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# Intra-arterial injection of *Mesobuthus tamulus* venom elicits cardiorespiratory reflexes involving perivascular afferents

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**Abstract**

Role of perivascular afferents for the cardiorespiratory alterations produced by *Mesobuthus tamulus* (BT) envenomation was examined in urethane-anaesthetized male rats. Blood pressure (BP), respiratory rate (RR) and heart rate (HR) were recorded after injecting BT venom/saline in the distal end of femoral artery for 60 min. In addition, paw oedema was also determined. Injection of venom produced an immediate (within 2 s) increase in RR followed by a decrease and finally a sustained increase up to 60 min. BP was increased (within 10 s) by 30–50%, which gradually declined but remained above the initial level up to 60 min. The bradycardiac response was late to occur (after 50 s) and the peak response was seen between 10 and 50 min, which remained at that level. There was oedema in the ipsilateral hind paw (venom injected side) as compared to contralateral side and saline control group. The oedema and cardiorespiratory changes were maximal at 1.0 mg/kg of venom. Pretreatment with indomethacin significantly attenuated the venom-induced responses and also blocked the paw oedema. Present experiments reveal that BT venom in a segment of an artery produces oedema by involving prostaglandins to sensitize the nociceptors present in perivascular tissues to evoke the cardiorespiratory reflexes.


*Keywords:* Perivascular nerve endings; Indian red scorpion venom; Prostaglandins; Indomethacin; Vasosensory reflexes

## 1. Introduction

Indian red scorpion (*Mesobuthus tamulus*; BT) envenomation produces alterations in cardiorespiratory system resulting in lethality (Murthy et al., 1991; Tiwari and Deshpande, 1993; Deshpande and Alex, 2000; Pandey and Deshpande, 2004). Cardiovascular manifestations include dysrhythmias, myocarditis, ischemia and infarction-like pattern (Murthy and Yeolekar, 1986; Murthy and Zare, 1998). In experimental animals, the toxicity involves the augmentation of J-reflexes associated with the production of pulmonary oedema (Deshpande et al., 1999; Bagchi and Deshpande, 1998, 1999). In the above studies, sublethal concentration of venom was used whereas, the lethal concentration of venom produced immediate apnoeic and bradycardiac responses elsewhere (Deshpande and Alex, 2000; Pandey and Deshpande, 2004). The immediate apnoeic response is associated with an increase in blood pressure (BP) followed by a progressive decline (Pandey and Deshpande, 2004). These changes occurred within 2 s and the animals died within 30 min of exposure to venom. In these experiments, the decrease in BP was not associated with an increase in HR; rather there was a progressive decrease in HR. This indicates the existence of mechanisms other than those mediated by baroreflexes. Thus, the systemic toxicity produced by venom cannot be fully accounted to the effects of venom on autonomic nervous system or the activation of J-receptors (Pandey and Deshpande, 2004; Deshpande et al., 1999, 2005; Bagchi and Deshpande, 1998, 1999). Hence, additional mechanisms

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for the action of BT venom are speculated. Such mechanisms may be due to the chemicals (5-HT, histamine, bradykinin, peptide toxins, etc.) present in the venom (Chhatwal and Habermann, 1981; Nassar et al., 1992; Galvez et al., 1990; Wudayagiri et al., 2001; Basu et al., 1990; Deshpande et al., 2005). The earlier studies have shown that venom/histamine sensitizes the afferent vagal discharges (Bagchi and Deshpande, 1999; Anand and Paintal, 1988). Such sensitization is also expected from the other chemicals present in the venom, which are either inflammatory mediators or induce inflammation (Evans et al., 2000; Julieus and Basabaum, 2001). In a study elsewhere, the presence of nociceptive agents (capsaicin, anandamide or α,β-MeATP) in the vascular tree evoked cardiorespiratory reflexes (McQueen et al., 1998; Smith and McQueen, 2001). It is thus expected that nociceptive agents, either present in the venom or released after envenomation, may also produce cardiorespiratory changes involving vasosensory afferents as produced by capsaicin/anandamide (Smith and McQueen, 2001). Therefore, this study was planned to evaluate the involvement of vasosensory responses for the BT venom-induced cardiorespiratory alterations. The experimental model was designed in such a way that the venom was injected in a local segment of an artery, so that the vasosensory afferents could be excited precisely and the mechanism could be ascertained.

