EFFECT OF *IN VIVO* ELECTRICAL STIMULATIONS ON PROTEIN FRACTIONS OF DENERVATED GASTROCNEMIUS MUSCLE OF FROG *RANA HEXADACTYLA* (LESSON)

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ABSTRACT

Muscle protein fractions were studied in frog. Sciatic denervation resulted in decrement in the myofibrillar proteins while electrical stimulation to the denervated muscle elevated myofibrllar protein fractions. The results are discussed.

INTRODUCTION

ELECTRICAL stimulation is widely employed for inducing exercise into the muscles[1]. Various parameters suitable for inducing muscular exercise and training in amphibian muscle have been standardized[2]. Sciatectomy and disuse of the muscle lead to muscle wasting and atrophy[3]. In denervation atrophy the muscle suffers disuse affecting work performance and resulting in blood flow and altered membrane permeability[4]. Thus electrical stimulation and disuse result in altered protein fractions. In the present study the impact of a standardized programme of electrical stimulation on the denervated muscle of frog was analysed.

MATERIALS AND METHODS

Male frogs, *Rana hexadactyla* (Lesson) (35 ± 2 g) collected from ponds near Tirupati were maintained in clean aquaria and fed with cockroaches *ad libitum*. Water in the aquaria was regularly changed once a day. Frogs acclimatized to laboratory conditions were divided into three groups of six animals each: I : Normal sham-operated frogs (C). II: Sciatic denervation—denervated control (DC). III: Electrical stimulation for denervated animals for 10 days—denervated stimulated (DS).

Sciatic denervation

Sciatic denervation was performed under aseptic conditions. The sciatic nerve supplying the shank was separated and about 2 cm length of the nerve was cut at the posterior part of the thigh. Sham-operated normal animals were maintained as controls.

Electrical stimulation

A special plastic chamber was designed to restrain the animals during electrical stimulation. After 10 days of sciatectomy the gastrocnemius muscles of sciatectomized frogs were stimulated for 30 min daily for 10 days using an electrical stimulator (INCO/CSIO Research Stimulator, Ambala, India). Rectangular pulses of 5 V, 2 cycles/sec and 100 milli sec pulse duration were used to stimulate the muscles.

After appropriate exercises, the three groups of frogs were double-pithed and the gastrocnemii were isolated and chilled rapidly by keeping them in freezing mixture. These muscles were used to determine the biochemical parameters.

Soluble and insoluble proteins[5], sarcoplasmic protein fractions[6] and structural protein fractions[7] were estimated in the muscles.

RESULTS AND DISCUSSION

The data presented in table 1 indicate the changes in protein fractions of skeletal muscle after sciatectomy and induced exercise programme.

DC muscle

The decrease observed in soluble proteins of skeletal muscle after 10 days of sciatectomy agreed with the decrease reported in sarcoplasmic proteins of denervated muscle in chicks[8], rats and pigeons[9]. Similar decrease was also observed in structural proteins of denervated muscles. This indicates the deranged structural and dynamic levels of organization of the DC muscle after 10 days of sciatectomy leading to denervation atrophy. The decrease in the sarcoplasmic proteins is due to the decrease in albumins and globulins. Similarly among structural proteins the actin and myosin levels of the DC muscle were decreased. However, the collagen content increased in DC muscle in comparison to that of control. Increased collagenation reported in denervated muscles supports the increase observed in collagen content. The present results on protein composition in DC muscle showed the deranged structural and dynamic levels of organization leading to muscular atrophy within 10 days of sciatectomy.

Table 1 *Levels of soluble proteins, albumins, α, β-globulins, γ-globulins and albumins; Globulins ratio; and levels of structural proteins, actin, myosin and collagen in the gastrocnemii of 10 days denervated control and denervated stimulated frogs in relation to control frogs*

