectables was kept at 0.10 ml and the room temperature was maintained at 25°C ± 0.5°C throughout the experiment.

In the second group of experiments, i.a. injection of des-Arg (10 ng/kg) was given, and the cardiorespiratory parameters were recorded at the interval of 5 min up to 15 min. Further, the venom was injected in the same peripheral end of the femoral artery, and the cardiorespiratory parameters were recorded at the interval of 5 min up to 60 min.

In the third group of experiments, the animals were pretreated with Hoe-140 (10 ng/kg) which was followed by injection of venom and the cardiorespiratory changes were recorded as above.

Analysis of Data and Statistics
The values at a given time were pooled and were presented as a mean ± standard error of mean (SEM); the MAP, HR, and RR responses before venom were taken as initial responses. The comparisons of various groups were made by using the two-way analysis of variance (ANOVA) followed by Student’s t-test wherever required. A P < 0.05 was considered significant.
Results

In our earlier reports, we have shown that 1 mg/kg of venom produced optimal responses on cardiorespiratory parameters. Therefore, this concentration was chosen as a tool for the elicitation of vasosensory reflex responses in this study.

Individual and mean ± SEM values of RR, MAP, and HR are given after the injection of venom (1 mg/kg) only and after the injection of venom in des-Arg and Hoe-140 pretreated groups [Figures 1 and 2] and the original tracings are also shown in the figures.

Effect of des-Arg on Venom-induced Cardiorespiratory Changes

An increase in the rate and depth of respiration was seen immediately after the administration of venom in venom only group (RR from 74 ± 5.1 to 104 ± 10.8/min), and it was about 40% of the initial. RR was then decreased below the initial level within 5 min (45 ± 10.9/min). Subsequently, a gradual sustained increase in RR was noticed up to 60 min [Figure 1].

BT venom-induced respiratory changes in des-Arg pretreated animals were similar to the venom only responses during the entire period of observation [Figure 1].

Administration of venom in venom only group produced an immediate fall in MAP (from 90 ± 4.9 to 87 ± 6.7 mm Hg) followed by a rise and reached the peak (131 ± 6.8 mm Hg) within 5 min. Subsequently, it decreased but still remained above the initial level [Figure 1]. In des-Arg pretreated animals, the immediate depressor response was accentuated as compared to venom only group (from 80 ± 12 to 68.6 ± 10 mm Hg), but the pressor response was attenuated markedly up to 60 min [Figure 1]. The responses were significantly different from the corresponding values in venom only group (P < 0.05, two-way ANOVA).

Figure 1: Des-Arg (10 ng/kg) pretreatment attenuated cardiovascular changes (mean arterial pressure and heart rate) induced by intra-arterial injection of venom. Original tracings of respiration (Resp), ECG and blood pressure are shown in the top left panel. The dotted line at “0” indicates the time of injection of venom (1 mg/kg). The horizontal line indicates 5 s for Resp and ECG, 50 s for blood pressure. The mean ± standard error of mean values from six experiments are shown in line graphs. Arrows indicate the point of administration of venom. An asterisk (*) indicates P < 0.05 (two-way analysis of variance) as compared to “venom only” group from des-Arg pretreated group (des-Arg + venom)
Singh and Deshpande: B1 receptors modulate vasosensory reflexes

There was no change in HR immediately after the administration of venom in venom only group. Within 5 min, HR began to decrease and the decrease was maximal at 30 min (151 ± 18.9 beats/min), which remained nearly same up to 60 min [Figure 1]. In des-Arg pretreated animals, the venom-induced decrease in HR began earlier and reached maximal level at 10 min (from 320 ± 4 to 175 ± 32.6 beats/min). Subsequently, increase in HR was observed but was much lower than the initial. The responses were significantly different from the venom only group [Figure 1; P < 0.05, two-way ANOVA].

**Effect of Hoe-140 on Venom-induced Cardiorespiratory Changes**

In Hoe-140 pretreated group, the venom-induced changes in RR were not statistically different from the venom only group but showed an increasing trend in the rate of respiration in late phase after 30 min [Figure 2]. The immediate depressor response was accentuated as compared to venom only group (from 83 ± 10 to 65 ± 13 mm Hg) and the pressor response was similar to venom only group up to 30 min. However, after 30 min, the pressure response was lower than the venom only group response [Figure 2]. The responses were significantly different from the corresponding values in venom only group from 30 to 60 min (P < 0.05, two-way ANOVA). In Hoe-140 pretreated animals, the decrease in HR began immediately after venom and the decrease was about 55% at 5 min (from 302 ± 31 to 135 ± 24.5 beats/min), which remained at the same level up to 60 min [Figure 2]. The responses up to 15 min were significantly different from the venom only group (P < 0.05, Student’s t-test for unpaired observations).