## 2. Methods

### 2.1. Animals and anaesthesia

All the experiments were performed according to the guidelines given by the ethical committee of the Institute of Medical Sciences, Banaras Hindu University, Varanasi for conducting animal experiments. Healthy male albino rats (Charles–Foster strain; $253 \pm 8.3$ g) were anaesthetized with an intra-peritoneal injection of urethane (1.5 g/kg). A maintenance dose (50–100 mg) of anaesthesia was given as required.

### 2.2. Dissection and recording

Under the anaesthetic effect, tracheal cannulation was done to keep the respiratory tract patent. The femoral artery was dissected and freed from the surrounding tissue. The proximal and distal ends were cannulated with cannulae (P50 size) filled with heparinized saline (20 IU/ml). The proximal cannula was connected to a Stathum Strain Gage pressure transducer (Bio-Devices, Ambala, India) and the distal cannula was utilized for the administration of venom/drug/saline.

The respiratory excursions were recorded by securing the skin over xiphisternum and connecting it to a force displacement transducer via a thread. The electrocardiographic potentials were recorded by using needle electrodes, connected in standard limb lead-II configuration. The BP, respiratory movements and ECG were recorded on a chart recorder (Bio-devices). The mean arterial pressure (MAP) was calculated using the appropriate calibrations. The respiratory rate (RR) and heart rate (HR) were computed by counting the number of deflections in a given strip of recording. The latent period for all these responses was also calculated.

### 2.3. Determination of paw oedema

At the end of experiment, both the hind paws were dissected, skin was cut open and removed. It was then weighed and dried in an electric oven (90 °C) to a constant weight ($>48$ h). The difference in wet weight and dry weight of the paw provided the water content and was expressed as a percentage of wet weight.

### 2.4. Experimental protocol

#### 2.4.1. Determination of concentration–response of BT venom

Animals were allowed to stabilize for 30 min after dissection. Then the initial recordings of BP, ECG and respiration were taken. To ascertain the effect of stretch/volume on vessel wall, 0.1 ml of normal saline was injected in the peripheral segment of femoral artery (i.a.) and the recordings of the cardiorespiratory parameters were made for 15 min at every 5 min intervals. Following this, crude BT venom (0.5–5.0 mg/kg), keeping the volume of injection constant at 0.1 ml, was injected intra-arterially and cardiorespiratory parameters were recorded for 60 min at every 5 min intervals. In a given experiment, the animal was exposed to a single concentration of BT venom. At the end of the experiment, both the hind paws were dissected and were kept in an electric oven for determining water content.

#### 2.4.2. Effect of indomethacin on venom-induced changes

After stabilization, the initial recordings of BP, ECG and respiration were taken. Indomethacin (10 mg/kg, i.a.) or vehicle was injected and the recordings of cardiorespiratory parameters were made for 30 min at every 5 min intervals to see the effect of indomethacin/vehicle alone. Subsequently, crude BT venom (1.0 mg/kg, i.a.) was injected in these animals and the cardiorespiratory parameters were recorded for 60 min at every 5 min intervals.

#### 2.4.3. Effect of normal saline on cardiorespiratory parameters

In a separate series of experiments, normal saline (0.1 ml, i.a.) was injected and the responses (BP, RR and HR) were recorded for 60 min at every 5 min intervals. These animals served as time-matched control group.

### 2.5. Drugs and solutions

Crude BT venom was obtained from Haffkine Institute, Mumbai, India. Stock solutions of BT venom (10 mg/ml) were prepared in distilled water and refrigerated. Subsequent dilutions were made in normal saline at the time of experimentation. Indomethacin was obtained from Sigma Chemical Company, St Louis, MO, USA and was dissolved in absolute alcohol (25 mg/ml). Subsequent dilution of indomethacin was made with normal saline at the time of experimentation. Heparin (1000 IU/ml) was obtained from Biological Evans Ltd, Hyderabad, India and heparinized saline (20 IU/ml) was used to fill the transducer and cannula.