Component	Control muscle		Denervated control muscle		Denervated stimulated muscle
Soluble proteins (mg/g wet weight)	85 48 ± 5.0	− 64.54 $P < 0.001$	30.31 ± 1.5	− 3.96 NS	82.09 ± 4.3
Albumins (mg/g wet weight)	29.18 ± 1.5	− 62.95 $P < 0.001$	10.81 ± 0.8	− 3.46 NS	28.17 ± 1.7
α, β-globulins (mg/g wet weight)	8.92 ± 0.5	− 51.9 $P < 0.001$	4.29 ± 0.5	− 7.52 $P < 0.05$	8.21 ± 0.5
γ-globulins (mg/g wet weight)	14.76 ± 0.83	− 48.03 $P < 0.001$	7.67 ± 0.5	− 7.52 $P < 0.05$	13.65 ± 2.0
A/G	1.23 ± 0.05	− 26.74 $P < 0.001$	0.901 ± 0.07	5.69 NS	1.30 ± 0.2
Structural proteins (mg/g wet weight)	175.63 ± 5.0	− 59.93 $P < 0.001$	70.37 ± 5.4	− 8.5 $P < 0.001$	160.61 ± 6.9
Actin (mg/g wet weight)	83.28 ± 4.5	− 66.53 $P < 0.001$	27.87 ± 1.3	− 6.21 $P < 0.05$	78.1 ± 7.8
Myosin (mg/g wet weight)	92.19 ± 5.46	− 61.04 $P < 0.001$	35.91 ± 2.0	− 5.42 $P < 0.05$	87.19 ± 5.1
Collagen (mg/g wet weight)	14.29 ± 1.2	+ 40.79 $P < 0.001$	20.12 ± 1.2	+ 6.36 NS	15.2 ± 1.1

Each value represents the mean of six individual observations; Mean ± SD; + and − indicates increase and decrease over that of control muscle; *P* denotes level of significance, and NS denotes non-significant.

DS muscle

The insignificant change in water soluble proteins of DS muscle in comparison to control muscle indicates the arrest of protein degradations seen in the denervated muscle. Similarly maintenance of almost control level of structural proteins even in denervated muscle denotes the active addition of proteins in DS muscle over DC muscle. The increase reported in the rate of incorporation of labelled amino acids into proteins in trained muscles[10] indicates the possibility of active protein synthesis in DS muscle. Protein fractions such as albumins, α, β-globulins and γ-globulins constitute the majority of enzymatic proteins of glycolytic cycle. The increase in this fraction suggests improved glycolytic efficiency and thus the muscular contractile capacity of the DS muscle. The active increase in the structural proteins in DS muscle as a result of induced exercise can support the possible elevation in the contractile machinery of the

muscles. This shows improvement in myofibrils in DS muscle than in DC muscle. Thus the increase in sarcoplasmic and myofibrillar proteins indicates an overall increase in non-collagenous proteins. However, the insignificant change observed in the collagen content of the DS muscle from control suggests possible inhibition on the collagen accumulation in the denervated muscles as a result of induced exercise. The decrease in collagen content also leads to improved muscular efficiency.

In conclusion, it can be stated that sciatectomy leads to muscular atrophy. The training programme of electrical stimulation can be employed for the treatment of muscle atrophy and the ensuing muscle damages.

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NEWS

DECLARATION OF TALLOIRES: POLIO CAN BE ERADICATED!

The Task Force for Child Survival, whose members are the World Health Organization (WHO), UNICEF, the World Bank, the United Nations Development Programme (UNDP), and the Rockefeller Foundation, met in Talloires, France, during 10–12 March 1988.

Seventy-five of the world's leading experts on child and maternal health, learned about the progress achieved during the last decade in the fields of immunization, diarrhoeal disease control, family planning and resource mobilization.

Participants expressed admiration for the efforts made by developing countries to combat dangerous threats to the health of infants and children through primary health care. They also discussed the desirability of health development aid in the 1990s pursuing and expanding the initiatives aimed at protecting the world's children.

A number of targets were discussed as being achievable by the year 2000, despite being challenging. Polio should be eradicated from the globe. Deaths from neonatal tetanus should disappear. Measles and diarrhoea deaths should be reduced by 95% and 70% respectively, and childhood and maternal mortality reduced by half. There should be universal primary education and 80% female literacy. Severe malnutrition should be eliminated.

The Declaration of Talloires commits the Organizations who are sponsors of the Task Force to these goals, which are seen as making essential contributions to human and national development and to the attainment of health for all by the year 2000. (For details please write to: The Carter Presidential Center, Inc., One Copenhill, Atlanta, Georgia 30307. OR Media Service, World Health Organization, 1211 Geneva 27, Switzerland.)