FUNCTIONAL AND MORPHOLOGICAL TRANSFORMATION OF THE LATISSIMUS DORSI MUSCLE DURING MUSCLE CONDITIONING BY MULTICHANNEL STIMULATION

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SUMMARY

This study was undertaken to survey the changes in force and morphology of the Latissimus dorsi muscle (LDM) during the transformation into a fatigue–resistant muscle by multichannel stimulation via the thoracodorsal nerve. Therefore,in six sheep a silicon chamber connected to a pressure transducing system was implanted under the left LDM. Muscle biopsies from the left and right LDM were harvested at the beginn, at the end of Phase I and of Phase II of our conditioning protocol. At the end of Phase I the LDM contained 90% Type I muscle fibers with the highest level of mean maximum pressure (140,3mmHg). At the end of the conditioning protocol (Phase II) the LDM contained 100% Type I fibers and reached a mean maximum pressure of 92mmHg. The increase of the frequency of the muscle contractions during Phase II resulted only in the reduced power of an completely transformed fatigue-free skeletal muscle. Concerning muscle power a 90% transformation of the LDM seems to offer distinct advantages compared with a total transformation to Typ I fibers.

STATE OF THE ART

Various approaches have been made to rule out the optimal stimulation protocol for the conditioning of the LDM to a fatigue-resistant muscle by means of FES in the past. The fatigue-resistant LDM wrapped around the ventricles of the heart or around an artificial or biological neoventricle should then serve as a cardiac assist device. Concerning the resulting hemodynamic data clinically and experimentally a distinct loss of contractility of the transformed LDM is evident. In order to evaluate a new developed conditioning protocol with multichannel stimulation a functional and histomorphological analysis of the LDM was carried out in the time-course of the experiment.

MATERIAL AND METHODS

6 female sheep were used for this experiment.

Surgical procedure:
During operation the sheep were placed in right side position to perform a lateral incision on the left side. The left thoracodorsal nerve was prepared carefully and four ring-shaped electrodes

were sutured to its epineurium in different position to perform carousal stimulation. The electrode leads were led out percutaneously. A silicon chamber connected to a pressure-transducing system was placed under the left LDM. This configuration was designed to measure the pressure produced by the muscle under varying the stimulation conditions.

Muscle Conditioning:

Two weeks after the implantation the stimulation protocol was started. Muscle conditioning was performed by multichannel (carousel) burst stimulation of the thoracodorsal nerve. Eight bipolar standardized combinations of electrodes were formed with the four stimulation electrodes (Table 1). Stimulation parameters were: burst stimulation, burst duration 660 ms, pulse frequency 28,8 Hz and pulse width 540 µs. The demanded amperage to achieve maximum tetanic tension seperately evaluated for each electrode combination. At least six combinations of equal contraction strength were selected. Amperage was adjusted to slightly submaximal levels for performance of carousal stimulation. The stimulation threshold of each electrode combination was determined every week and the amperage was readjusted if necessary. The electrode combinations were changed also if necessary.

Our stimulation protocol contained of two Phases: In Phase I of the stimulation protocol we started with 10 min/h work and 50 min/h rest. The duty circles (”on ” periods) were increased according to the fatigue resistance of the muscle until 10 contractions/min could be performed chronically around the clock. In Phase II of our stimulation protocol the frequency of the contractions was increased from 10 to 70/min. During the conditioning program the changes of muscle force (= mean maximum pressure= MMP) were monitored by the silicon balloon system.

Synopsis of the Eight Standartized Combinations of Electrodes Used for Multichannel Stimulation of the Latissimus Dorsi Muscle:

Electrode	1	2	3	4
Combination 0	0	0	-	+
Combination 1	0	0	+	-
Combination 2	0	-	0	+
Combination 3	0	+	0	-
Combination 4	-	0	0	+
Combination 5	+	0	0	-
Combination 6	-	0	0	+
Combination 7	+	0	0	-

+ = electrode used as a positive pole
- = electrode used as a negative pole
0 = electrode not used

Histomorphological analysis:

Muscle biopsies were harvested from the cranial, the caudal and scapular part of left LDM at the begin of the stimulation protocol, at the end of Phase I and at the end of the stimulation protocol (end of Phase II). The biopsies were immediately snap-frozen at –80 C in isopentane, cooled by dry ice and stored at –80 C until use. Serial transverse cryosections of 10µm thickness were stained with actomyosin ATPase after alkaline (ph 10,2) and acid (ph 4,3) preincubation according to Guth and Samaha. All stained sections were examined by light microscopy at a 100-fold magnification. The resulting fields were displayed on the monitor of a personal computer by means of a video camera mounted on the microscope. The analysis was performed with a pen linked to the personal computer by one experienced investigator. More than 300 muscle fibers were evaluated in each section. After comparison of the serial sections stained for actomyosin ATPase with alkaline (ph 10,2) and acid (ph 4,3) preincubation, the muscle fibers were divided into Type I, Type II and Type IIc.

