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Injection of *Mesobuthus tamulus* venom in distal segment of femoral artery evokes hyperventilatory and hypertensive responses in anaesthetised rats

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ABSTRACT

Intra-arterial (i.a.) injection of Indian red scorpion (*Mesobuthus tamulus*; BT) venom produces cardiorespiratory changes by involving perivascular receptors. The afferents involved in mediating these reflex responses are not known. The present investigation was conducted to examine the afferents mediating the vasosensory reflexes evoked by i.a. injection of BT venom in the peripheral end of femoral artery in urethane anaesthetised rats. Blood pressure (BP), ECG (for heart rate), and respiratory movements (for rate and depth) were recorded for 30 min after the i.a. injection of venom. Minute ventilation (MV) was computed by using appropriate calibrations for depth and rate of respiration. After the injection of venom, there was immediate hyperventilatory, intermediate hypertensive and delayed bradycardiac response. Equal volume of saline (0.10 ml, i.a.) did not produce any cardiorespiratory changes thus, eliminating the possibility of stretch mediated responses. Sectioning of ipsilateral sciatic and femoral nerves attenuated the hyperventilatory and hypertensive responses produced by venom significantly. After the neurotomy, the latency of bradycardiac response was shortened significantly. Even the time to reach the peak bradycardiac response was also shortened. The data provide evidences for the partial involvement of somatic nerves in mediating the vascular reflexes.

Indian red scorpion (*Mesobuthus tamulus*; BT) envenomation produces severe cardiorespiratory abnormalities [1–4,10,11]. A significant changes in the mean arterial pressure (MAP), respiratory rate (RR) and heart rate (HR) are seen after envenomation [3,12,13]. All these alterations cannot be attributed to a single factor or mechanism. The envenomation syndrome produces ECG changes mimicking acute coronary insufficiency [11]. It has been shown that the ischemic manifestations in conditions like migraine, angina, embolism and myocardial infarction involve vasosensory reflexes [7,9]. Hence, the involvement of vasosensory reflexes in BT envenomation is also expected. In our earlier study, we have shown that intra-arterial injection of BT venom evokes cardiorespiratory changes via vasosensory reflexes [14] but the afferents involved in mediating these reflex responses are not yet identified. Therefore, the present study was conducted to examine the involvement of afferents in somatic nerves for mediating the vasosensory reflexes.

Experiments were performed on healthy male albino rats (Charles–Foster strain), weighing between 200 and 300 g after obtaining the clearance from the Institute Ethical Committee for Animal Experimentation. The animals were housed in the 12:12 h light/dark cycle and were provided with *ad libitum* food (Hindustan Lever Ltd.) and water. Animals were anaesthetised with urethane (Merck, Germany), with an initial dose of 1.5 g/kg body weight, intra-peritoneally. A maintenance dose of anaesthesia (50–100 mg) was given, if required.

The tracheal cannulation was performed to keep the respiratory tract patent. The length of the tracheostomy tube was kept minimum (<2.5 cm) to avoid unnecessary dead space. Tracheal secretions were aspirated from time to time by gentle suction through a fine polyethylene tube. The femoral artery in femoral triangle was dissected and skeletonized by clearing the tissues and fascial attachments by blunt dissection. Cannula filled with freshly prepared heparinized saline (20 IU/ml) was inserted in to the femoral artery through a small nick and secured firmly. The cannula was in turn connected through a three-way stop cock to Statham strain gauge pressure transducer (Biodevices, Ambala). The distal end of the same artery was also cannulated for injection of saline/venom, etc. The blood 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 was taken as a parameter throughout the study. The instrument was calibrated regularly.

Femoral nerve was dissected out on the ipsilateral side during the dissection of femoral artery. The sciatic nerve was dissected by making an incision on the point between ischial tuberosity

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Table 1
Injection of BT venom in peripheral segment of femoral artery and its effect on mean arterial pressure (MAP), minute ventilation (MV) and heart rate (HR) in naïve and neurotomised (NX) rats

Time (min)	MAP (mm Hg)			MV (ml/min)			HR (beats/min)		
	Saline	Venom only	Venom + NX	Saline	Venom only	Venom + NX	Saline	Venom only	Venom + NX
Before	90.8 ± 4.7	93.4 ± 6.1	91.6 ± 4.0	185.3 ± 13.0	150.3 ± 12.2	153.4 ± 19.6	310 ± 15.7	301 ± 13.0	307 ± 11.0
After									
0	96.8 ± 4.2	86.3 ± 5.7	80.8 ± 6.8	184.5 ± 10.4	250.4 ± 29.3	201.6 ± 20.5	305 ± 14.0	303 ± 14.4	307 ± 11.0
5	96.1 ± 5.4	131.2 ± 6.7*#	105.0 ± 7.8	177.6 ± 16.6	158.6 ± 20.7	106.3 ± 16.3#	300 ± 13.0	271 ± 14.9	151 ± 23.0*#@
10	98.3 ± 5.0	125.5 ± 6.3*#	105.3 ± 7.1@	169.3 ± 5.1	142.9 ± 11.4	136.2 ± 17.3	305 ± 12.0	218 ± 22.5*#	168 ± 24.0*#
15	94.8 ± 6.8	122.2 ± 5.7*#	102.4 ± 5.7@	174.1 ± 10.7	170.6 ± 12.4	156.3 ± 19.2	307 ± 11.4	184 ± 24.7*#	176 ± 27.0*#
20	97.3 ± 4.6	116.6 ± 5.6*#	100.4 ± 8.0	171.1 ± 6.0	207.7 ± 13.3	152.8 ± 15.4@	308 ± 9.8	158 ± 22.5*#	171 ± 21.0*#
25	98.2 ± 4.5	113.3 ± 4.4*#	100.2 ± 9.0	166.9 ± 5.5	204.9 ± 12.6*#	170.7 ± 17.3	306 ± 10.3	151 ± 18.9*#	178 ± 17.8*#
30	94.6 ± 5.6	110.4 ± 5.6*	100.2 ± 9.0	171.1 ± 4.2	231.7 ± 20.6*#	119.2 ± 11.3#@	307 ± 11.4	153 ± 17.8*#	171 ± 24.3*#

