Indian red scorpion venom modulates spontaneous activity of rat right atria through the involvement of cholinergic and adrenergic systems

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The effect of Indian red scorpion (Mesobuthus tamulus concanensis, Pocock; MBT) venom was investigated on isolated rat right atrial preparations. MBT venom (0.001-3.0 µg/ml) exhibited a peculiar concentration-response pattern with respect to rate. The venom concentrations between 0.001-0.01 µg/ml increased the atrial rate (phase I), followed by a relative decrease with 0.03-0.3 µg/ml (phase II), and then an abrupt increase with 0.6-3.0 µg/ml (phase III). On the other hand, the force was unaltered by venom at phases I and II, while an increase was seen at phase III (3.0 µg/ml). Propranolol (0.1 µM) completely blocked the cardiostimulant action of venom at phase III. Further, this stimulant action of venom was absent in atria obtained from reserpinized animals. Pretreatment with atropine (0.3 µM), produced tachycardia at concentrations 0.1-0.3 µg/ml of venom. But, hexamethonium (30 µM) had no influence on the venom (0.1 µg/ml)-induced alterations in rate. However, MBT venom increased the acetylcholinesterase (AChE) activity (2-3 fold) in a concentration-dependent manner. Tetrodotoxin (2 µM), did not block the increase in rate produced by 0.01 µg/ml of venom. Results suggest that, MBT venom-induced alterations of cardiac rhythm are mediated through cholinergic as well as adrenergic mechanisms depending upon the concentrations. The modulation of atrial rate at very low concentrations may be due to the direct action of venom on the atrium.

Indian red scorpions (Mesobuthus tamulus concanensis, Pocock; MBT) pose a serious health hazard with a high mortality rate in children1-3. The most common abnormalities encountered after envenomation are acute myocarditis, dysrhythmias, several degrees of heart block, hemodynamic changes and pulmonary edema4-6. These cardio-vascular changes are explained on the basis of increased adrenergic5,7,9 or cholinergic activity5,10 or due to the alterations in endocrine functions11,12 or even due to the sensitization of cardio-pulmonary reflexes12,13.

Reasons for the manifestation of a particular type of dysrhythmia after envenomation are still not known. Further, earlier reports do not isolate the independent action of venom on heart, as tachycardia/bradycardia can also be produced by several factors operating on the heart in vivo, such as, various cardio-respiratory reflexes, hormonal influences, effect of temperature, etc. Therefore to isolate these factors, an in vitro study has been undertaken to examine the effect of venom on the rhythmic activity of spontaneously beating right atrium, in terms of rate and force. Further, involvement of cholinergic/adrenergic systems in the venom-induced alterations has also been explored.

Materials and Methods

Recording of right atrial potentials—Experiments were done on adult male albino rats (Charles Foster strain) weighing 125-250 g. Animals were sacrificed by cervical dislocation and exsanguination. Thorax was opened, the heart was carefully dissected out and placed in a petri dish containing Kreb's Ringer at 4°C, bubbled with 100% O2. The right atrium was separated out and fixed to a sylgard bottomed organ bath (volume = 5 ml), containing the physiological solution at 28°C±1°C and was bubbled continuously with 100% O2. The potentials were amplified and recorded either on a chart recorder or on a computer with the help of an AD converter.

Recording of right atrial force—In selected experiments, contractions of spontaneously beating right atrium were recorded. The atrium was dissected out as described earlier and mounted vertically by securing one end to a glass tube placed in an organ bath (volume = 25 ml) containing Kreb's Ringer kept at 28°C±1°C and bubbled continuously with 100% O2.
The other end of the atrium was fastened firmly by a fine thread to a force displacement transducer. The physiological solution was changed at 15 min intervals unless otherwise mentioned. The right atrial preparation was kept under an initial resting tension of 0.1 g and was allowed to equilibrate for 30 min before making the control recordings. The isometric contractions were recorded on a chart recorder.

