



VASOSENSORY REFLEX RESPONSES ARE MEDIATED THROUGH THE AFFERENTS ORIGINATING FROM VASCULAR PLEXUSES IN ANAESTHETISED RATS

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ABSTRACT

In the neurotomised animals, venom-induced mean arterial pressure (MAP) and respiratory rate (RR) changes were markedly attenuated but not the heart rate (HR) changes indicating the existence of additional afferent pathway. Hence, further sets of experiments were performed to explore the afferents. Albino rats were anaesthetised with an intra-peritoneal injection of urethane (1.5 g/kg). Tracheostomy was performed to keep the airway patent. Femoral artery was cannulated proximally for the recording of blood pressure and distally to inject the chemicals/venom. The effect of venom on MAP, HR and RR were recorded at every 5 min for 60 min and presented as mean \pm SEM. Intra-arterial injection of *Mesobuthus tamulus* venom elicits vasosensory reflexes producing immediate hyperventilatory, intermediate hypertensive and delayed bradycardiac responses in anaesthetised rats. After ipsilateral nerve sectioning, the RR and MAP changes were attenuated significantly but not the HR changes. Pre-treatment with neurotomy plus vagotomy plus local anaesthetics, blocked all the venom-induced cardiorespiratory responses viz changes in RR, MAP and HR. The observations of this study provide evidence for afferent pathways in somatic nerves and also through the perivascular nerve plexuses.

KEY WORDS: Nociception, Vasosensory Afferents, Vascular Plexuses and Cardiorespiratory Changes.



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Received on: 07-03-2017

Revised and Accepted on: 30-03-2017

DOI: <http://dx.doi.org/10.22376/ijpbs.2017.8.2.b585-592>

INTRODUCTION

The involvement of vascular nociceptors in the pain associated with angina, myocardial infarction, migraine and intermittent claudication have been shown earlier^{1,2}. It has also been shown that the medium sized peripheral blood vessels play important role in the modulation of cardiorespiratory parameters⁷. Further, peripheral vascular disorders are also implicated in the long term cardiovascular changes and other behavioural changes^{3,4}. These changes are believed to be secondary to the autonomic changes. It has been shown that intra-arterial (i.a.) injection of *Mesobuthus tumulus* (BT) venom elicits reflex cardiorespiratory changes which last longer than the other nociceptive agonists (capsaicin/anandamide/ $\alpha\beta$ Me-ATP) -induced responses⁵⁻⁸. These changes were categorized as immediate-hyperventilatory, intermediate-hypertensive and delayed-bradycardiac responses⁵. Further, it was shown that the responses are mediated by prostaglandins⁵. It has been shown earlier that nociceptive vascular reflex responses evoked by BT venom modulate cardiorespiratory parameters involving transient receptor potential vanilloid 1 (TRPV1)⁹ and the efferents are located in the sympathetic and vagal parasympathetics¹⁰. The role of TRPV1, 5-HT₃ receptor and B1-kinin receptor is also well established in the venom-induced vasosensory reflex responses^{9,11}. Since, the venom-induced mean arterial pressure (MAP) and respiratory rate (RR) changes were markedly attenuated in the neurotomed animals but not the HR changes¹². Therefore, further sets of experiments were performed to explore the afferent pathways mediating the HR changes. Therefore, this study was conducted to reveal the course of sensory afferents originating from the peripheral blood vessels which mediate the vasosensory reflex responses modulating the cardiorespiratory parameters.

MATERIALS AND METHODS

Animals and Anaesthesia

All the experiments were performed after getting approval from the Institute Ethical Committee (Ref No. Dean/13-14/CAE/189). Experiments were performed on healthy albino rats (Charles-Foster strain), weighing between 200-300 g. Animals were anaesthetised with urethane (Merck, Germany), with an initial dose of 1.5 g/kg body weight, intra-peritoneally and was maintained by injecting the urethane as required. The animals were exposed to the 12:12 h light/dark cycle to keep diurnal variations intact and were provided with ad libitum animal food (Hindustan Lever Ltd.) and water.