The saline data provides the time-matched responses for all parameters. The values are mean ± S.E.M. from six different experiments in each group. An asterisk (*) indicates $P < 0.05$ as compared to before values; #$P < 0.05$ as compared to saline group; @$P < 0.05$ as compared to venom only group (Newman–Keuls–Students test for multiple comparisons).

and greater trochanter down to the popliteal region on the ipsilateral side. A thread was passed under the nerves and loops were made.

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 was calculated from these recordings. The depth of the respiration was calibrated by introducing a known volume of air (1 or 2 ml) in the lung immediately after the death of the animal. The minute ventilation (MV) was calculated with the rate and depth of the respiration.

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

Crude BT venom was obtained from Haffkine Institute, Mumbai, India. Stock solution of BT venom (2 mg/ml) was prepared in distilled water and refrigerated. Heparin was obtained from Biological Evans Ltd., Hyderabad, India.

After the stabilization of animal for 30 min, the control recording of BP, ECG and respiration were obtained. Then the saline/venom (0.1 ml/1 mg/kg) was injected in the peripheral end of femoral artery and the cardiorespiratory parameters were recorded at every 5 min intervals for 30 min. In the second group, the protocols mentioned above were repeated after ipsilateral neurotomy (sectioning of sciatic and femoral nerves).

The results were presented as mean ± S.E.M. values. The MAP, MV or HR responses before venom were taken as initial responses. The values after the injection of venom at every 5 min up to 30 min were computed. The significant difference between the two groups was assessed by two-way ANOVA followed by Student–Neuman–Keuls test for multiple comparisons. A P-value < 0.05 was considered significant.

The data for the effect of BT venom on MAP, MV and HR before and after sectioning of sciatic and femoral nerves are given in Table 1 and Fig. 1. Intra-arterial injection of BT venom produced immediate hyperventilatory, intermediate hypertensive and delayed bradycardiac responses. After injecting the venom, there was immediate decrease (about 10% of initial) in MAP followed by sustained increase up to 30 min. The increase was more than 40% of initial at 5 min which decreased gradually and remained above the initial level up to 30 min (Table 1). There was instantaneous hyperventilation which returned to initial level at 5 min. After 10 min, ventilation increased again and at 30 min the increase was 50% above the initial (Table 1). The bradycardiac response began at 5 min, reached its peak around 25 min (Table 1 and Fig. 1) and remained at that level up to 30 min.

