

Adrenaline improves endurance of rabbit gastrocnemius: A study with continuous high frequency stimulation

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Abstract: Beta adrenoceptor agonists are well known for their potentiating effects on peak twitch and tetanic tension and defatiguing effects on skeletal muscles. Adrenaline (ADR) is one of these agonist which is known for inotropism but less described for fatigue. In addition, studies on high frequency stimulation (HFS) of skeletal muscles are scarce and not available for tetanization fatigue related with endurance and recovery under the influence of ADR. We hypothesized that ADR can maintain peak tetanic tension (PTT) produced by mammalian skeletal muscles for longer period as well as help in recovery from fatigue on continuous HFS. Gastrocnemius muscles (medial Belly) from both limbs were isolated from Rabbits (*Oryctolagus cuniculus*) and continuously stimulated at High frequency of 80Hz for 20Sec. Tetanic tensions were recorded digitally with the measurement of PTT at different time points during this stimulation. Time (T_{50}) was also noted at which muscle force was reduced to 50%. At 20Sec of continuous stimulation, mean PTT(% of initial) was declined significantly in both the ADR treated and control CTL muscles being greater in CTL ones. T_{50} was found 74.9% greater in ADR than CTL, being significant. When muscles, which were fatigued with same stimulation protocol, were allowed to recover with and without adrenaline, the PTT recovers by 3.4 folds in ADR and about 2 folds only in CTL. Significant differences between CTL and ADR treated-continuously stimulated high frequency fatigued muscles confirm the hypothesis that in mammalian muscles ADR increases the endurance by delaying the high frequency fatigue and helps in its recovery.

Keywords: Fatigue, adrenaline, tetanic tension, gastrocnemius.

INTRODUCTION

Continuous activation or stimulation of muscles results in progressive depression in mechanical output which is termed as fatigue. Fatigue is considered as peripheral when its etiology is found in the muscle itself. There is no single cause of fatigue and its etiology includes several ionic as well as metabolic alterations like elevation in extracellular potassium (Clausen *et al.*, 1998; Overgaard *et al.*, 1999), intracellular accumulation of lactate and H^+ ions (Sahlin *et al.*, 1981), Accumulation of inorganic phosphate (Dahlstedt *et al.*, 2001) lack of ATP, etc.

Mechanical output of the muscles is not absolute; rather it is dynamic and affected by pattern of stimulation which changes the behavior of the muscles accordingly. Edwards (Edwards *et al.*, 1977) has described 2 classes of fatigue depending on the pattern of stimulation. When muscles are fatigued with high frequency, they recover rapidly by reducing the frequency (Jones *et al.*, 1979) and this type of fatigue is known as high frequency fatigue (Edwards *et al.*, 1977). When muscles are stimulated continuously, ionic concentrations across the membrane are changed to the greater extent such that, Na^+ is accumulated inside and K^+ is accumulated outside of the membrane. Thus reduced conduction of action potential is proposed (Cairns and Dulhunty, 1995). This type of fatigue is improved by

increasing extracellular Na^+ and augments by increasing extracellular K^+ (Cairns and Dulhunty, 1995). When muscles are stimulated with low frequency, they undergo low frequency fatigue and these muscles require much greater time to recover (Edwards *et al.*, 1977). Force decline during low frequency stimulation, results from failure of excitation contraction coupling and less Ca^{++} release from sarcoplasmic reticulum (Jones, 2003; Westerblad *et al.*, 1993).

Beta adrenergic agonist are the known inotropic agents in skeletal muscles which not only enhance the peak twitch and tetanic tensions (Brown *et al.*, 1948; Cairns and Dulhunty, 1993a) but also help the muscles to stand against fatigue and improves its endurance (Cairns and Dulhunty, 1994). But this latter effect of beta receptors agonist is less described in the literature than inotropism especially for the physiological agonist adrenaline. Like other agonist adrenaline enhances twitch and tetanic tensions, improves rate of contractions, relaxation and fatigue. These effects of adrenaline on twitch tension are reported in both fast twitch fatigued and non fatigued muscles. In fresh muscles adrenaline is reported to enhance peak incomplete and complete tetanic tensions (Bowman and Zaimi, 1958; Cairns and Dulhunty, 1993b) but not known if similar effects are found in fatigued muscles. In slow twitch muscles beta adrenergic agonists show contradictory results. Earlier studies describe that beta agonist reduces the active state and peak tension in

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slow twitch muscles (Al-Jeboory and Marshall, 1978; Bowman *et al.*, 1962). Later Cairns (Cairns and Dulhunty, 1993b) demonstrated the effects of beta agonist in slow twitch muscles similar to the fast twitch fibers. Since the classification of fatigue by Edwards *et al.* (1977), few studies have described the effects of adrenaline on fatigue and endurance when isolated muscles are stimulated at high frequency. However effects of other beta adrenoceptor agonist are reported. Cairns and Dulhunty (1994) has described that salbutamol maintains the peak force 10-20% better than control during continuous high frequency stimulation. Furthermore, ample evidences are available for the influence of adrenaline on submaximal (Bowman and Zaimi, 1958; Serratos *et al.*, 1981) and maximal (Cairns and Dulhunty, 1993b) tetanic tension in fresh muscles; same effects are not reported in fatigued muscles.