### 2.6. Analysis of data and statistics

The results were presented as mean ± SEM values. The MAP, RR or HR responses before venom were taken as initial responses. The values after the injection of venom at every 5 min up to 60 min were normalized to the initial responses. The statistical analysis for various groups at different intervals was performed using tests mentioned elsewhere (Littell et al., 1998). The analysis of variance (ANOVA) followed by Student–Newman–Keuls test for multiple comparisons were done using Sigmastat software. Student's *t*-test was also used wherever required. A *P*-value $<0.05$ was considered significant.

## 3. Results

Intra-arterial injection of BT venom produced changes in RR, BP and HR and were monitored up to 60 min. Injection of equi-volume of saline (0.1 ml) did not produce any changes in RR, MAP and HR up to 60 min (Figs. 1 and 2) indicating the lack of effect of stretch/volume on vessel wall for the observation time.

### 3.1. Effect of venom on respiratory rate

BT venom produced concentration-dependent changes in RR. There was immediate increase in rate and depth of respiration (within 1.5–2.5 s) after the administration of BT venom (Figs. 1 and 2). The immediate tachypnoeic response was maximal with 1.0 mg/kg of venom with a shortest latency. The increase in RR was followed by a decrease. The decrease was then recovered to initial level (within 15–20 min) and subsequently increased above the initial level. The decrease in RR was maximal with 5.0 mg/kg of venom.

### 3.2. Effect of venom on mean arterial pressure

After administration of BT venom, the MAP began to rise (~10 s) and reached the peak level within 5 min. After reaching the peak, the pressure decreased gradually and remained above the initial level up to 60 min. The increase

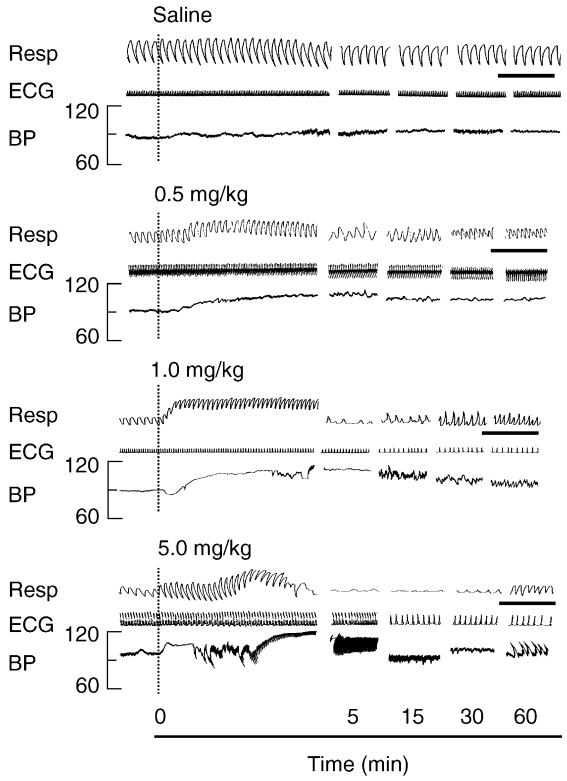


Fig. 1. Actual recordings showing the effect of different concentrations of BT venom on respiration (Resp), ECG and blood pressure (BP) and compared with normal saline. The responses after venom/saline are shown at different time intervals as indicated in the lower panel. The point of injection is indicated by dotted line. The unit for calibration of BP is in mm Hg. The horizontal line in each panel is 5 s for respiration and ECG; and 50 s for BP.

in MAP was significantly greater than the time-matched saline group ($P<0.05$, Two-way ANOVA) or from the initial at 0.5 and 1.0 mg/kg venom ($P<0.05$, One-way ANOVA). The effects on MAP were maximally seen with 1.0 mg/kg of venom (Figs. 1 and 2). However, with 5.0 mg/kg of venom, the fluctuations in BP were greater and were not different from the initial level ($P>0.1$, One-way ANOVA; Fig. 2). The initial basal responses before venom at different concentrations were not significantly different from one another (Fig. 2).