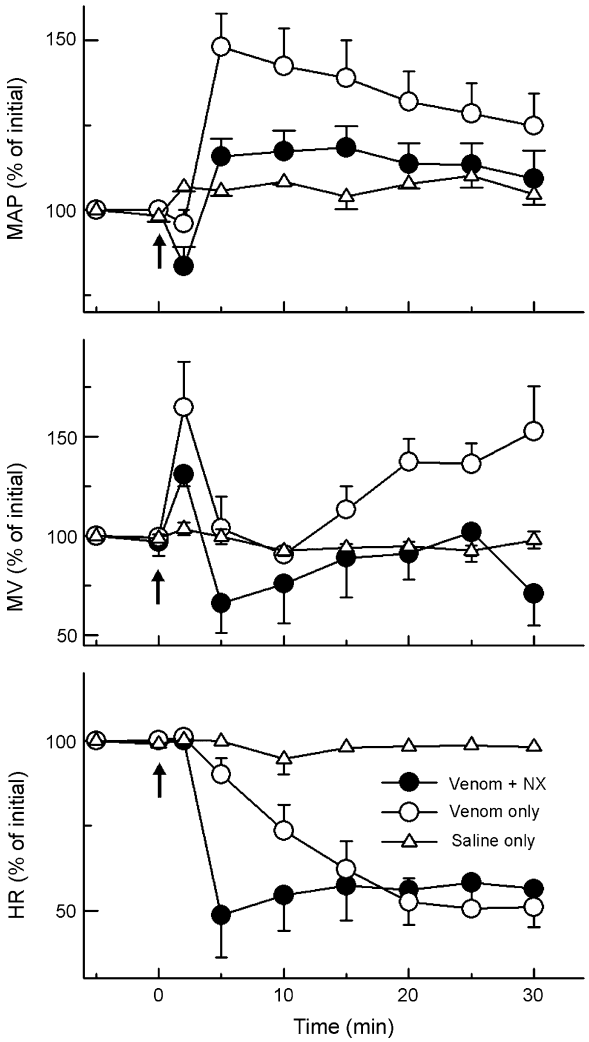

Fig. 1. Time-response relationship of BT venom (i.a.) on cardiorespiratory parameters in naïve and neurotomised rats. The MAP, MV and HR after saline, venom only and venom after the sectioning of sciatic and femoral nerves on the ipsilateral side are shown in all panels. The values are mean ± S.E.M. from six experiments in each group. The responses after venom are significantly different from the saline group ($P < 0.05$, two-way ANOVA). The responses of venom in neurotomy (NX) group are significantly different from the venom only group for MAP and MV ($P < 0.05$, two-way ANOVA) whereas, for HR the differenecs were seen up to 10 min ($P < 0.05$, Neuman–Keuls–Student test). An arrow indicates the point of injection of venom/saline.

I.a. injection of equi-volume of saline did not alter the cardiorespiratory parameters. The changes were only about 5–6% of initial and were not significantly different ($n = 6$, Table 1 and Fig. 1).

Neurotomy *per se* did not alter the cardiorespiratory parameters from the initial (Table 1). In neurotomy group, it was observed that the immediate hyperventilatory response produced by venom (i.a.) was attenuated significantly and the delayed hyperventilatory response was blocked ($P < 0.05$, two-way ANOVA; Table 1 and Fig. 1). The venom-induced MAP was attenuated significantly at all time intervals ($P < 0.05$, two-way ANOVA). In case of HR after neurotomy, the onset of bradycardiac response was earlier than venom alone group (Table 1). The magnitude of peak bradycardiac response was not altered but occurred much earlier (at 5 min) than the venom only group (Table 1).

The present observations demonstrate the presence of afferents in sciatic and femoral nerves for mediating the vasosensory reflexes elicited by injecting BT venom in an arterial segment. These afferents modulate ventilatory and pressure responses. In this study, we selected 1 mg/kg of venom as this concentration produced optimal responses as shown in our earlier study [14]. Intra-arterial injection of equi-volume of saline did not produce the significant changes in the cardiorespiratory parameters excluding the possibility of stretch/volume in mediating these responses.

The response pattern after venom can be categorized broadly into two phases, viz. early phase between 0 and 5 min and delayed phase between 5 and 30 min. Early phase (because of short latency) represent the activation of nociceptors present in the perivascular area by the chemicals (nociceptive agents) present in venom while the late phase appears to involve a time-related cellular actions.

In the studies elsewhere, involvement of early phase has been shown by injecting capsaicin/anandamide in the common iliac artery [7,15] where they observed hyperventilatory and hypotensive responses occurring within 10 s after the injection of agonists. We also observed a transient hypotensive response (after a latency of 10 s) after the injection of venom but the magnitude was <10% of the initial [14]. However after neurotomy, the hypotensive response was accentuated. Thus, indicating the absence of afferents for this response in somatic nerves. However, the previous reports suggest that the initial/early phase of response induced by capsaicin or other nociceptive agents activate nociceptors present in the somatic afferents [8,15].

The activation of nociceptors by an agonist involve number of cell signalling pathways that may involve G-protein or other membrane bound ligands [6]. These in turn may activate the generation of cGMP, phospholipase, arachidonic acid metabolites, leukotrienes, etc. and the generation of these molecules require time. Our observations in the delayed phase between 5 and 30 min are consistent for such actions. Since, we extended the time of observation up to 30 min, we were able to detect the delayed phase of response. In our previous study, using similar protocol, the involvement of prostaglandins has been demonstrated for the delayed phase [14]. The blockade of hyperventilatory/hypertensive responses at this phase after neurotomy indicate that these actions are mediated through the afferents in somatic nerves. Thus, the delayed phase may involve the intracellular cell signalling pathways that activate the nociceptors present on somatic afferents. The signaling pathways include adenylyl cyclase, cAMP, protein kinase C, phospholipase C and D, diacylglycerol, inositol triphosphate, etc. as suggested for inflammatory pain [5].

The bradycardiac response in intact animal (non-neurotomy group) indicates that the afferents in somatic nerves functionally prevent the sudden decrease in HR which may be a protective phenomenon. The advancement of bradycardiac response in neurotomy group indicate that the regulatory offsetting pathways are carried through the somatic nerves. However, the mechanisms for delayed phase of bradycardiac response requires to be investigated.

In conclusion, the BT venom in a segment of a blood vessel stimulate the nociceptors, to produce hyperventilatory and hypertensive responses. The responses are mediated by the direct activation of nociceptors (early phase responses) or by the activation of intracellular signalling molecules (delayed phase responses) as indicated by the latency. The data provide evidences for the involvement of afferents in somatic nerves to produce venom-induced vasosensory reflexes.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.neulet.2008.04.037.

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