Vol. 157, No. 1, 1988 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
November 30, 1988 Pages 225-231

OBSERVATION OF FATIGUE UNRELATED TO GROSS ENERGY RESERVE OF SKELETAL MUSCLE DURING TETANIC CONTRACTION -AN APPLICATION OF ^{31}P-MRS-

S. Takata, H. Takai, T. Ikata, and I. Miura*

Department of Orthopedic Surgery
School of Medicine, the University of Tokushima
3-18-15, Kuramoto-cho, Tokushima 770, Japan

*Bioenergetics Res. Center, Tokushima Res. Inst.
Otsuka Pharmaceutical Co., Ltd.,
463-10, Kagasuno, Kawauchi-cho,
Tokushima 771-01, Japan

Received September 26, 1988

The mechanism of muscle fatigue was studied by ^{31}P-MRS. During tetanic contraction for 2 minutes(min), the tension measured with a strain gauge and Phosphocreatine(PCr)/ Inorganic phosphate(Pi)+ Phosphomonoester(PME) ratio decreased to 31.5±4.4% of the control value and 0.6±0.1, respectively. The intracellular pH(pH) also decreased to 6.62±0.04. Toward the end of the stimulation, the tension decreased to 25.3±1.9% of the control value. However, during 20min stimulation, the PCr/(Pi+PME) ratio increased to 2.5±0.5 and the pH to 6.91±0.04. These results show that muscular fatigue is ascribable not to a decreased level of high energy metabolites required for actomyosin ATPase, but to an increase in the threshold intensity of excitation in excitation-contraction coupling.

Previous studies using ^{31}P-MRS indicated decrease in tension associated with decrease in the level of high energy metabolites(1,2), or decrease in PCr concentration during tetanic or non-tetanic contraction(3-10).

But our preliminary studies on human forearm and rabbit skeletal muscles showed that decrease in the PCr/(Pi+PME) ratio, was not correlated with muscular fatigue(11). In this study muscular fatigue is defined as decrease in tension.

Theoretically, ATP hydrolysis in skeletal muscles is an indication of actomyosin ATPase activity. So it is doubtful if decrease in the PCr/(Pi+PME) ratio during exercise is an indication of muscular fatigue.

0006-291X/88 $1.50

If the oxygen supply is maintained at a normal level, reduction in the level of PCr may be an indication of ability of muscle performance. Therefore, muscular fatigue due to tetanic contraction may be attributable to inability to hydrolyze ATP in large enough quantities to maintain the initial tension.

In order to confirm this, we studied the relationship between energy metabolism measured by ^{31}P-MRS and muscular fatigue measured with a strain gauge. The right sciatic nerve of WKY rats was stimulated electrically to perform tetanic contraction, which is reported to induce a similar metabolic demand to that of voluntary contraction(12).

MATERIALS AND METHODS

Animals. Male WKY rats (n=10), weighing 370-385g, were used in this study. About 84% of the hindlimb muscles of rats consist of fast twitch muscles(13).

Operation procedures. General anesthesia was induced with sodium pentobarbital (60mg/kg, intraperitoneally). The right sciatic nerve was exposed and a small bipolar electrode was placed in contact with it. The tendons of the right gastrocnemius, plantaris and soleus muscles were isolated at the ankle, tied together, cut free from the heel and connected to a strain gauge at a tension of 50 dynes.

Stimulation. The sciatic nerve was stimulated electrically with a train of 100 pulses at a intervals of 15 milliseconds(ms) (approximately 67Hz), a voltage of 4v and a main interval of 2 seconds(s). In this way the muscles were stimulated electrically to maintain a 1.5s tetanic contraction every 2s. This type of stimulation was continued for 20min for ^{31}P-MRS. In other rats, the stimulation voltage was increased to 8v after 10min stimulation at 4v.

Recording of tension development. Tension development during isometric exercise was recorded with a strain gauge. The time course of changes in tension was given by the following equation:

$$\frac{\text{Tension acquired in each subsequent 2min stimulation}}{\text{Tension acquired in the first stimulation}} \times 100\%$$

Magnetic resonance spectroscopy. The ^{31}P-MRS spectra were recorded with a BEM 250/80 spectrometer (Phospho-Energetics) operating at 32 MHz. The spectrometer was operated in the Fourier transform mode with a pulse duration of 15μs, and a pulse interval of 2s. The acquisition time was 2min. The hindlimb was placed in a 2cm diameter solenoid coil. The peak of PCr was assigned a chemical shift of zero. The tissue levels of creatine phosphate(PCr), inorganic phosphate(Pi) and β-ATP were estimated from the areas under individual peaks. The intracellular pH was estimated from the chemical shift(δ) of Pi peaks and calibrated by the following equation:

$$pH=6.90-\log\{(\delta-5.805)/(3.290-\delta)\}$$ (14)

RESULTS

A series of ^{31}P-MRS spectra during exercise and subsequent recovery is shown in Fig.1.