### 3.3. Effect of venom on heart rate

After i.a. injection of venom, there was decrease in HR in a concentration-dependent manner (Figs. 1 and 2). With 0.5 mg/kg, the bradycardiac response was slow to occur and reached maximum at 50 min. In the case of 1.0 mg/kg of venom, the maximal decrease occurred at 30 min while with the 5.0 mg/kg of venom, the decrease was seen within 10 min. The bradycardiac response produced by venom never returned

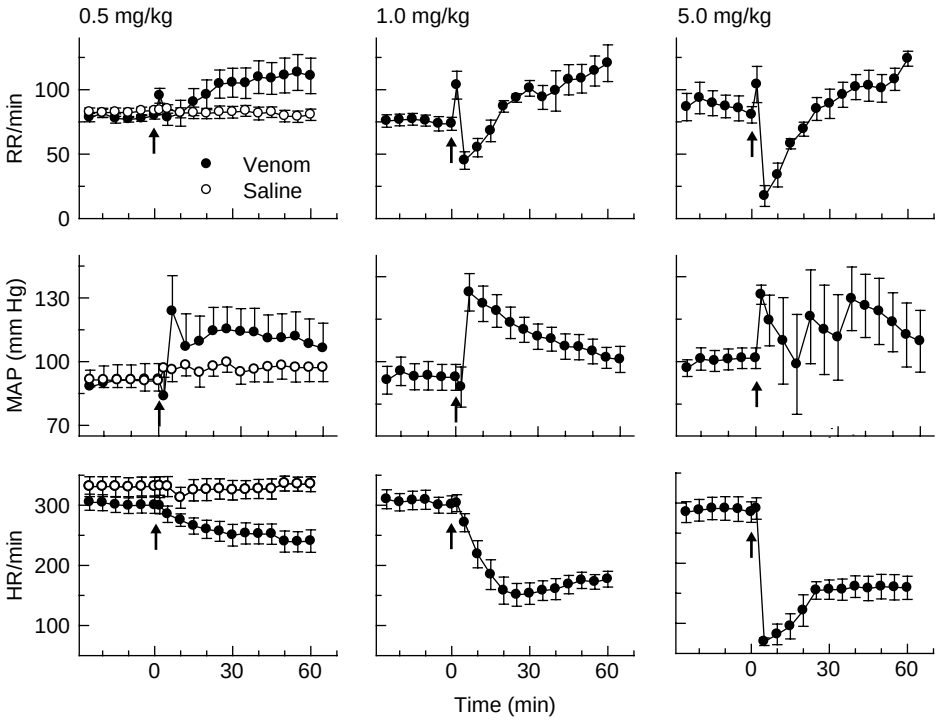


Fig. 2. Time–response relationship of different concentrations of BT venom on respiratory rate (RR), mean arterial pressure (MAP) and heart rate (HR). The time-matched response after saline ($n=6$) are shown in each row at 0.5 mg/kg column. The values are mean ± SEM from 6 to 9 experiments for each concentration. The responses after venom are significantly different from the saline group at all concentrations ($P<0.05$, Two-way ANOVA) except MAP response at 5.0 mg/kg. An arrow indicates the point of injection of venom.

to initial level (Fig. 2). The venom-induced bradycardiac responses at all the concentrations were significantly different from saline group ($P<0.05$, Two-way ANOVA).

In the present study, 50% mortality of animals was observed with 5.0 mg/kg of venom whereas no mortality was observed with 0.5 or 1.0 mg/kg of venom.

### *3.4. Effect of venom on latency of the cardiorespiratory parameters*

The time of onset of response was different for different parameters but it was shortest for tachypnoeic response. The latencies for tachypnoea were 2.7 ± 0.33, 1.5 ± 0.22 and 2.2 ± 0.48 s for 0.5, 1.0 and 5.0 mg/kg of venom, respectively. The hypertensive changes were next to occur and the latencies were 12.7 ± 2.19, 9.3 ± 0.67 and 7.0 ± 0.86 s for 0.5, 1.0 and 5.0 mg/kg of venom, respectively. The bradycardiac changes were last to occur and the values were 850 ± 120, 475 ± 81.4 and 48 ± 5.2 s for the respective concentrations mentioned earlier.