Experimental protocol—After exposing the atrium to a concentration of venom, the potentials were recorded at every 5 min intervals up to 30 min. A steady response was observed at 20 min and hence the responses of the corresponding time were normalized with the initial rate (control) for comparison purposes. The concentrations of venom used in the present study ranged from 0.001-3.0 μg/ml. In preliminary experiments, a lack of response to a subsequent higher concentration of venom after the first exposure was observed, therefore, only a single concentration of venom was used in a given experiment.

In a separate series, the concentration-response of venom was performed in atria pretreated (10-15 min; before) with atropine (0.3 μM) or propranolol (0.1 μM). Pretreatment experiments (10-15 min) with hexamethonium (30 μM) or tetrodotoxin (2 μM) were performed with either 0.1 or 0.01 μg/ml of venom, respectively.

In another set of experiments, animals were injected with reserpine (4 mg/kg; ip) 24 hr prior to the experiment. On the day of experimentation, animals were sacrificed and the atria were separated out for recording the atrial potentials as described earlier. Then, they were exposed to 3.0 μg/ml of venom.

The atrial force was recorded in selected concentrations of venom viz. 0.01, 0.1 and 3.0 μg/ml, representing each phase of the concentration-response curve. The amplitude of contraction recorded at 20 min was normalized with that of the control (initial). Tetrodotoxin (2 μM), atropine (0.3 μM) and propranolol (0.1 μM) were also added to find out their effect on force at 0.01, 0.1 and 3.0 μg/ml of venom, respectively.

Estimation of acetylcholinesterase (AChE) activity—AChE activity was measured by the spectrophotometric method described elsewhere. Briefly, the right atrium was dissected out and rinsed several times with cold physiological solution and homogenized with 2 ml phosphate buffer (pH 8). The homogenate was centrifuged and the AChE activity (control) was assayed at 412 nm, in both the supernatant and particulate fractions. The total activity (supernatant + particulate) is represented in absolute units and one unit of activity is defined as the amount of substrate (in micromoles) hydrolysed per ml per min. Specific activity of the enzyme was obtained as units of activity/mg of protein.

To obtain the AChE activity in venom treated group, the atria were incubated for 20 min with different concentrations of venom in Kreb's Ringer and the AChE activity was assayed as described before.

Drugs and solutions—Kreb's Ringer solution had the following composition (mM): NaCl, 137; KCl, 2.68; CaCl2, 1.8; MgCl2, 6H2O, 0.88; NaH2PO4, 2H2O, 0.36; NaHCO3, 7 and glucose 11. Crude MBT venom was obtained from Haffkine's Institute, Bombay, India. Atropine sulfate, propranolol, reserpine, hexamethonium bromide, and tetrodotoxin were obtained from Sigma Chemical Company, St.Louis, MO, USA. Reserpine was dissolved in 0.1 N glacial acetic acid. Stock solutions of all other drugs were prepared in distilled water and final dilutions were made in Kreb's Ringer.

Analysis of data—All the values are presented as means ±SE. Differences between various groups were compared by using one way/two way analysis of variance (ANOVA). Student's t test or Student-Newman-Keuls test were also done, as required. A P value < 0.05 was considered significant.

Results

Effect of venom on atrial rate and force of contraction—MBT venom at different concentrations (0.001-3.0 μg/ml) produced a peculiar response pattern regarding the atrial rate. At lower concentrations (0.001-0.01 μg/ml; phase I), an increase in atrial rate was observed. At a concentration as low as 0.001 μg/ml, the venom increased the atrial rate by 12% and at 0.01 μg/ml, there was about 40% increase in rate from the initial value (Fig. 1). In the intermediate concentrations of venom (0.03-0.3 μg/ml; phase II), the rate was found to be significantly lesser than that observed at 0.01 μg/ml (P < 0.05; Fig. 1). In higher concentrations of venom (> 0.3 μg/ml, phase III), there was greater increase in atrial rate (Fig. 1).
The atrial force remained unaltered in phases I and II (Fig. 2). In phase I, the force of contraction after venom (0.01 µg/ml) was 96±4% of the initial and in phase II, it was 108±10% of the initial. However, in phase III (3.0 µg/ml), a greater increase in atrial force (160±18%) was observed, similar to that of rate (Figs 2 and 1).