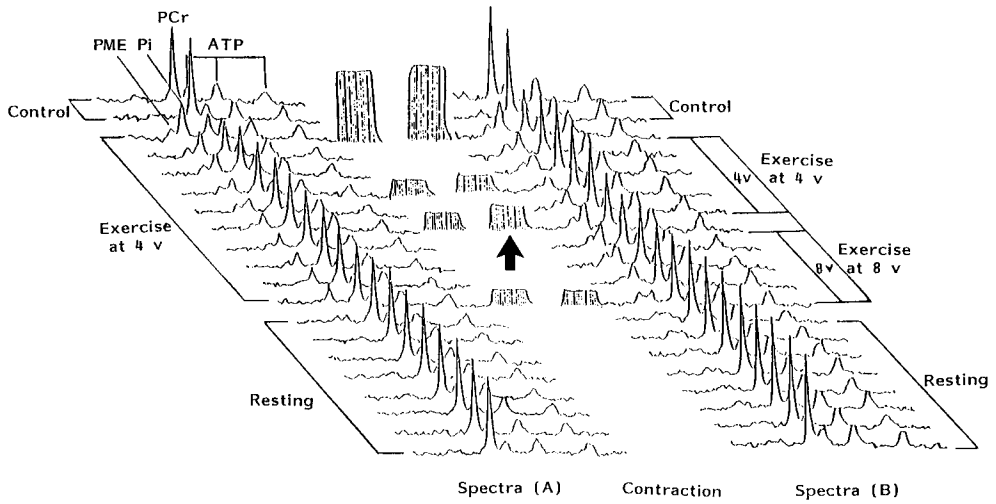

Fig.1.Typical spectra and corresponding contractions(inserts).
(A)Stimulation at constant voltage
(B)Effect of stimulation at increased voltage
On increasing the stimulation voltage to 8v, the tension increased and ATP was hydrolyzed in larger quantity.(Arrow)
PME;phosphomonoester, Pi;inorganic phosphate,
PCr;phosphocreatine, ATP;adenosine triphosphate

PCr/(Pi+PME)(Fig.2). The PCr/(Pi+PME) ratio decreased to 0.6±0.1 in the first 2min of the stimulation. Later, it gradually increased, reaching to 2.5±0.5 at the end of the 20min stimulation, and returning to the control value in 6min of rest.

Intracellular pH(Fig.2). The intracellular pH decreased more rapidly to 6.62±0.04 in the first 2min of the stimulation, indicating significant acidosis. At the end of stimulation it had increased to 6.91±0.04, and gradually returned to the control value in 8min of rest.

Tension development(Fig.3). Tension as an index of muscular function decreased rapidly to 31.5±4.4% of the control level during stimulation for 2min and did not increase again during further stimulation. Toward the end of the stimulation period the tension decreased to 25.3±1.9% of the control value. On the contrary, the PCr/(Pi+PME) ratio increased gradually to 2.5±0.5. Thus although the muscle was still rich in high energy metabolites at the end of the stimulation, the muscle function did not recover. However, the tension returned to 72.4±6.7% of the control value when stimulated in 4min of rest. These results indicated that muscular fatigue was not due to decrease in a level of high energy metabolites required for actomyosin ATPase.