Dissection and Recordings

The tracheal cannulation was done to keep the respiratory way patent. Trachea was exposed by making 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 by a thread. Tracheal secretions were aspirated by gentle suction through a fine polyethylene tube. Femoral triangle was exposed by making an incision along the course of femoral artery. The femoral artery was dissected by clearing the tissues and fascia

surrounding it. The femoral vein and femoral nerve were separated out from the artery. Freshly prepared heparinised saline (20 IU/ml) was loaded in the syringe attached with appropriate sized cannula. A small nick was made in the femoral artery proximally and the cannula was inserted and secured firmly with thread. Later on, the cannula was connected through a tri-way stop cock to the Statham Strain Gage pressure transducer (Biodevices, Ambala). After cannulation of proximal segment of the femoral artery, the distal segment of the same artery was cannulated to inject the drugs/venom/saline in a local segment of the vessel. The placement of cannula was confirmed by injecting 0.10 ml saline. The pressure transducer was filled with the heparinised saline and connected with tri-way stop cock which in turn was connected with artery through cannula. The free pulsatile flow of blood was obtained after releasing the thread clamp. The pressure transducer was connected to a bridge amplifier via galvanometer. The galvanometer deflections were recorded on a chart paper with the help of writing pen. The mean arterial pressure (MAP) was computed from the recording and was considered as the parameter for blood pressure throughout the study. The instrument was calibrated in between the experiments as per the need. The needle electrodes were connected as per the standard limb lead-II configuration for the recording of electrocardiogram (ECG). The electrocardiographic potentials were recorded on a chart recorder. Heart rate (HR) was computed manually from R-R intervals of the ECG. The force displacement transducer was connected to the chest with the thread by securing skin over xiphisternum. The respiratory movements were recorded on a chart recorder via a bridge amplifier. Respiratory rate (RR) and minute ventilation (MV) was computed from the respiratory excursions. After the dissection, 30 min was given for the stabilization of the vital parameters which was followed by the initial recording of BP, ECG and respiratory movements. Then, 0.10 ml of normal saline was injected in the peripheral segment of femoral artery and the cardiorespiratory parameters were recorded at every 5 min up to 20 min as initial recording. This was followed by the injection of venom (1.0 mg/kg) in the peripheral segment of the same femoral artery and the cardiorespiratory parameters were recorded at every 5 min up to 60 min.

Drugs and Solutions

Crude BT venom was purchased from the Haffkine Institute, Mumbai, India. 2 mg/ml stock solution of BT venom was prepared in the distilled water and was refrigerated. 1 mg/kg BT venom was freshly prepared from the stock solution and injected to stimulate the perivascular nociceptors as it produces optimal responses on the cardiorespiratory parameters. Heparin was obtained from Biological Evans Ltd., Hyderabad, India.

Experimental Protocol

In this study, twenty four (24) rats were used and were divided in to four groups (n = 6). In the first group, BT venom was administered intra-arterially and the BP, ECG and respiratory movements were recorded at every 5 min up to 60 min. These responses were considered as the venom only / control responses. In

the second group, rats were pre-treated with femoral nerve sectioning (NX) along with bilateral vagotomy (B/L VagX) and the venom-induced cardiorespiratory parameters were recorded at every 5 min up to 60 min. In the third group, rats were pre-treated with NX plus B/L VagX plus intra-arterial injection of lignocaine (0.1 ml; 2%) and cardiorespiratory parameters were recorded. In the fourth group, ipsilateral femoral artery and vein were severed in addition to the pre-treatment of the second group and the cardiorespiratory parameters were recorded. All the chemicals/venom/saline was injected in the peripheral segment of femoral artery as the proximal segment was connected with the Statham Strain Gage pressure transducer for the BP recording. Volume of the injectables was kept constant (0.10 ml) to avoid the effect of stretch on the vessel wall and the room temperature was maintained at $\sim 25^{\circ}\text{C}$ throughout the experiment.

Analysis of data and statistics

The results were presented as mean \pm SEM values. The MAP, HR and RR responses before venom were considered as initial responses. The comparisons of both groups were done by using the two-way analysis of variance (ANOVA) test. Statistical Analysis was done by using Graph Pad Prism version-6. Student's *t*-test was also done wherever required. A *p*-value < 0.05 was considered as significant.

RESULTS

In this study, BT venom (1 mg/kg) was injected in the peripheral segment of femoral artery to stimulate the perivascular nociceptors as this concentration produced optimal responses on the cardiorespiratory parameters in the anesthetised rats⁵. Individual and mean \pm SEM values of MAP, HR and RR are given after the injection of venom only and after the injection of venom in pre-treated groups (Fig 1-4).

