EFFECT OF MONO-VALENT ANIONS ON ACTIVE, PASSIVE TENSIONS AND TENSION EQUILIBRIUM LENGTH OF GASTROCNEMII OF UROMASTIX HARDWICKII

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Background: The length tension relationship has been used to determine the contractile and elastic state of the muscle. However, the shape of the active and passive tensions has been found to vary from muscle to muscle and in different animals as well. It depends upon the muscle architecture and specific function it performs. The change in the state of a skeletal muscle produced under the influence of chemical agents is not evaluated for the parameters obtained from the length tension relation. **Methods:** In the present study an attempt has been made to observe the effects of mono-valent anion on the contractile characteristics of isolated Gastrocnemius muscles of Uromastix. **Results:** The results demonstrated that both the active and passive tensions changed on treatment with mono-valent anions with a shift in their curves. This change was statistically significant for active tensions. Further, Tension equilibrium length (TEL) also affected significantly. **Conclusion:** It is concluded that length tension parameter, TEL < resting length (Lo) is also a useful indicator of muscle state representing dominant elasticity under the influence of mono-valent anions. It can be used to express the state of different contractile and elastic characters of the skeletal muscle.

Key Words: Monovalent ions**,** Active & passive tensions, Tension Equilibrium length, Resting Length, Uromastix, gastrocnemius muscle.

INTRODUCTION

Length tension relationship provides interpretation of sliding filament theory, tested by measuring the tension of muscle fiber at different sarcomere length.[1] In addition, length-tension relationship of whole skeletal muscle has been used for the explanation of its contractile and elastic state by the measurements of parameters like, active tension, passive tension, resting length.

However, these parameters are not evaluated with respect to a change in the contractile and elastic state of muscle in different physiological conditions or under the influence of various chemical agents. The physiological effects of mono-valent anions on skeletal muscle have been extensively reported earlier. It has been reported[2] that mono-valent anions potentiate twitch tension. It is evident that the cause of twitch potentiation is the specific action of each anion, which may enter the sarcoplasm and act at the internal membrane. It was suggested by[3] that nitrate cause potentiation by increasing the capacity of membrane depolarization to release calcium from bound sites (sarcoplasmic reticulum) and thus facilitate the excitation-contraction coupling. It is expected that such anions may also affect the shape of the length tension curve due to a change in the active and passive tension generation ability of the treated muscles. Further, the change that is expected to produce in the L-T parameters may be referred to a particular state of the muscle, which can be used as an indicator of that muscle state.

In the present study, the active and passive tension along with the muscle length at which both the active and passive tensions are equal, i.e., the Tension equilibrium length (TEL), has been determined from the length-tension relation according to[4] from the muscles treated with normal reptilian buffer solution and a buffer solution having chloride replaced with mono-valent anions.

MATERIAL AND METHODS

Both the sexes of adult reptile Uromastix *hardwickii* (100-150gm) were used in all the experiments reported here.

The reptilian buffer solution[5] used in this study was having the following composition: NaCl, 100mM; KCl, 3.8mM; $CaCl_2$, 1.8mM; KH_2PO_4, 1.2mM; Na_2HPO_4, 5.8mM. The Mono-valent anion buffer solution was prepared having the following composition, in which Chloride was replaced by mono-valent anions. NaI, 100mM; KI, 3.8mM; $Ca(No_3)$, 1.8mM; KH_2PO_4, 1.2mM; Na_2HPO_4, 5.8mM. The experimental muscles used for mono-valent anion treatment were soaked in this solution for 35 minutes before recordings.

The animals were killed by decapitation that conforms the ethical standard. Then, gastrocnemii of both limbs were dissected out immediately. These gastrocnemius muscles were dissected out along with knee joint and Achilles tendon and immediately transferred into muscle chamber containing temperature (20^0C) regulated reptilian buffer.

The circulator thermostatic bath model RMT6 (Cat No: 232-030) was used for temperature regulation of muscle bath.