### *3.5. Effect of venom on the water content of hind paw*

The mean ± SEM values of water content in ipsilateral (venom/saline injected) and contralateral (control) hind paw are given in Fig. 3. The water content (% of wet weight) in

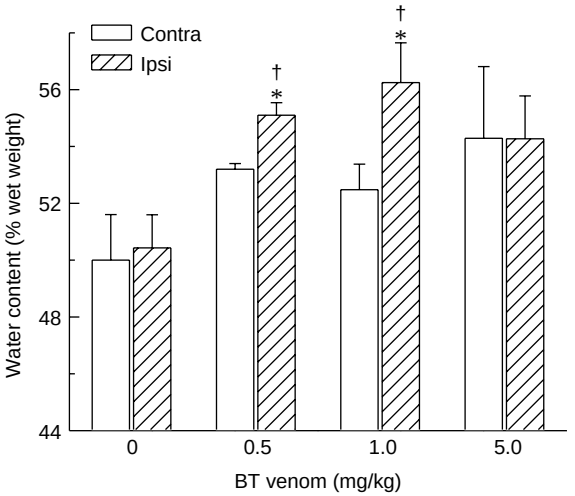


Fig. 3. Histograms showing the water content of ipsilateral (Ipsi) and contralateral (Contra) hind paw after i.a. injection of different concentrations of venom. The values at 0 indicate saline only group. The values are mean ± SEM from 4 to 5 different experiments for each concentration. An asterisk (*) indicates significant difference from contralateral side ($P<0.05$, Student's $t$-test for paired observations) and † indicates $P<0.05$ as compared to the corresponding paw in saline only group.

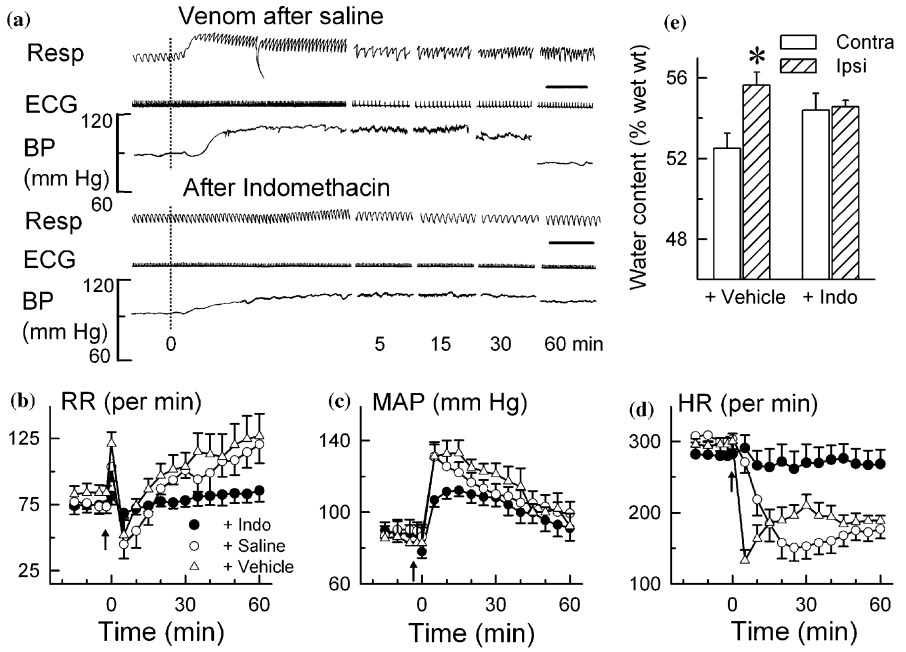


Fig. 4. Indomethacin blocked the venom-induced responses. (a) Actual recordings showing the effect of indomethacin on BT venom (1.0 mg/kg, i.a.)-induced changes on respiration (Resp), ECG and blood pressure (BP) and compared with venom only group. The point of injection of venom is indicated by dotted line. The horizontal line is 5 s for respiration and ECG and 50 s for BP. The time–response relationship of vehicle/indomethacin (+Indo) pretreated animals and venom only group on RR, MAP and HR are shown in (b–d), respectively. The RR and HR responses are significantly different from the vehicle control/venom only group ($P<0.05$, Two-way ANOVA). The MAP responses are significantly different up to 30 min from vehicle control group ($P<0.05$, Student–Newman–Keuls test for multiple comparisons). Histograms in (e) show the water content of ipsilaterlal (Ipsi) and contralateral (Contra) hind paws after exposure to BT venom (1.0 mg/kg) in indomethacin/vehicle-pretreated animals. An asterisk (*) indicates $P<0.05$ as compared to the contralateral side (Student's *t*-test for paired observations).

