Investigations of Cardiac Sarcolemmal ATPase Activity in Rabbits with Acute Myocarditis Produced by Scorpion Venom (Buthus Tamulus)

Kari Radha Krishna Murthy, M.B., B.S., M.D.

SUMMARY
Acute myocarditis is produced in rabbits with scorpion (Buthus tamulus) (a common scorpion found in South India) venom. Acute myocarditis is confirmed by changes in the ECG taken before and after venom injection. The atrial and ventricular sarcolemmal Na⁺–K⁺ ATPase, Mg⁺⁺ ATPase, and Ca⁺⁺ ATPase activities are assayed in control and venom injected rabbits. Atrial and ventricular sarcolemmal ATPase activities are similar in control animals. A significant reduction in atrial Ca⁺⁺ ATPase activity is seen in venom treated rabbits. Animals injected with 2 mg/Kg venom exhibited significant increases in Mg⁺⁺ ATPase and Ca⁺⁺ ATPase activities in the ventricular sarcolemma. However, significant reductions in Na⁺–K⁺ ATPase and Ca⁺⁺ ATPase activities are observed in ventricular tissue from rabbits treated with 4 mg/Kg of venom.

Additional Indexing Words:
Na⁺–K⁺ ATPase Mg⁺⁺ ATPase Ca⁺⁺ ATPase Heart membranes Scorpion venom

CARDIAC sarcolemma plays an important role in maintaining appropriate intracellular levels of sodium, potassium, magnesium, and calcium ions and thereby participates in contraction and relaxation of the myocardium.¹ This is by virtue of the presence of Na⁺–K⁺ ATPase, Mg⁺⁺ ATPase, and Ca⁺⁺ ATPase enzymes located on the sarcolemma.² Scorpion (Buthus tamulus) stings in South India are known to produce many cardiovascular effects with acute myocarditis, leading to death.³,⁴ The exact mechanism behind the production of acute myocarditis is not known.⁵ Myocardial contraction is due to cellular depolarization and Ca⁺⁺ plays an important role in
excitation contraction coupling. Subcellular Ca\textsuperscript{++} transport in different areas of the dog heart has been reported recently. However, there have been no investigations showing the effects of scorpion venom on sarcolemmal ATPase enzymes of cardiac muscle. The present study correlates the alterations in cardiac sarcolemmal ATPase activity with ECG abnormalities in rabbits with acute myocarditis, produced by injection of different doses of scorpion venom (Buthus tamulus). A preliminary report of the part of this study was presented earlier.

**Materials and Methods**

Rabbits (1–1.5 Kg) were anesthetized with pentobarbitone sodium (30 mg/Kg I.V.) before induction of myocarditis. In one group of rabbits scorpion venom was injected intramuscularly in a dose of 2 mg/Kg; another group received 4 mg/Kg. Control animals received injections of normal saline. The standard limb lead II ECG was recorded before and within 2–4 hours after venom injection. Control and experimental animals were deprived of food overnight prior to being sacrificed. Sarcolemmal ATPase activities were assayed 12–16 hours after venom injection.

*Isolation of the cardiac sarcolemmal fraction and assay of ATPase activities:*

The rabbits were sacrificed by cervical dislocation. The hearts were removed quickly and placed in ice-cold tris-HCl (10 mM, pH 7.4), in the presence of either 2 mM dithriothreitol (DTT) or 0.2 mM 2 mercaptoethanol (2 MER). Atria and ventricles were separated at the coronary sulcus. Cardiac sarcolemma from atria and ventricles was isolated from control and experimental animals simultaneously under identical conditions. The procedure will be described briefly; a detailed description was published by Tomlinson et al. After homogenisation, the cardiac tissue was centrifuged at 2,500×g for 30 min (Janetzki K24 cold centrifuge). The sediment was suspended with stirring for 10 min in tris-HCl (10 mM, pH 7.4 to 8.0) and centrifuged a second time at 2,500×g for 30 min. The hypotonic treatment was repeated 5 times. The residue was then treated with 0.4 M lithium bromide (LiBr) for 45 min and centrifuged as before. After washing, the pellet was treated with 0.6 M potassium chloride (KCl) for 15 min. After centrifugation and repeated washings, the sediment was suspended in 1 mM tris-HCl (pH 7.4) and was used immediately for assays of sarcolemmal ATPase activity. The complete isolation procedure was carried out at or below 4°C. Aliquots containing 70–150 µg protein were used for assays of Na\textsuperscript{+}–K\textsuperscript{+} ATPase, Mg\textsuperscript{++} ATPase, and Ca\textsuperscript{++} ATPase, with 4 mM tris-ATP as substrate. The inorganic phosphate liberated due to enzymatic action was measured according
to Fiske & SubbaRow. The sarcolemmal protein was estimated according to Lowry et al. All the assays were performed in triplicate. The results were analysed statistically with Student’s ‘t’ test.