The following histomorphometric parameters were determined for the left LDM:

- the percentage of Type I, Type II and IIc muscle fibers in relation to the number of muscle fibers counted
- the equivalent diameter of Type I, Type II and Type IIc muscle fibers in µm
- the percentage of perimysial and endomysial connective tissue in relation to the measured area of the section

RESULTS

At the beginning of the stimulation the LDM performed a MMP of 112,7 mmHg in the silicon balloon. The histomorphological analysis revealed 78,7 $\pm$ 10,5% Type I, 3,1 $\pm$ 3,4% Type II and 3,1 $\pm$ 3,4% Type IIc fibres at the beginning of the stimulation protocol. The eqiuivalent diameter of the Type I fibers revealed 55,6µm , of the Type II fibers 57,1µm and of the Type IIc fibers 57,1µm . The percentage of the peri-and endomysial connective tissue was 12,5%.
At the end of Phase I the MMP was 140,3 mmHg. At this time the LDM contained 90,1 $\pm$ 14,9% Type I, 7,6 $\pm$ 8,2% Type II and 2,2 $\pm$ 0% Type IIc fibres. The eqiuivalent diameter of the Type I fibers revealed 49,9µm , of the Type II fibers 58,7µm and of the Type IIc fibers 56,8µm . The percentage of the peri-and endomysial connective tissue was 14,6%.
At the end of Phase II, i. e. the end of the stimulation protocol, the MMP was 92 mmHg. Histomorphometrically we found a completely transformed LDM with 100% Type I fibres. The equivalent diameter of the Type I fibers was 46,7µm. The percentage of the peri-and endomysial connective tissue was 16,1%.

DISCUSSION

The results clearly demonstrate that the LDM containing 90% of Type I fibres increased the MMP from 112,7 to 140,3 mmHg at the end of Phase I. At the end of Phase II the LDM revealed 100% of Type I fibres but decreased the MMP from 140,3 to 92 mmHg. In Phase I of our stimulation protocol the distribution of the duty circles to 60 sec offers an overall stimulation-

frequency of 1,2 Hz. This low frequency stimulation of a sheep LDM resulted in the transformation to a fatigue-free muscle at the highest achievable power-level. The increase of the frequency during Phase II resulted only in the reduced power of an completely transformed fatigue-free skeletal muscle. Concerning muscle power a 90% transformation of the LDM seems to offer distinct advantages compared with a total transformation to Typ I fibers.
Taking into consideration that the LDM used in cardiomyoplasty or aortomyoplasty has to perform up to 50 contractions per minute depending on the stimulation mode, the question arises whether a stimulation protocol should be finished at contraction rates of 10 or 70 contractions per minute around the clock to obtain optimal muscle performance in cardiac assist.

REFERENCES

1. Salmons S, Jarvis JC. Cardiac assistance from skeletal muscle: A critical appraisal of the various approaches. Br Heart J1992; 68:333-338
2. Salmons S, Vrbova G. The influence of activity on some contractile characteristics of mammalian fast and slow muscles. J Physiol (Lond) 1969; 201: 535-549
3. Girsch W, Koller R, Gruber H, Holle J, Liegl Ch, Losert U, Mayr W, Thoma H. Histological assessment of nerve lesions caused by epineural electrode application in rat sciatic nerve. J Neurosurg 1991; 74:636-41
4. Koller R, Girsch W, Liegl Ch, Gruber H, Holle J, Losert U, Mayr W, Thoma H. Long-term results of nervous tissue alterations caused by epineural electrode application: An experimental study in rat sciatic nerve. PACE 1992;15:108-15
5. Salmons S, Hendrikson J. The adaptive response of skeletal muscle to increased use. Muscle Nerve 1981;4:94-101
6. Magovern JA, Furnary AP, Christlieb IY, Kao RL, Magovern GJ. Bilateral latissimus dorsi cardiomyopathy. Ann Thorac Surg 1991; 52:1259-65
7. Chachquez JC, Grandjean P, Schwartz K, Mihaileanu S, Fardeau M, Swynghedauw B, Fontaliran F, Romero N, Wisnewsky C, Perier P, Chauvaud S, Bourgeois I, Carpentier A. Effect of latissimus dorsi cardiomyoplasty on ventricular function. Circulation 1988;78:203-15
8. Carpentier A, Chachquez JC, Grandjean P, eds. Cardiomyoplasty. Mount Kisco, NY:Futura Publishing, 1991
9. Chagas AC, Moreira LF, Protasio L, Camarano GP, Leirner A, Stolf NAG,Jatene AD. Stimulated preconitioned skeletal muscle cardiomyoplasty. An effective means of cardiac assist. Circulation 1989;80:202-8
10. Guth L, Samaha FJ: Procedure for the histochemical demonstration of actomyosin ATPase . Exp-Neurol. 1970 Aug; 28(2): 365-7

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