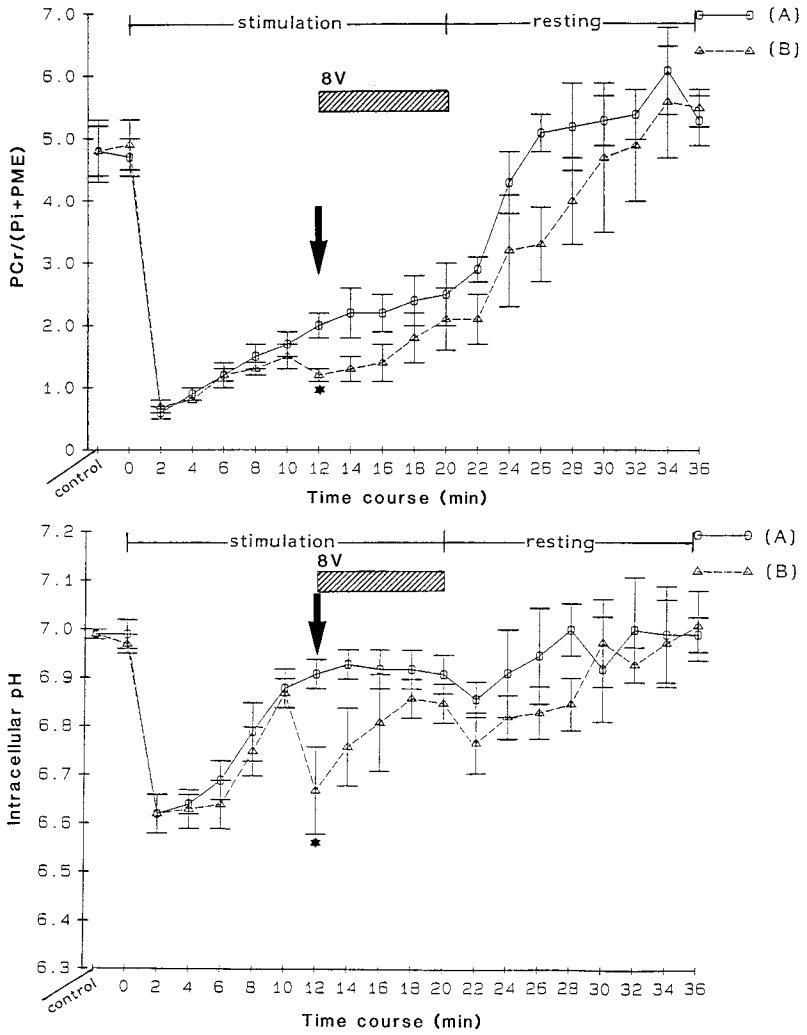

Fig.2.Changes in the PCr/(Pi+PME) ratio and intracellular pH. Increase in the voltage of stimulation produce significant changes.(*P< 0.05 by the t-test)
At the arrow the voltage was increased to 8v. The shaded bars indicate stimulation at 8v. Values are $\bar{X} \pm$ S.E.;n=5.
(A)Stimulation at constant voltage
(B)Effect of increase in voltage of stimulation

Stimulation at increased voltage(Fig.2,3). This stimulation produce significant change. During 2min stimulation at 8v, the PCr/(Pi+PME) ratio decreased to 1.2±0.1 and the pH to 6.67±0.09, while the tension increased to 35.3±2.2% of the control value.

DISCUSSION

The present study indicated that there were two types of muscular fatigue: a)fatigue associated with ATP hydrolysis in

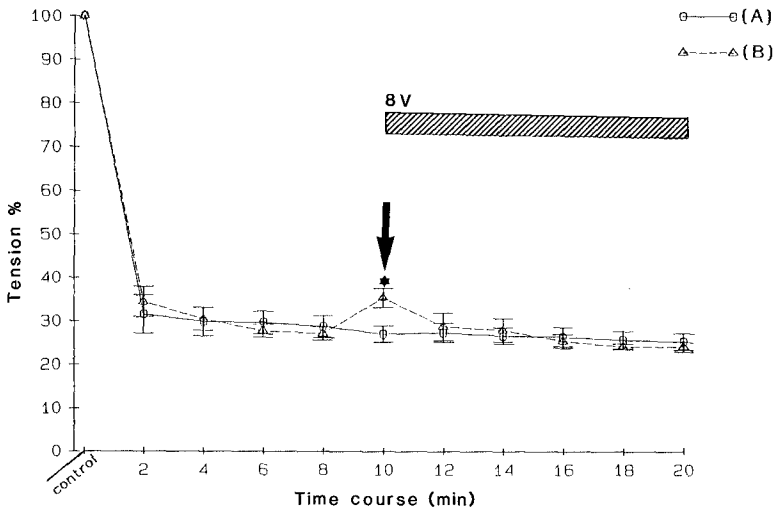

Fig.3.Changes in tension from the control value. Increase in the voltage of stimulation resulted in significant increase in the tension. At the arrow, the voltage of stimulation was increased to 8v. The shaded bar indicates stimulation at 8v. Significantly different from value on constant stimulation. (*P<0.05 by the t-test)
(A)Stimulation at constant voltage
(B)Effect of stimulation at increased voltage

large quantitiy, or b)fatigue associated with ATP hydrolysis in small quantity.

In the first 2min of stimulation, the tension decreased rapidly to 31.5±4.4% of the control value and the PCr/(Pi+PME) ratio also decreased to 0.6±0.1. The Intracellular pH also decreased to 6.62±0.04 at the same time. During exercise lactate is known to increase with decrease in bicarbonate(15).

Present ^{31}P-MRS studies have shown that muscular fatigue during tetanic or non-tetanic contraction was associated with decrease in high energy metabolites(1,2). Results have shown that ATP was hydrolyzed adequately to allow muscle contraction, and that consequently muscular fatigue was associated with decrease in high energy metabolites required for actomyosin ATPase activity.

In the present work we found that in the period from 4min after the beginning of stimulation to the end of the stimulation, reduced muscle tension was associated with increase in the PCr/(Pi+PME) ratio, and by the end of the stimulation period, the PCr/(Pi+PME) ratio had returned to 2.1±0.5 and the pH to 6.91±0.04. This recovery of the PCr/(Pi+PME) ratio could be due to increase in production of ATP or decrease in its consumption.