ipsilateral and contralateral paws of saline-treated animals was similar. But with 0.5 and 1.0 mg/kg of venom, the water content in ipsilateral side was significantly greater than the contralateral side ($P<0.05$, Student's *t*-test for paired observations) as well as the saline control group ($P<0.05$, Student's *t*-test for unpaired observations). This indicates the paw oedema on ipsilateral side. At 5.0 mg/kg of venom, the water content in both the limbs was greater than the saline-treated group but differences between ispilateral and contralateral side were not different from each other ($P>0.9$, Student's *t*-test for paired observations). The magnitude of paw oedema was greatest with 1.0 mg/kg of venom.

The effect of BT venom on all parameters was consistent and greater with 1.0 mg/kg. Hence, this concentration was chosen for subsequent comparisons.

### *3.6. Indomethacin attenuated BT venom-induced alterations*

Pretreatment with indomethacin (10 mg/kg, i.a.)/vehicle *per se* did not change the resting MAP, RR and HR up to 60 min. However, indomethacin blocked the venom (1.0 mg/kg)-induced cardiorespiratory changes. The RR changes in these animals after venom remained more or less at the initial level up to 60 min (Fig. 4). The blocking effect of indomethacin was significantly different from the vehicle control group/venom only group ($P<0.05$, Two-way ANOVA and Student–Newman–Keuls test for point to point comparisons). The hypertensive changes induced by venom were also attenuated significantly in indomethacin-treated animals ($P<0.05$, Two-way ANOVA; Fig. 4). The responses after 30 min were similar to vehicle control group ($P>0.1$, Student–Newman–Keuls test). The bradycardiac changes induced by BT venom were blocked by indomethacin ($P<0.05$, Two-way ANOVA and Student–Newman–Keuls test for multiple comparisons; Fig. 4). In the vehicle control group, the bradycardiac response occurred earlier than the venom only group but the magnitude of response was similar to venom only group (Fig. 4).

The water content in the ipsilateral and contralateral hind paw in indomethacin/vehicle-treated groups after venom is given in Fig. 4. There was no oedema in the ipsilateral hind paw as compared to the contralateral side in indomethacin-treated animals ($P>0.9$, Student's *t*-test for paired observations). However, in vehicle-treated group, the water content was significantly greater on the ipsilateral side ($P<0.05$, Student's *t*-test for paired observations).

## 4. Discussion

The observations of this study demonstrate that BT venom in a peripheral segment of artery evoked cardiorespiratory changes unrelated to the volume/stretch on the vessel wall. The cardiorespiratory changes were seen as immediate hyperventilatory (within 2 s); intermediate hypertensive (after 10 s) and delayed bradycardiac (occurring after 50 s) responses. In addition, local paw oedema was also observed.

In reports elsewhere, the vasosensory reflexes were elicited by injecting the agonists in the common iliac artery at its bifurcation (Smith and McQueen, 2001; McQueen et al., 1998). In the present study, the methodology was further improvised by injecting the venom into the peripheral end of femoral artery so as to restrict the distribution of venom to only one side (ipsilateral). This provides an opportunity to identify the pathophysiological changes occurring in a local segment of a vessel. Further, the blood pressure was recorded from the central end of the same femoral artery, eliminating the consequences of carotid arterial cannulation. This is very critical because the cardiorespiratory reflex centres are located in the pontomedullary region and carotid cannulation in acute conditions may compromise the blood supply to these structures. The time-matched saline control group (Figs. 1 and 2) eliminates the possibility of local ischemic responses modulating the cardiorespiratory reflexes.

The cardiorespiratory responses are unlikely to be due to the systemic effects of venom resulting from its entry into the circulation. This was achieved by keeping the volume of injection to 0.1 ml in all groups. Even with this minimal volume, certain spillage/entry of venom in the circulation may occur. If the responses are due to the spillage-induced systemic toxicity, then maximal response is expected at the highest concentration (5.0 mg/kg) of venom. In our study, the latency of tachypnoeic response was shortest (1.5 s) at 1.0 mg/kg. Similarly, at this concentration, maximal hypertensive response was observed (Fig. 2). The paw oedema was also greatest at this concentration. Since these responses were optimal at 1.0 mg/kg of venom without any mortality, these effects may not be due to the systemic toxicity.