Although activation of beta adrenergic receptors by different agonist seems to produce identical results but actually certain differences are reported in literature especially regarding the potencies of different agonist (Al-Jeboory and Marshall 1978; Holmberg and Waldeck 1977). The purpose of this study is to evaluate the influence of adrenaline on isolated gastrocnemius muscles of rabbit for their: (i) endurance and (ii) recovery from fatigue.

METHODOLOGY

Adult rabbits of both sexes were sacrificed and medial belly of gastrocnemius muscles of both limbs were isolated rapidly and incubated in normal Krebs-Henseleit buffer solution having the following composition (mM) as follows: NaCl; 118, KCl; 4.8, CaCl₂; 2.5, MgSO₄; 1.2, Na₂HCO₃; 27.2, KH₂PO₄; 1 and Glucose; 10. pH was maintained throughout experiment at 7.4. Buffer solution was thermostatically maintained at 30°C and was continuously bubbled with oxygen.

Mechanical recording

Muscles from both limbs were fixed in horizontal chamber in such a way that one of their end (origin) was fixed by using a stainless steel pin inserted in the piece of knee joint and their farther end (insertion tendon) was attached with isometric force transducer with the help of a piece of thread. Muscle chamber was connected with thermostatic controlling bath through which experimental temperature was fixed to 30°C. Muscles were fixed at their resting or optimal length at which maximal tension is obtained. Tetanic stimulations of 50V and frequency of

80Hz were given to the muscles for 20sec continuously by using Harvard-6002, stimulator. Tetanic tensions were recorded and analyzed digitally with computer based power lab system (AD Instruments). Maximal tetanic tensions were calculated per cross sectional area (Kg/cm²). Cross sectional area was calculated by dividing muscle weight (grams) by the product of resting length (cm) and specific density (1.056). However, for comparison purpose, tetanic tension was expressed as % of initial rather values in Kg/cm².

Adrenaline treatment and experimental groups

Muscles from both limbs were selected randomly for adrenaline treatment such that one muscle from each animal served as CTL and other as ADR treated. Adrenaline was added directly in the buffer present in the muscle chamber with the final concentration of 1×10^{-5} mM in which ascorbic acid (1 mM) was also added in order to prevent the oxidation of ADR (Clausen *et al.*, 1993). For obtaining the influence of ADR on endurance and recovery of fatigue, two different experimental sets were designed. In the first set, fresh muscles were treated with adrenaline (incubation time 30 min) and endurance of the muscles was observed as decline in PTT (% of Initial) by giving continuous stimulation for 20sec. In the second set, already fatigued muscles were treated with ADR (30min incubation) and recovery from fatigue was observed as % increment from the tetanic tension which was earlier calculated at the time of fatigue.

STATISTICAL ANALYSIS

Data was analyzed on Microsoft excel. Percent changes in the initial tensions were expressed as mean $\pm$ standard error. 0.05 was considered as significant level.

RESULTS

Effects of adrenaline on the endurance of muscles

Continuous tetanic stimulation of muscles at 80Hz for 20 sec results in decline of isometric force to about 20% of initial. On pre-incubation of muscles in adrenaline for 30min, muscle endurance was found significantly greater than control. When % tetanic tension was plotted against time points, as shown in fig. 1, adrenaline shift the curve upward and at the end of stimulation, tetanic tension was almost 2 folds greater in ADR than CTL. Effects of adrenaline started to appear significantly at about 6sec of stimulation where tetanic tension was about 12% greater in ADR than CTL, and reach to maximum at 14sec where difference was about 26%. Beyond 14sec the difference

Table 1: P values, determined by student's t test, representing statistical difference b/w CTL and ADR at each time point during 20sec of continuous stimulation

Time (sec)	2	4	6	8	10	12	14	16	18	20
P values	0.176	0.098	0.043	0.017	0.005	0.16	0.013	0.013	0.02	0.02

was found reduced. table 1 shows significance level between tetanic tension of CTL and ADR treated muscles at all time points. Adrenaline increases the time to fatigue as muscles with adrenaline took 30% more time to fatigue to half of the initial tension. As shown in fig. 2, mean value for T_{50} (in sec) was $13.6 \pm 1.57(6)$ with adrenaline as compared to $10.2 \pm 1.4(5)$ in CTL ($P > 0.05$).