However, tension decreased to 25.3±1.9% of the control value, the recovery of the PCr/(Pi+PME) ratio was probably due to decrease in consumption of ATP.

In preliminary studies, we found that the result of isotonic exercise at a tension of 50 dynes or 100 dynes was similar to that of isometric exercise in this study. This finding indicates that this type of muscular fatigue is ascribable to be inability to hydrolyze sufficient ATP for adequate contraction. This conclusion is supported by our finding that on stimulation at higher frequency (1.5ms-intervals), the tension decreased more than on stimulation at 15ms-intervals.

In some rats, the stimulation was increased to 8v for the last 10min of stimulation. During this intensified stimulation, the tension was increased by hydrolysis of ATP in larger quantity in the 2min of stimulation. This finding indicates that the threshold intensity of excitation in excitation-contraction coupling increases gradually , and consequently, the tension cannot be maintained at the control value.

Based on our results, we propose the following two possible mechanisms of muscle fatigue in tetanic contraction :

1)Fatigue is due to decrease in the quantal release of acetylcholine from the terminal end of motor neurons; e.g., as a result of decrease in the Ca^{2+} permeability of the terminal end of the motor neuron, because Ca^{2+} permeability is responsible for the amount of acetylcholine released(16).

2)Fatigue is due to decrease in Ca^{2+} release from terminal cisterns of the sarcoplasmic reticulum as the result of an increased threshold intensity of excitation of the end plate or sarcolemma, or as the result of insufficient release of Ca^{2+} itself(17,18). Ca^{2+} is thought to be very important in controlling excitation-contraction coupling and in triggering glycogenolysis (19). A decrease in ACh release or Ca^{2+} release may be ascribed to decrease in ATP production by mitochondria located in neuromuscular junctions. Further studies are required to examine these possibilities.

REFERENCES

1. Dawson,M.J.,Gadian,D.G.,Wilkie,D.R.(1980).J.Physiol.299,465-484.
2. Challiss,R.A.J.,Hayes,D.J.,& Radda,G.K.(1987).Biochem.J.246, 163-172.
3. Dawson,M.J.,Gadian,D.G.Wilkie,D.R.(1977).J.Physiol.267,703-735.

4.Chance,B.(1984).J.Trauma.24,154-166.
5.Arnold,D.L.,Matthews,P.M.,Radda,G.k.(1984).Magn.Reson.Med.1, 307-315.
6.Chance,B.,Eleff,S.,Leigh,Jr.J.S.,Sokolow,D.,& Sapega,A.(1981). Proc.Natl.Acad.Sci.USA.78,6741-6718.
7.Chance,B.,Eleff,S.,Bank,W.,Leigh,Jr.J.S.& Warnell,R.(1982). Proc.Natl.Acad.Sci.USA.79,7714-7718.
8.Hoult,D.I.,Busby,J.W.,Gadian,D.G.,Radda,G.K.,Richards,R.E.,& Seeley,P.J.(1974).Nature.252,285-287.
9.Lunt,J.A.,Allen,P.S.,Brauer,M.,Swinamer,D.,Treiberg,E.O.,Belc-asro,.,Eccles,R.,& King,E.G.(1986) Magn.Reson.Med.3,946-952.
10.Tanokura,M.,& Yamada,K.(1984).FEBS Lett.171,165-168.
11.Koga,K.& Miura,I.(In preparation)
12.Shenton,Jr.D.W.,Heppen,R.B.,Chance,B.,Glasgow,S.G.,Schnall, M.D., & Sapega,A.A.(1986).J.Orthop.Res.4,204-211.
13.Armstrong,R.B.,& Laughlin,M.H.(1983).J.Physiol.344,189-208.
14.Flaherty,J.T.,Weisfeldt,M.L.,Bulkley,B.H.,Gardner,T.J.,Gott, V.L., & Jacobus,W.E.(1982).Circulation.65,561-571.
15.Schardt,F.W.,& Wiegand,M.K.(1986).Fed.Proc.45,2930-2932.
16.Ganong,W.F.(1981).Review of medical physiology.10th edition.77 -88 edition.
17.Dawson,M.J.(1986).NMR in Biology and Medicine, Ed.by Shu Chien and Chien Ho.185-200.
18.Chiba Foundation Symposium 82(1981): Human Muscle Fatigue: Physiological Mechanisms, Ed. R.Porter and J.Whelan, Pitman Medical,London.
19.Ebashi,S.,& Endo,M.Calcium Ion and Muscle Contraction.Pro. Biophys.Mol.Biol.,Pergamon Press,Oxford,18,144-159.