_Tetrodotoxin did not block the venom response at lower concentrations—_The lower concentrations (0.01 µg/ml) of venom, produced an increase in atrial rate (123±8% of the initial) without changing the force of contraction. The venom-induced increase in rate observed at this concentration was not altered in presence of tetrodotoxin (2 µM; 125±11% of the rate after TTX; n=4). However, tetrodotoxin by itself reduced the rate and force by 30% of the initial (P < 0.05; unpaired t test).

_Atrazine potentiated the atrial rate at intermediate concentrations of venom—_Atrazine (0.3 µM), potentiated the venom-induced increase in atrial rate at concentrations between 0.1-0.3 µg/ml. The increase in atrial rate at these concentrations was significantly higher than the venom alone group (P < 0.001; two way ANOVA, Student-Newman-Keuls test; Fig. 3). But, at higher concentrations (≥ 0.6 µg/ml) the rate was similar to that of the venom alone group (Fig. 3).

However, atropine could not potentiate the force of contraction significantly, although, a 20% increase in force was seen at 0.1 µg/ml of venom (P > 0.3; paired t test; Fig. 2).

_Lack of effect of hexamethonium on venom action—_Hexamethonium (30 µM) was used to block the nicotinic ACh receptors present in the atria.
Venom (0.1 μg/ml), in presence of hexamethonium increased the rate by 11±6%, which is not significantly different from the initial rate (P > 0.9; paired t test) or from the venom alone response (P > 0.9; unpaired t test). However, hexamethonium alone increased the rate by 10±4% (P > 0.9; paired t test).

Propranolol blocked the MBT-venom response at higher concentrations—Since, the venom-induced increase in atrial rate at higher concentrations was not influenced by the cholinergic agents, involvement of β-adrenoceptors at these concentrations was investigated. After pretreatment with propranolol (0.1 μM for 15 min), the concentration-response of venom upto 0.1 μg/ml remained unaltered as compared to that of venom alone group (Fig. 4). But at higher concentrations (1 and 3 μg/ml), the venom-nauced increase in rate and force of contraction were blocked completely by propranolol (Figs 4 and 2; P < 0.05, t test).

Reserpination blocked the cardio-stimulant action of venom at higher concentrations—The control atrial rate in the reserpine treated group was 110±7 beats per min (n = 5) and was not different from the untreated group (105±17 beats per min; n = 6). The mean atrial rate in response to venom (3.0 μg/ml) in the untreated group was 129±10%, whereas in the reserpine treated group it was 93±9% of the initial. The values in the reserpine treated group were significantly less than the untreated group (P < 0.05, unpaired t test).

Effect of venom on atrial acetylcholinesterase activity—The results are shown in Table 1. The AChE activity of the atrial tissue without venom (control) was 0.12 units, and it increased with increasing concentrations of venom. The AChE activity in the venom treated groups was significantly higher than the control group (P < 0.05, unpaired t test).
activity in the stock solution of crude MBT venom (10 μg/ml) was only 0.01 units.

**Discussion**

The observations of this study indicate that the MBT venom stimulates both parasympathetic and sympathetic systems present in the right atrium. Further, the present data demonstrate that the MBT venom has a triphasic action on atrial rate and a biphasic action on atrial force, depending on the concentrations. The lower concentrations (0.001-0.01 μg/ml) of venom have a direct action, intermediate concentrations (0.03-0.3 μg/ml) act through cholinergic mechanisms, while higher concentrations (>0.3 μg/ml) involve the adrenergic system.

The involvement of autonomic storm in envenomed animals has been reported and such autonomic overactivity will be influenced by factors such as, basal vagal/sympathetic tone, hemodynamic status of the animal, cardiac and pulmonary factors, etc. On the contrary, in isolated preparations as in this study, the atrial tissue is isolated from all external innervations keeping the intrinsic plexuses intact. The present results show the effect of activation of these neuronal plexuses by venom at different concentrations. Our results, do not dispute the autonomic storm produced by the venom, but reveal that the venom activates the muscarinic receptors at lower concentrations and adrenergic receptors at higher concentrations in *in vitro* conditions.