Venom-induced changes in cardiorespiratory parameters

In the stabilized animals after the injection of venom, immediate- tachypnoeic (latency \sim 2s), intermediate-hypertensive (latency \sim 40 s) and delayed- bradycardiac responses (latency \sim 5 min) were observed (Fig 2-4). The respiratory changes exhibited immediate increase in RR (73 ± 5.06 to 103 ± 7.2) followed by a decrease (73 ± 5.06 to 45 ± 6.7) which returned to the initial level within 15 min and continued to increase up to 60 min (Fig 2-4). The hypertensive response began within 40 s, reaching its peak within 5 min which exhibited a decreasing trend up to 60 min but never reached to the initial level (Fig 2-4). The bradycardiac response began within 5 min and the maximal bradycardia was seen at 25 min which remained at that level up to 60 min (Fig 2-4).

Neurotomy (NX) plus B/L Vagotomy (VagX) pre-treatment attenuated the venom-induced RR and MAP changes

After ipsilateral NX + VagX, there was significant decrease in the resting level of RR. In these rats there was slight increase in RR immediately after the administration of venom (from 43.5 ± 2.87 to 48 ± 2.45

per min). The latency of the response remained same (< 2 s). The RR decreased at 5 min (43.5 ± 2.87 to 24.5 ± 2.94 per min) and remained at that level up to 60 min (Fig 1-2). The responses were significantly different from the corresponding values in control group ($P < 0.05$, two way ANOVA; *t*-test for unpaired observations). There was no change in resting MAP after NX + VagX. In these rats the immediate depressor response was accentuated after venom as compared to control (from 92.8 ± 4.3 to 80.2 ± 7.14 mm Hg) and the pressor response was attenuated (131 ± 6.77 to 122 ± 2.13 mm Hg; Fig 1-2). After 5 min the MAP began to decrease and the trend continued up to 60 min. The responses were significantly different from the control group ($P < 0.05$, two way ANOVA; *t*-test for unpaired observations). Hering-Traube waves appeared at 15 min after the i.a. injection of venom and remain prominent up to 60 min and these were associated with bradycardiac response (Fig 1). After NX + VagX, there was increase in the resting HR. In these animals, the bradycardiac response was not different than the venom only group (Fig 1-2).

Neurotomy (NX) plus B/L vagotomy (VagX) plus i.a. xygnocaine (Xyl) pre-treatment attenuated the venom-induced RR, MAP and HR changes

After NX+VagX+Xyl pre-treatment, the resting RR was decreased significantly. In these rats, there was no change in RR after the administration of venom during the entire period of observation (Fig 1 and 3). The responses were significantly different from the corresponding values in control group ($P < 0.05$, two way ANOVA; *t*-test for unpaired observations). After NX+VagX+Xyl, here was a transient fall in the resting MAP. In these animals after venom, there was slight immediate depressor response (from 91.5 ± 8.73 to 83.2 ± 8.3 mm Hg) followed by an attenuated pressor response (131 ± 6.77 to 118.9 ± 5.6 mm Hg) up to 60 min (Fig 1 and 3). The responses were significantly different from the control group ($P < 0.05$, two way ANOVA; *t*-test for unpaired observations). Hering-Traube waves were prominent after 15 min after venom (Fig 1). After NX+VagX+Xyl, the resting HR was increased. In these animals, the bradycardiac response was delayed and less severe in comparison to the control group (Fig 1 and 3). The responses were significantly different from the corresponding values in control group ($P < 0.05$, two way ANOVA; *t*-test for unpaired observations).

Neurotomy and femoral vessel sectioning (NX+VasX) plus B/L vagotomy (VagX) pre-treatment attenuated the venom-induced RR, MAP and HR changes.