The proximal end of the isolated muscle having knee joint was fixed in the pin provided in the muscle bath. Its distal end having Achilles tendon was fixed in a hook and thread passing through a pulley for attachment with Isometric Force Transducer (Cat-No 50-7913). For the stimulation of muscle, a pair of stimulating electrode was placed beneath the muscle. The stimulating electrode was connected to D.C stimulator (Harvard Cat No: 50-7459) (50v, 0.5mS, 1Hz).

The recording of twitch tensions at various muscle lengths from gastrocnemii placed in normal reptilian buffer and reptilian buffer solution (chloride replaced with mono valent an-ions) was obtained and the isometric active and passive tensions were calculated.

For experimental purpose, the muscle length was increased from flaccid to fully stretched length stepwise (1mm each) by using macro-manipulator. During this stretching, muscle was stimulated (50V, 5msec, 1pulse/sec) at each step. Pen deflection was measured and calculated as passive tension (Kg/cm^2). While, the pen deflection obtained on twitch stimulation was measured and calculated as active tension (Kg/cm^2). Later, the values of calculated active and passive tensions were plotted against muscle length to obtain the length-tension curve.

Individual length-tension curves were then used to calculate the tension equilibrium length (TEL) according to[4] at which the active and passive tensions were equal.

The data was analyzed by standard statistical method. In addition, student's T-test was performed for the comparison of two sets of data with the determination of significance level at 0.05

RESULTS

The average value of maximum active tension as shown in Fig.1 was found to decrease after treatment with mono-valent anion solution being 48% lesser than its average value obtained in normal solution. This difference was statistically significant ($P<0.005$). In addition, the passive tension was higher after Mono-valent anion treatment being 51% than its average value obtained in normal solution (Fig.1). This difference was however statistically non-significant ($P>0.05$). The average value of tension equilibrium length (TEL) presented in Fig.2 has been found to reduce significantly ($P<0.05$) in mono-valent anion solution when compared with its average value obtained in normal solution.

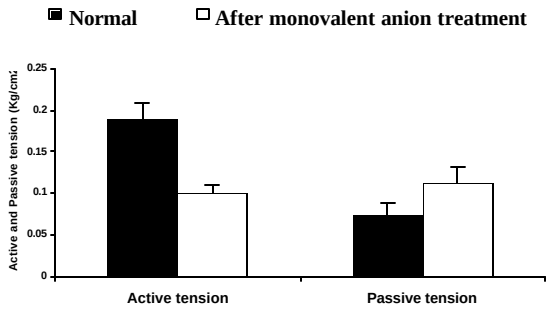

Fig-1. Effect of Monovalent anions on Active & Passive Tensions (Uromastix Gastrocnemii)

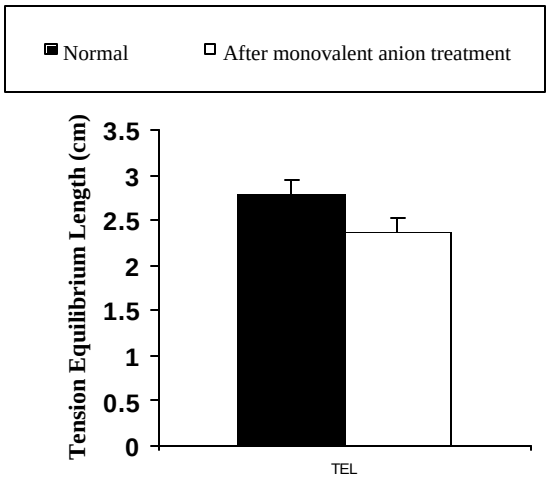

Fig- 2. Effect of Mono-valent anions on Tension Equilibrium Length (Uromastix Gastrocnemii)