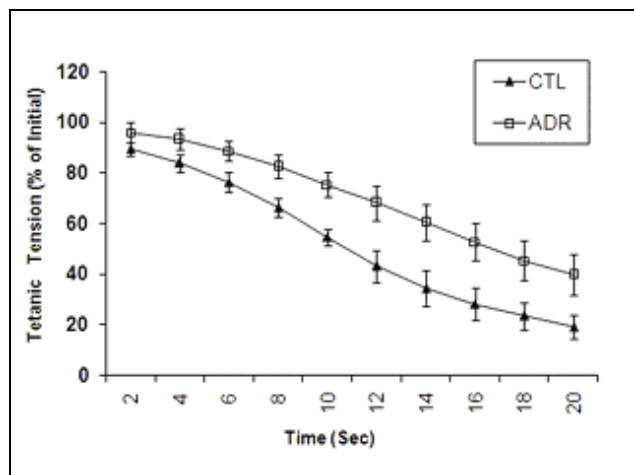


Fig. 1: Comparison of decline in tetanic tension during fatigue in ADR treated and control gastrocnemii.

Effects of adrenaline on recovery from fatigue

According to fig.3 after 20sec of stimulation PTT was declined to about 19% of initial. Later, when these muscles were allowed to recover, PTT was found to increase significantly, in both the CTL and ADR treated muscles. However, ADR treated muscles showed 20% significantly greater force than CTL ones.

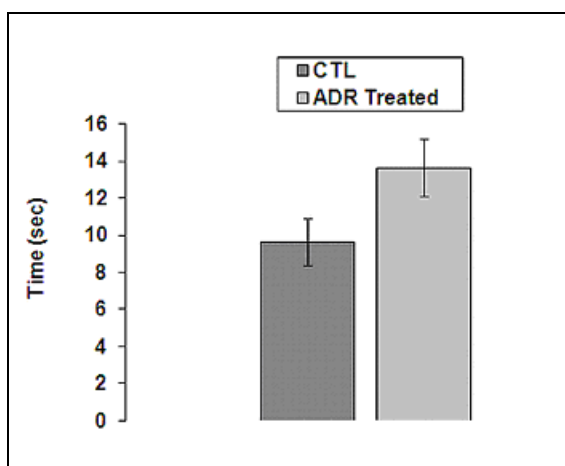


Fig. 2: Comparison of T_{50} between ADR treated and control gastrocnemii.

DISCUSSION

Effects of Adrenaline on the Endurance of Muscles

According to the results presented above, after HFS for 20sec duration, the muscle force was found about 20%

greater than control under the influence of adrenaline and this effect was similar to that of terbutaline (Cairns and Dulhunty, 1994).

No such mechanism is reported, to our knowledge, with the intermittent tetanic stimulation pattern in actively contracting muscles, using adrenaline. However, it has been reported in literature that in K^+ depressed muscles, beta receptors agonist can improve muscle contractility (Clausen *et al.*, 1993). Further, the muscle force generation has been explained to be dependent on Ca^{++} released by sarcoplasmic reticulum during excitation-contraction coupling. Additionally, increased Ca^{++} transient from sarcoplasmic reticulum was also proposed earlier for the resistances develop in muscles against fatigue (Cairns and Dulhunty, 1993a; Cairns and Dulhunty, 1994). But it should be noted that development of resistance to fatigue and maintenance of tension for longer period may be the result of 2 different possibilities. The muscle tension that declines during fatigue is either increased by adrenaline or the adrenaline not let the force to decline.

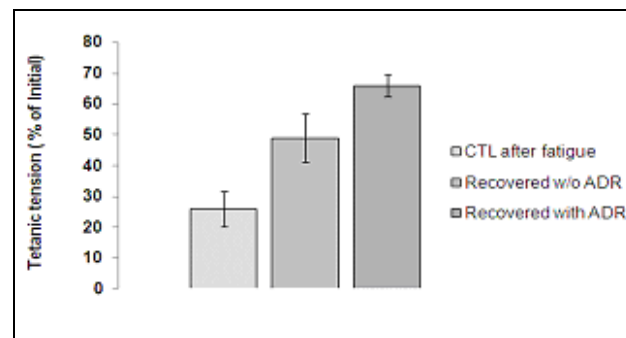


Fig. 3: Comparison of peak tetanic tension after recovery between ADR treated and control gastrocnemii.

Although the outcome of both possibilities is same but both include different mechanisms. If muscle tension is increased by adrenaline it means that adrenaline is directly acting on the Ca^{++} transient or similar processes. If adrenaline not let the force to decline, it means that development of fatigue related biochemical alterations become slow, that results in increase in T_{50} which is true in our study (fig. 2). In this scenario further investigations are required at muscle cell level, as adrenaline is reported to correct many alterations at many places from sarcolemma to contractile apparatus (Chasiotis and Hultman, 1985; Clausen and Flatman, 1977; Clausen and Flatman, 1980).