After pre-treatment, there was significant decrease in the resting RR. In these animals, there was no immediate increase rather there was decrease in RR after the administration of venom within 5 min (from 55.5 ± 2.3 to 36.5 ± 5.9 per min). After 5 min RR remained at the same level up to 60 min (Fig 1 and 4). The responses were significantly different from the corresponding values in control group ($P < 0.05$, two way ANOVA; *t*-test for unpaired observations). After pre-treatment, there was no change in the resting MAP. In these animals, there was slight immediate depressor response (from 77 ± 6.3 to 72.6 ± 6.9 mm Hg) followed by attenuated pressor response (131 ± 6.7 to $113.7 \pm$

3.8 mm Hg) up to 60 min (Fig 1 and 4). After 5 min, the MAP continued to decrease and reached even below the initial level up to 60 min. The responses were significantly different from the control group ($P < 0.05$, two way ANOVA; t -test for unpaired observations). Hering-Traube waves were prominent after 15 min after venom (Fig 1). An increase in resting HR was observed

after pre-treatment which was not significant statistically. In these animals, the decrease in HR began after 5 min which was much less than the control (Fig 1 and 4). The responses were significantly different from the corresponding values in control group ($P < 0.05$, two way ANOVA; t -test for unpaired observations).

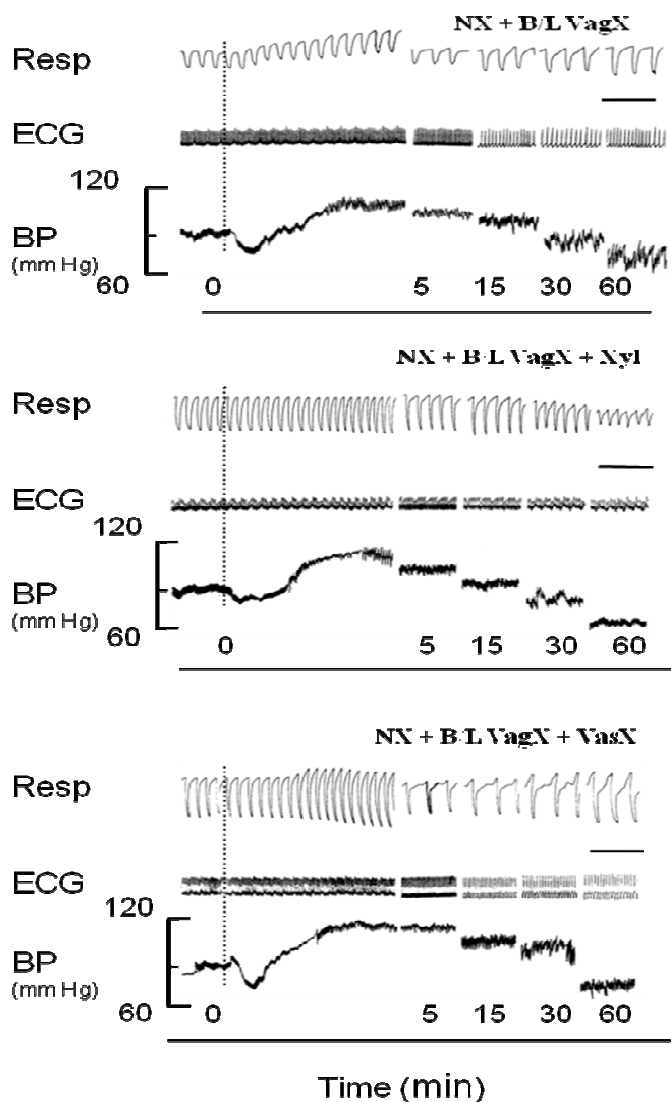


Figure 1

Original recording showing the effect of intra-arterial injection of 1 mg/kg of *Mesobuthus tumulus* venom in peripheral segment of femoral artery on respiration, ECG and blood pressure after in naive and pre-treated animals. NX = Ipsilateral neurotomy, B/L VagX = Bilateral vagotomy, Xyl = Xylocaine (lignocaine 2%) and VasX = Femoral vessel sectioning. The point of injection is indicated by dotted line. The horizontal line = 5 s for respiration and ECG and 50 s for blood pressure.