In contrast to lower concentrations, 5.0 mg/kg of venom produced highly fluctuating MAP responses, greater apnoeic response and severe envenomation syndrome with 50% mortality. The greater degree of MAP fluctuations at this concentration of venom indicates the systemic actions of venom as reported elsewhere (Rowan et al., 1992). Even though the water content in ipsilateral paw was not different from contralateral side at this concentration, it was greater than the saline control group (Fig. 3). The increase of water in both sides, though not significant from the saline group, indicates the systemic toxicity. These findings indicate that the responses at 5.0 mg/kg may be due to the systemic toxicity in addition to the local effects. Thus, the effects at lower concentrations (0.5 and 1.0 mg/kg) of venom may not be due to the systemic changes produced by the venom.

HR responses were late to occur with lower concentrations and reached their peak after 30 min. The decrease in HR with these concentrations of venom cannot be correlated with the pressure changes. The peak pressure response was seen at 5 min and at that time the HR changes were minimal at lower concentrations. However, with 5.0 mg/kg, the pressure changes were lesser than 1.0 mg/kg but the decrease in HR was much greater. Thus, the HR changes are independent of pressure response (baroreflex mechanism). In addition, the HR changes cannot be due to the anoxic or hypercapnoeic reflex alterations because under such circumstances, one expects an increase in HR rather than the decrease.

In this study, the observation period was extended up to 60 min after injecting venom in a segment of artery. This enabled us to identify the intermediate and delayed responses evoked by the venom. The responses by and large exhibited a definite pattern in all the parameters with various concentrations (0.5–5.0 mg/kg) of venom except for the intermediate hypertensive response at 5.0 mg/kg. The immediate tachypnoeic response occurred within 2 s and was transient. Such quick response pattern favours the involvement of neural components. While the intermediate hypertensive and delayed bradycardiac responses persisted up to the period of observation (60 min) suggesting the involvement of humoral factors (inflammatory mediators) in addition to the neuronal factors.

The paw oedema observed in this study can result from the direct effect of venom on the capillary endothelium or by the activation of tissue macrophages to release the inflammatory mediators or may be due to the chemicals present in the venom (Chhatwal and Habermann, 1981). In the earlier works from this laboratory, the action of venom is shown to involve kinin, an inflammatory mediator (Bagchi and Deshpande, 1998, 1999; Deshpande et al., 1999, 2005). Kinins are known to mediate their actions by involving PGs (Davis and Perkin, 1994). Further, the prostaglandin synthetase inhibitor prevented the production of pulmonary oedema induced by BT venom (Bagchi and Deshpande, 1998). In the present study, indomethacin blocked the paw oedema produced by venom and also attenuated the cardiorespiratory reflexes (immediate, intermediate and delayed). Thus, the PGs are involved in mediating these actions of venom.

The PGs, notably PGE2, are known to sensitize the peripheral nociceptors (Julieus and Basbaum, 2001) by lowering the firing threshold of nociceptive sensory neurons (Ferreira et al., 1978; Schaible and Schmidt, 1988; Nicol and Cui, 1994; Evans et al., 2000). Further, PGs augment the release of chemicals/transmitters from these nerve terminals and also increase capillary permeability to produce oedema (Julius and Basbaum, 2001). Thus, it is proposed that the venom in the vascular tree at first stimulate nociceptors by its components followed by the production of oedema.

The local tissue oedema in turn excites the nociceptor afferents to produce the delayed reflex changes. It has been shown that even the micro-oedema in the lung parenchyma around the 'J' receptors can sensitize the pulmonary C-fibres (Deshpande et al., 1999; Anand and Paintal, 1988; Paintal, 1973). On the basis of the above mechanism, one can expect that the tissue-oedema may further sensitize the free nerve endings around the blood vessels.

In summary, the venom in a segment of artery evokes immediate tachypnoeic, intermediate hypertensive and delayed bradycardiac responses involving PGs. The immediate responses are suggestive of neural involvement and the intermediate and the delayed responses are mediated via the inflammatory process and oedema. Further experiments are required to identify the afferents and the mediators modulating these vasosensory reflexes.

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