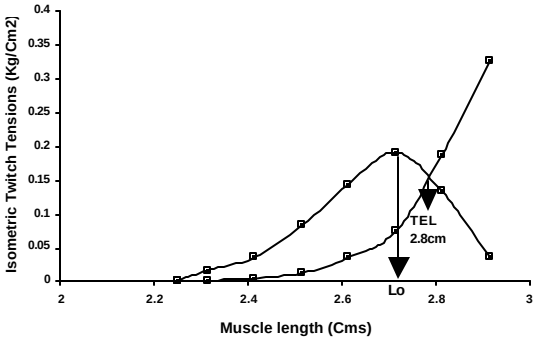

Fig. 3a. Length Tension curve Uromastix Gastrocnemii Normal reptilian buffer Solution

DISCUSSION

In the present study, a significant fall was observed in the average value of maximum active tension after 35 minutes of treatment with mono-valent anion (Fig.1). In our opinion, a protracted contracture[6] has probably resulted in our experiments that has probably

decreased the active tension. The an-ion treatment had been reported to enhances the availability of calcium[7, 8] for cross bridge interaction. According to[9] mono-valent anion affect calcium-pumping ability of skeletal muscle. These anions also significantly depress the active binding of calcium in the sarcoplasmic reticulum by effectively replacing calcium with magnesium at binding sites. Thus, elevates the sarcoplasmic calcium levels and enhancing mechanical output of skeletal muscle. However, when muscle was soaked in Mono-valent anion solution for 35 minutes, a massive release of calcium overloads the muscle. According to[10], calcium overload may cause development of contracture, which is the state of sustained excess contraction. In the present study it is suggested that the same contracture has reduced the maximum active tension significantly in gastrocnemius muscle after mono-valent anion treatment (Fig.1). It has also resulted in a shift of active tension curve in length-tension relation shown in Fig 3a & b.

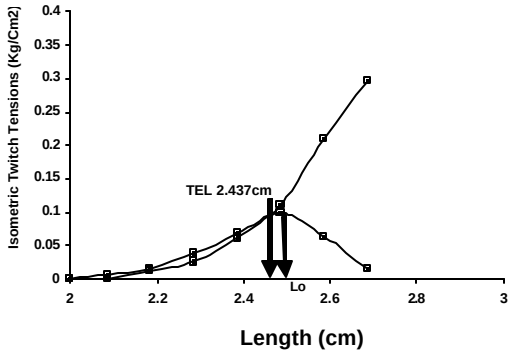

Fig-3b. Length Tension Curve Uromastix gastrocnemii Monoalent anions solution

Mono-valent anion treatment for 35 minutes also increased the passive tension non-significantly (P>0.05) in our experiments as shown in (Fig. 1). This rise in passive tension may be due to swelling phenomenon or due to disrupted sarcomeres [11], because when the muscle was soaked in mono-valent anion solution, the rigidity and swelling was observed visually in the present study, after 35 minutes that was mainly responsible to increase passive tension at resting length. It has also resulted in a shift of Passive tension curve in length-tension relation shown in Fig 3a & b.

A significant fall in the average value of tension equilibrium length was also observed in gastrocnemius muscle after 35 minutes treatment with mono-valent anion (Fig. 2). According to[4] a change in the tension equilibrium length reflects a concomitant change in contractile and elastic behaviour of experimental muscle. In the present study, when muscle was soaked in mono-valent anion solution for 35 minutes, contracture was responsible to decrease maximum active tension significantly (Fig. 1) and on the other hand swelling was responsible to increase passive tension (Fig. 1). Therefore, in the present study elastic elements are dominated over contractile component (Fig. 3a & b). Hence, changes in both the contractile and elastic behaviour have resulted in a significant decrease in tension equilibrium length (Fig.2). This change represents a dominant elastic state of muscle, i.e., tension equilibrium length falling ahead of resting length (TEL < I_o) as shown in Fig. 3b conforming the proposal given by[4]. It is therefore concluded that Tension equilibrium length is a parameter obtained from length-tension relation can be used an indicator of muscle's contractile and elastic states.

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