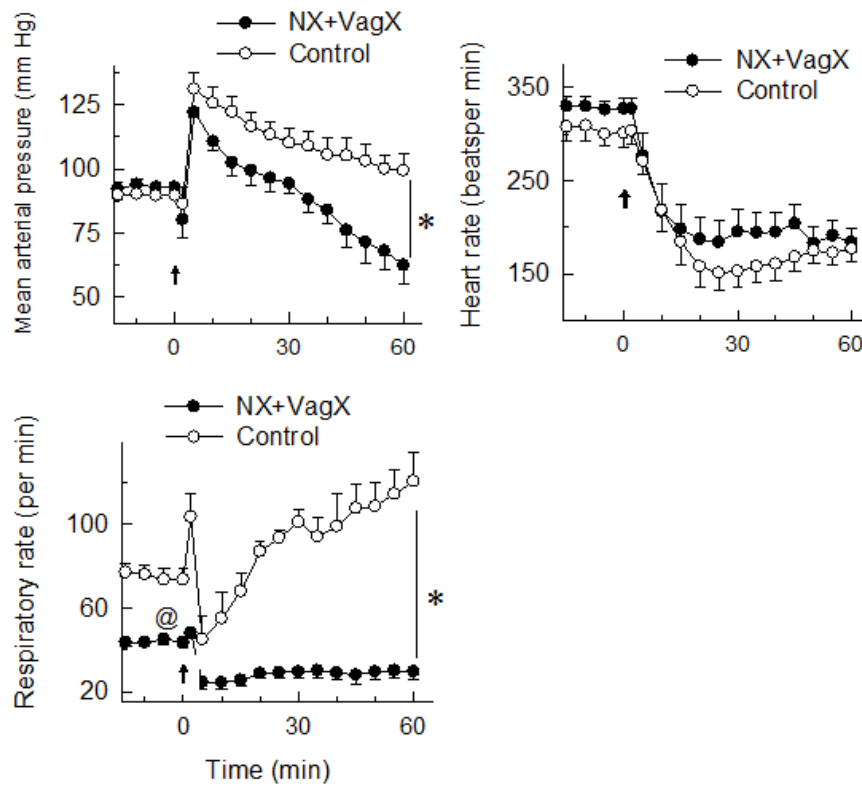


Figure 2

Time-response relationship of BT venom (1 mg/kg) on mean arterial pressure (MAP), heart rate (HR) and respiratory rate (RR) in NX + B/L VagX pre-treated animals. Venom-induced cardiorespiratory responses without any pre-treatment of the animals are considered as the control responses. NX = Ipsilateral neurotomy and B/L VagX = Bilateral vagotomy. The arrow indicates point of injection of the chemicals/drugs/venom. “@” = $p < 0.05$ from control (t -test for unpaired observations); “*” = $p < 0.05$ from control responses (two way ANOVA).

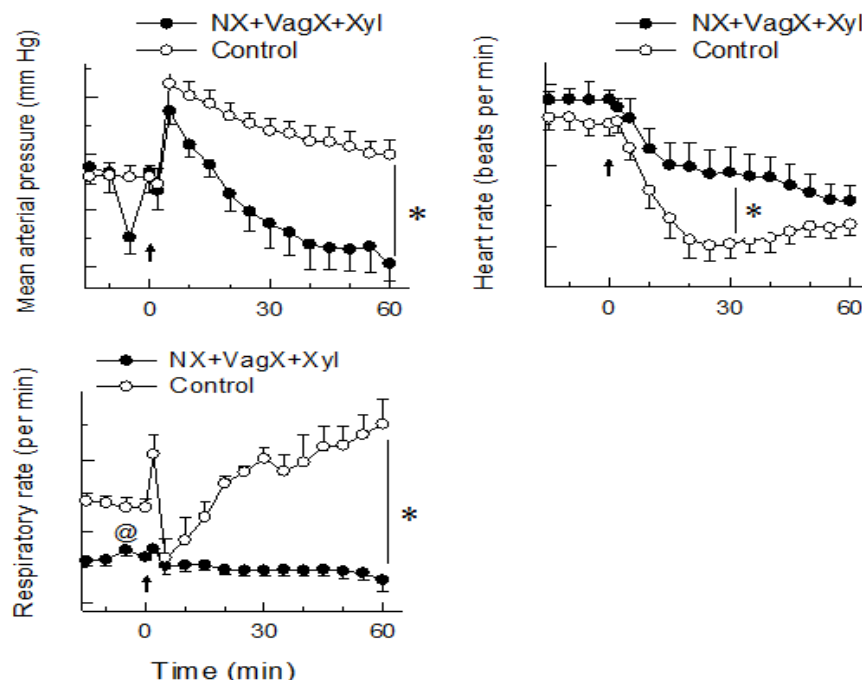


Figure 3

Time-response relationship of BT venom (1 mg/kg) on mean arterial pressure (MAP), heart rate (HR) and respiratory rate (RR) in NX + B/L VagX + i.a. Xyl pre-treated animals. Venom-induced cardiorespiratory responses without any pre-treatment of the animals are considered as the control responses. NX = Ipsilateral neurotomy, B/L VagX = Bilateral vagotomy and Xyl = Xylocaine (lidocaine 2%). The arrow indicates point of injection of the chemicals/drugs/venom. “@” = $p < 0.05$ from control (t -test for unpaired observations); “*” = $p < 0.05$ from control responses (two way ANOVA).