**Discussion**

In our previous studies, venom-induced vasosensory reflexes altering the cardiorespiratory parameters were demonstrated. The afferents were found to be present in the ipsilateral
somatic nerves and in nerve plexuses around the blood vessels.[10] The involvement of TRPV1 and serotonin receptors (5HT3) in mediating the vasosensory reflex responses is also shown earlier.[17] Further, it has been shown that inflammatory mediators are involved in nociceptive processing at the perivascular nerve endings as the cardiorespiratory parameters of the reflex responses are blocked by the PG synthesis inhibitor.[14] These mediators in turn modulate the TRPV1 and serotonin receptors (5-HT3) to bring about the actions.[13,14]

BT venom contains serotonin, histamine, BK potentiating factor, peptide toxins, etc., and these chemicals are potential nociceptive agents.[12,13,14] Intra-arterial injection of venom thus stimulates vast population of nociceptors. Furthermore, venom also activates the kinin and PG synthesis endogenously.[2,17,18] Involvement of kinin and PG to noxious stimulation has been shown.[19] BK is an inflammatory mediator and produces the actions via BK-1 (B1) and BK-2 (B2) receptors.[13] The B1 receptors are normally dormant and are expressed after exposure to toxic chemicals/tissue injury products such as cytokines, substance P, neuropeptides, capsaicin, or after repeated exposure to BK.[13] The B1 receptors are involved in inflammatory hyperalgesia.[20] The B2 receptors, on the other hand, are involved in the production of inflammatory edema. Local application of exogenous kinins produces pain by stimulating B2 receptors in human skin[21] or in the rat tissues.[22] However, our results suggest that B2 receptors mediate late phase of pressor responses and immediate phase of HR responses produced by venom. The B2 receptors mediate their actions via inflammatory edema which activate the vasosensory afferents. The edema sets in after a time period thus the delayed phase of the pressor responses may be mediated via this mechanism. Our results also reveal that B2 receptors prevent a sudden decrease in HR produced by venom.

The evidence suggests that B1 receptors control PG production locally[23] and PGs are known hyperalgesic substances. The analgesic profile of B1 receptor antagonist resembles to that of nonsteroidal anti-inflammatory drugs,[24] which are agents known to inhibit the fatty acid, cyclooxygenase necessary for PGs synthesis. Thus, a chain of mediators (cytokines, kinins, and eicosanoids) may be involved in these reactions. The B1 receptors which are located on nonneural cells (fibroblast, mast cells, endothelial cells, etc.) produce PGs as a secondary mediator and in turn sensitize the nerve endings in the periphery. This has been demonstrated to sensitize vagal C-fibers.[14]

The B1 receptor antagonist pretreatment attenuated the venom-induced BP and HR responses [Figure 1]. These observations are consistent for the B1 receptor involvement in the nociceptive reflexes. Thus, B1 receptors mediated actions may be via PGs as discussed earlier. Our observation elsewhere with indomethacin is consistent for the attenuation by B1 receptor antagonist.

On the respiratory responses, there was no statistical difference between B1/B2 receptors antagonist-treated groups as compared to venom only groups. However, the B2 receptor antagonist increased the RR in the late phase. This may be due to the fact that respiration is influenced by BP changes. Consistence with this, the BP was decreasing in the late phase of response. Thus, the difference in the respiratory responses in B1 and B2 receptor antagonist groups may be due to baroreflex driven increase in RR. Further, it is known that the B1 receptors are expressed after latency as mentioned for the nociceptive actions[20] and respiratory responses occurred earlier than their expressions.

Conclusions

BT venom-induced vasosensory reflexes modulating cardiovascular parameters were mediated mainly via B1-kinin receptors and partially via B2-kinin receptors in anesthetized rats. The former involve hyperalgesic responses and the later involve inflammatory edema-dependent mechanisms.

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Nil.

Conflicts of Interest

There are no conflicts of interest.

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