Results

After venom injection, the animals exhibited pain (in unanesthetized rabbits), general shock, lacrimation, discharge from the nose and mouth, and increased bowel movements, resulting in defecation and frequency of micturition. The ECG changes in animals treated with 2 and 4 mg/Kg venom are shown in Figs. 1 and 2. The Na⁺–K⁺ ATPase, Mg⁺⁺ ATPase, and Ca⁺⁺ ATPase activities in atria and ventricles from control animals are shown in Tables I and II.

The atrial sarcolemmal ATPase activities in experimental animals treated with 2 and 4 mg/Kg venom were similar. These values are compared with sarcolemmal enzyme activities from control animals in Table I. All the ATPase activities were reduced, and a significant reduction in Ca⁺⁺ ATPase activity was seen in experimental animals.

Ventricular sarcolemmal ATPase activities from rabbits injected with 2 mg/Kg venom were compared with values from control animals (Table II). The Mg⁺⁺ ATPase and Ca⁺⁺ ATPase activities increased significantly. The results of ventricular sarcolemmal ATPase activities obtained from rabbits treated with 4 mg/Kg venom are also compared with those of control animals in Table II. There was a significant reduction in Na⁺–K⁺ ATPase and Ca⁺⁺ ATPase activities.

Discussion

Acute myocarditis due to a scorpion (Buthus tamulus) sting is very common in South India and it is recognised from non-specific ECG changes

Fig. 1. Standard lead II ECG from a rabbit. The paper speed is 50 mm/sec. From left to right: normal sinus rhythm (before venom) (HR 260/min), sinus tachycardia (HR 335/min), and sinus tachycardia (HR 600/min).
Fig. 2. A: hyperacute injury pattern with ST elevation, B: ventricular tachycardia, C: sinus tachycardia with ST-T changes, D: acute infarction, E: sinus arrest, F: infarction like pattern (paper speed 100 mm/sec), G: infarction (paper speed 50 mm/sec), H: runs of ventricular premature beats, I: multifocal ventricular premature beats.

(Figs. 1, 2). The sarcolemmal preparations from cardiac muscle do not show consistent values for ATPase activities because of differences in the isolation procedures and different species of animals employed.1,2,9,15)-17) The hypotonic shock and LiBr and KCl salt treatments are known to remove myofibrils and earlier workers, using similar methods, demonstrated with electron microscopy that the sarcolemmal fraction is relatively free from other subcellular structures.2,9,16) Further, the purity of the sarcolemmal fraction is indicated by the assay of Na+-K+ ATPase, Mg++ ATPase, and Ca++ ATPase activities from the same membrane preparation.9,17) These results (Tables I, II) agree closely with those reported by Tomlinson et al.9)

Several anatomical and physiological differences exist between atrial and ventricular myocardial cells.7) Atrial cells are small, have neither T tubules
Table I. Effect of Scorpion Venom (Buthus tamulus) on Rabbit Atrial Sarcolemmal ATPase Activities
(µM Phosphorus/mg Protein/hour; Mean±S.E.)

<table>
<thead>
<tr>
<th>ATPase</th>
<th>Control (n 10)</th>
<th>Venom Treated@ (n 12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na⁺—K⁺ ATPase</td>
<td>5.23±0.94</td>
<td>3.68±0.04</td>
</tr>
<tr>
<td>Mg²⁺ ATPase</td>
<td>9.00±1.18</td>
<td>8.85±0.80</td>
</tr>
<tr>
<td>Ca²⁺ ATPase</td>
<td>9.81±0.55</td>
<td>7.22±0.64*</td>
</tr>
</tbody>
</table>

@ Results from 2 and 4 mg/Kg venom treated animals.

n indicates number of animals.

* p<0.05.

Table II. Effect of Scorpion Venom (Buthus tamulus) on Rabbit Ventricular Sarcolemmal ATPase Activities
(µM Phosphorus/mg Protein/hour; Mean±S.E.)

<table>
<thead>
<tr>
<th>ATPase</th>
<th>Control (n 10)</th>
<th>Venom Treated 2 mg/Kg venom (n 6)</th>
<th>Venom Treated 4 mg/Kg venom (n 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na⁺—K⁺ ATPase</td>
<td>5.18±0.69</td>
<td>4.67±0.12*</td>
<td>3.28±0.12*</td>
</tr>
<tr>
<td>Mg²⁺ ATPase</td>
<td>9.60±0.58</td>
<td>22.12±2.11*</td>
<td>10.00±0.82</td>
</tr>
<tr>
<td>Ca²⁺ ATPase</td>
<td>11.23±0.89</td>
<td>20.77±1.49*</td>
<td>8.45±0.35*</td>
</tr>
</tbody>
</table>

n indicates number of animals.