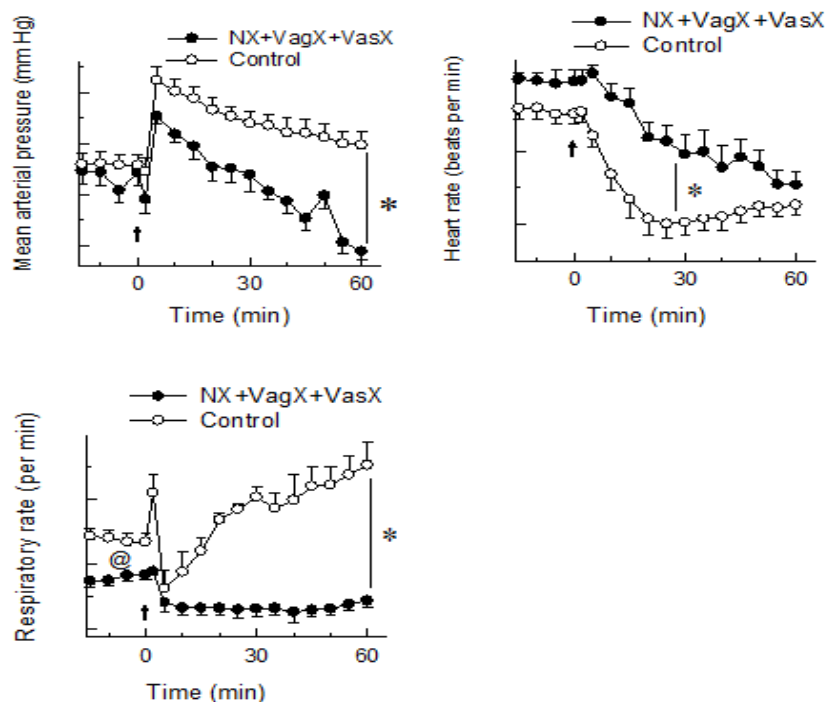


Figure 4

Time-response relationship of BT venom (1 mg/kg) on mean arterial pressure (MAP), heart rate (HR) and respiratory rate (RR) in NX + B/L VagX + VasX pre-treated animals. Venom-induced cardiorespiratory responses without any pre-treatment of the animals are considered as the control responses. NX = Ipsilateral neurotomy, B/L VagX = Bilateral vagotomy and VasX = Femoral vessel sectioning. The arrow indicates point of injection of the chemicals/drugs/venom. “@” = $p < 0.05$ from control (t -test for unpaired observations); “*” = $p < 0.05$ from control responses (two way ANOVA).

DISCUSSION

In the present study, cardiorespiratory responses are temporally dispersed. Intra-arterial (i.a.) injection of BT venom elicits vasosensory reflexes producing immediate hyperventilatory, intermediate hypertensive and delayed bradycardiac responses in anaesthetised rats⁵. After ipsilateral nerve sectioning, the respiratory rate (RR) and mean arterial pressure (MAP) changes were attenuated significantly but not the heart rate (HR) changes¹², indicating the existence of additional pathway modulating HR. Pre-treatment with i.a. injection of xylocaine, blocked all the venom-induced cardiorespiratory responses *viz* changes in RR, MAP and HR⁹. The respiratory alterations occurred in three different phases, immediate- hyperventilatory response; intermediate- hypoventilatory response; and delayed- hyperventilatory response. The immediate response has very short latency (< 2 s) and is a consistent feature. Similar result was observed with the capsaicin/anandamide in similar experimental settings^{7,13}. Immediate and intermediate changes were attenuated when the ipsilateral somatic nerves were sectioned^{7,8,12,13}. In the NX plus B/L vagX pre-treated group, venom-induced RR and MAP changes were attenuated significantly but not the HR changes. The local anaesthetic injection after neurotomy plus B/L vagotomy abolished the venom-induced respiratory response pattern completely. This substantiates the point that the afferents are not only restricted to the somatic nerves. The afferents from nociceptors are carried by somatic nerve, while those present in the perivascular plexuses, may be through the plexuses. In addition to efferent activity autonomic nerves also carry