One of the important corrections made by adrenaline already reported, is to maintain the ionic environment of the muscle fibers (Clausen and Flatman, 1980). Studies on sustained stimulation of muscles have been reported to disturb the ionic environment inside and outside the muscle fiber. Especially the potassium ions are

accumulated outside, that disturbs the membrane excitability to decline muscle tension (Clausen *et al.*, 1998). This decline has been related to the fact that Na^+/K^+ pump does not maintain pace with sustained stimulation. Adrenaline activates and upregulate the Na^+/K^+ pump (Clausen and Flatman, 1980) which make corrections in ionic disturbances and enhance the membrane excitability (Clausen *et al.*, 1993; Clausen and Flatman, 1977). Earlier studies (Clausen *et al.*, 1993) that demonstrate the relationship between adrenaline, excitability and Na^+/K^+ pump, utilize the same standard protocol that muscles are exposed to raised potassium to observe effects on contractility. The assumption that adrenaline does not allow the force to decline is supported by the evidence that when muscles are exposed to adrenaline prior to raised potassium very little force decline is observed. In contrast when muscles are exposed to adrenaline sometime after the raised K^+ buffer, force declines 1st and then recovers (Clausen *et al.*, 1993).

Therefore, according to our results obtained on HFS fatigue of the gastrocnemius muscles of rabbit, it is confirmed that adrenaline has the ability to resist the process of HFS fatigue by either improvements in Ca^{++} cycle at the contractile apparatus to enhance muscle force or slowed down the biochemical alterations that favors fatigue.

Effects of adrenaline on recovery from fatigue

In another set of experiments as mentioned in methodology, adrenaline was found to improve the contractility of fatigued muscles and enhance the peak tetanic tension by 3 folds in comparison with control. To our knowledge this is the 1st study which describes the influence of adrenaline on peak tetanic tension in fatigue muscles where muscles were stimulated continuously with the frequency of 80Hz. It is obvious from Fig. 3 that functional ability of fatigued skeletal muscles enhances in the presence of adrenaline. Goffart (Goffart and Ritchie, 1952) was the first scientist who studied and described in detail the effects of adrenaline on peak tetanic tension *in vivo*. He did not find any change in maximal tetanic tension when muscles were stimulated with the frequency of 100Hz or more. Later, it was supported by Bowman (Bowman and Zaimi, 1958). However Bowman (Bowman and Zaimi, 1958) found the change in sub-maximal tetanic tension when muscles were stimulated by lower frequencies. He also described that this change in submaximal tetanic tension under the influence of adrenaline is sensitive to frequency and both have the inverse relationship. But this negative relationship between frequency and potentiation in tetanic tension is negated by Cairns and Dulhunty (1993b) who described the marked potentiation in tetanic tensions at lower and higher frequencies and with different beta adrenergic agonist. Cairns and Dulhunty (1993b) also demonstrated increment in tetanic tension by 7% under the influence of

adrenaline. In the present study very marked effects of adrenaline are observed on maximal tetanic tension. In fact, already fatigued muscles were continuously stimulated with the frequency of 80Hz. This difference of procedure between Cairns and the present study clearly distinguished our results obtained by using fatigued muscles instead of fresh ones. Further, the matter of inotropism should also be differentiated when using fatigued instead of fresh muscles. What potentiation produced in fresh muscles upon exposure to adrenaline may be simply justified by enhanced Ca^{++} transient from sarcoplasmic reticulum and does not include the correction of any fatigue related alteration within muscles. It is suggested that when fatigued muscles are exposed to adrenaline, they undergo specialized type of inotropism i.e., the defatiguing effects, in addition to the increment in Ca^{++} transient. It is worth to note here that defatiguing effects are defined as removal of causative factors of fatigue so the tension prior to the fatigue can be achieved again. Most important of which is upregulation of Na^+/K^+ ATPase and lowering of inorganic phosphate (Clausen *et al.*, 1993; Nielsen and Clausen, 1996). Here corrections in several fatigue induced biophysical and biochemical alterations may be involved in the improvement in the contractility, in addition to the Ca^{++} transient. However, the whole of the defatiguing effect may not be completely adrenaline dependent, as in our study a defatiguing effect of a lower scale has been observed in the recovery of CTL muscles (fig. 3) being adrenaline independent.

CONCLUSION

Adrenaline increases the muscles endurance during high frequency tetanic stimulation and delays fatigue, i.e., improves endurance. In this perspective it is suggested that influence of adrenaline on HFS involves, corrections in fatigue induced biophysical and biochemical alterations like lowering of inorganic phosphate and clearing of extra cellular potassium through upregulation of Na^+/K^+ ATPase or metabolic enzymes, which are collectively responsible for peak tetanic potentiation in HFS fatigued muscles.

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