* p<0.05.

nor membrane triads, and generate less contractile force than ventricular muscle. The atria display greater mitochondrial Ca²⁺ transport than the ventricles, whereas Ca²⁺ transport in sarcoplasmic reticulum, which is relatively low in atrial muscle, is reported to be similar in atria and ventricles. In the present study, sarcolemmal ATPase activities are similar in atria and ventricles (Tables I, II).

Intracellular K⁺ was not measured in the present investigation. The ECG changes (Fig. 2) suggest that there is a reduction in intracellular ionic potassium. The relationship between cellular potassium and ECG changes are described by Schamroth. A slight loss of intracellular potassium causes reduction in the resting membrane potential. This results in alterations in the repolarization process in the ventricle, manifested as T wave changes in the ECG. The ST segment changes in the ECG are due to further reductions in the intracellular potassium level and the resting membrane potential. With a 50% loss of intracellular potassium, the myocardial cell is electrically dead, seen as a Q wave in the ECG. The loss of potassium, reflected in the ECG in the present study, is probably due to the decreased Na⁺—K⁺ ATPase activity observed in atria and ventricles of venom treated rabbits (Tables I, II).
Sarcolemma is a dynamic structure, which maintains constant ionic levels of intracellular potassium and extracellular sodium. This is by virtue of Na\(^+\)-K\(^+\) ATPase activity located in the sarcolemma. Activation of Na\(^+\)-K\(^+\) ATPase is dependent upon a definite ratio of Na\(^+\) inside and K\(^+\) outside the cell. It is not surprising, therefore, to find a significant decrease in Na\(^+\)-K\(^+\) ATPase activity in ventricular tissue from 4 mg/Kg venom treated animals, and a mild fall in the enzyme activity from atria and ventricles in rabbits treated with 2 mg/Kg venom (Tables I, II). Reduction in Na\(^+\)-K\(^+\) ATPase activity may result in an increase in intracellular sodium and extracellular potassium, and a loss of intracellular potassium due to a failure of the sodium-potassium pump. This is suggested by ECG changes in rabbits treated with 4 mg/Kg venom (Fig. 2).

The function of sarcolemmal Mg\(^++\) ATPase is unknown. It is suggested that Mg\(^++\) ATPase generates the energy required for maintenance of ionic gradients across the cell membrane. It is also suggested that Mg\(^++\) ATPase maintains intracellular Mg\(^++\). There are reports to suggest that reduced cellular Mg\(^++\) results in cellular loss of potassium and gain in Na\(^+\) resulting in reduction in the force of contraction. Thus, a Mg\(^++\) dependent loss of cellular potassium may result in a reduction in Na\(^+\)-K\(^+\) ATPase activity for which Mg\(^++\) ATP is the substrate. Therefore, the associated alterations in Na\(^+\)-K\(^+\) ATPase activities in atria and ventricles in 2 and 4 mg/Kg venom treated rabbits may be a reflection of altered Mg\(^++\) ATPase activity. However, Mg\(^++\) ATPase activity in 4 mg/Kg venom treated rabbits is not altered, despite electrically dead or necrotic tissue as shown by ECG changes (Fig. 2). Depressed Na\(^+\)-K\(^+\) ATPase activity without any changes in Mg\(^++\) ATPase activity was demonstrated in left ventricular hypertrophy and failure in rabbits with aortic constriction.

The function of Ca\(^++\) ATPase is perhaps to regulate the efflux or influx of Ca\(^++\) through the sarcolemma. Dhalla et al are of the opinion that Ca\(^++\) ATPase activity is involved in the entry of Ca\(^++\) into the cell, thereby participating in excitation-contraction coupling. A significant reduction in Ca\(^++\) ATPase activity in atria and ventricular tissue from 4 mg/Kg venom treated rabbits and a significant increase in Ca\(^++\) ATPase activity in ventricular tissue from 2 mg/Kg venom treated animals (Tables I, II) suggest that these animals suffer from intracellular deficits and an overloading of calcium ions, respectively. Alterations in Ca\(^++\) ATPase activity from the sarcolemma were shown in different types of heart failure.

It is suggested that acute myocarditis due to scorpion venom (Buthus tamulus) causes definite sarcolemmal defects. These are reflected in alterations in cardiac sarcolemmal ATPase activities. These sarcolemmal defects
may be responsible for the subsequent pathological conditions.

ACKNOWLEDGMENTS

The author thanks the Dean, L.T.M. Medical College, Sion, Bombay, for providing facilities to conduct the experiments. The author also thanks Dr. Dabholkar N. A., Dr. Billimoria F. R., and Dr. Gore A, for their suggestions.

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