the afferent information. These sensory fibres are reported to travel centrally in the sympathetic nerves to the sympathetic ganglion and cause highly localized reflex responses¹⁴. The present observations substantiate for the existence of afferents in perivascular plexuses. There was decrease in RR after the immediate hyperventilatory response. This was not accompanied by the parallel decrease in ventilation. Thus, the decrease in RR is not resulting from the CO₂ washout effect produced by hyperventilation. However after local anaesthetic pre-treatment, this phase was totally blocked. This again indicates the involvement of additional afferent pathway for modulating the respiration. In the absence of vagal control, the respiration was slow and deep but the regulatory or compensatory mechanisms were absent as indicated by lower ventilatory response in vagotomised rats. The delayed changes in RR are mediated through somatic inputs and these are also abolished by local anaesthetic. The delayed responses mostly are mediated by slow conducting fibres or by the neuro-humoral mechanisms evoked by pain. These include catecholamines, glucocorticoids, thyroxin etc¹⁵. The blood pressure responses can also be categorized as immediate- depressor, intermediate- pressor response and delayed- recovery phase. The hypotensive response was not very marked but in the absence of vagal activity or somatic neural transmission it became apparent. The latency was similar to that observed with hypotensive response seen with capsaicin⁷. Hypotension by venom can be produced by kinins. Venom or the nociceptive agonist stimulates kinin mechanism at the periphery¹⁵. The intermediate pressor response is a consistent feature which peaked within 5

min but was associated with parallel reduction in RR. The decreased RR may be due to the baro-apnoea. In the present study, the increase in pulse pressure was observed after 15 min (Fig 1). This suggests the activity of adrenaline. It can be proposed that the nociceptive stimulation result in the increased suprarenal activity releasing adrenaline. The effects of adrenaline further can be seen as the sensitization of respiratory centres as demonstrated by the appearance of Hering-Traube waves after 15-30 min (Fig 1). Thus, the pressor response in the early phase may be through the sympathetic neuronal activity and the later phase by the adrenal medullary activity. Progressive fall of pressure in the absence of somatic neural afferent activity and vagal activity (after NX + VagX; Fig 2) indicate a different mechanism, this may be due to the dominance of kinin mechanisms at this phase. Kinins are activated after 30 min to augment the reflexes and also to produce pulmonary oedema¹⁶⁻²⁰. During venomation, concentration of kinins continuously increases and brings about systemic fall in pressure. In such situation a simultaneous increase in HR is expected, but no such increase was observed. On the other hand, there was persistently lower HR. The mechanism of bradycardia in this phase is not clear but, may be due to the action of kinins on the pacemaker activity. Kinins mediate their actions by involving inositol triphosphate (IP3) mechanism, increasing the intracellular Ca⁺⁺, and thus slowing the Ca⁺⁺ influx. This in turn may slow down the pacemaker potential to decreases the HR. The bradycardiac response was slow to occur and cannot be accounted for the increased pressure and the resulting

baroreflex- induced bradycardia. These changes were not altered even after sectioning the somatic nerves. This particular observation however was difficult to explain and indicate the existence of additional pathway that specifically regulates the HR. In the NX plus B/L VagX plus local anaesthetics pre-treated group, venom-induced HR changes were also attenuated significantly indicating the existence of afferents in the blood vessels. The blockade of bradycardiac response after vasectomy further supports our previous findings for the existence of additional pathway modulating HR. Since, the parasympathetic vagal output was not involved in mediating these responses, the efferents for this reflex appears to mainly exist in the sympathetic system governing pacemaker activity.

CONCLUSION

In conclusion, BT venom in a segment of femoral artery evokes vasosensory reflex mediated cardiorespiratory changes modulating blood pressure, respiration and heart rate. The observations of this study provide evidence for afferent pathways in somatic nerves and also through the perivascular nerve plexuses. The somatic nerves govern mainly the respiratory and blood pressure responses and the perivascular afferents modulate primarily HR changes.

CONFLICT OF INTEREST

Conflict of interest declared none.

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We sincerely thank the above reviewers for peer reviewing the manuscript