Although, tachycardia or bradycardia have been reported after scorpion envenomation, the reasons for the appearance of a particular type of dysrhythmia are not known. Full concentration-response pattern of this study provides clues for such manifestations. The occurrence of bradycardia or tachycardia depends on the amount of venom injected per sting, which in turn depends on the age and moulting stage of the scorpion. Further, the toxic effect of the venom also depends on the age and body weight of the victims.

The earlier reports on the effects of MBT venom on heart were carried out using a single higher concentration of venom, and the concentration-response pattern was not identified. In this study, a triphasic action of venom on atrial rate was observed depending upon the concentrations used (Fig. 1). An initial increase in rate at the lower concentrations (phase I), followed by a relative decrease at intermediate concentrations (phase II), and a greater increase in rate at higher concentrations (phase III) were identified. However, the force of contraction remained unaltered at phase I and phase II concentrations. But in phase III, the force was increased similar to that of the rate (Figs 2 and 1).

The increase in atrial rate seen at phase I, was not sensitive to atropine or propranolol (*P > 0.52; unpaired t test*). Thus, it may be mediated through mechanisms independent of cholinergic and adrenergic systems. Moreover, the increase in rate seen at lower concentrations is not accompanied by an increase in force, points to the possibility of the direct activation of the pacemaker cells. This is further supported by the lack of effect of tetrodotoxin (TTX) on the venom-induced changes in rate and force at lower concentration.

Atropine (0.3 μM) increased the atrial rate and force of contraction at phase II, but had no effect at phase III (Fig. 2). This indicates that the phase II response is due to the greater stimulation of cholinergic nerve terminals by venom, producing relative bradycardia. Unlike the reports with other scorpions, a clear cut bradycardia was not observed. However, the potentiation of phase II response by atropine after venom points to the involvement of muscarinic receptors and the activation of cholinergic neurons as reported elsewhere.

The lack of response to hexamethonium indicates that the cholinergic action of venom is not mediated through the pre-ganglionic plexuses present in the atrium. Therefore, it is probable that the action of phase II concentrations of MBT venom is mediated through the post-ganglionic cholinergic mechanisms. Further, the increased AChE activity observed in this study rules out the possibility of lesser destruction of ACh and its effects. Increase in AChE activity is not

### Table 1—MBT venom increased the AChE activity in a concentration-dependent manner

<table>
<thead>
<tr>
<th>MBT venom (μg/ml)</th>
<th>Specific activity (units/mg of protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (control)</td>
<td>0.12±0.005</td>
</tr>
<tr>
<td>0.001</td>
<td>0.21±0.003*</td>
</tr>
<tr>
<td>0.010</td>
<td>0.24±0.003*</td>
</tr>
<tr>
<td>0.100</td>
<td>0.31±0.007*</td>
</tr>
<tr>
<td>3.000</td>
<td>0.34±0.006*</td>
</tr>
</tbody>
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* *P < 0.05, as compared with control, Student's unpaired t test. The concentration-response relation of AChE activity were also significantly different (one way ANOVA).*
due to the presence of AChE in scorpion venom, as the total AChE activity in the stock solution (10 μg/ml) of crude MBT venom was negligible (0.01 units).

Increased atrial rate and force at higher concentrations (phase III) were blocked by propranolol, while atropine had no effect (Figs 3 and 4). Thus, it can be inferred that the higher concentrations of venom act through β-adrenergic receptors. The experiments with reserpinized animals have further confirmed the involvement of the adrenergic system in the phase III cardio-stimulatory action of venom. Similar observations were reported with Tityus serrulatus venom, but at very high concentrations.23,24

In conclusion, the present observations indicate that MBT venom has both direct and indirect (plexus mediated) actions on the atrium depending on the concentrations. In lower concentrations, the venom acts directly on the membrane and concentrations higher than that, activates either cholinergic or adrenergic mechanisms, producing bradycardia or tachycardia, respectively. However, the role of increased atrial AChE activity in venom action requires further investigation.

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References
19 Vatanpour H, Rowan E G & Harvey A L, Toxicon, 31 1619.
21 Ismail M, Osman O H & El-Asmar M F, Toxicon. 11 (1973) 15.