The Contraction of Unweighted Fast and Slow Rat Muscles in Calcium-Free Solution

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Abstract

Twitch and tetanic contractions of slow-twitch soleus and fast twitch plantaris muscles of the rats were recorded after 2 weeks hindlimb suspension. Ca-free solution and Ca-channel blocker D600 were used in order to investigate the unweighting induced changes in initial part of excitation-contraction coupling (ECC) system. The estimations of myosin ATPase and cross-sectional area of muscle fibres were performed to assess the changes in muscle fibre type composition and fibre atrophy occur in parallel to Ca-requirements properties. It was found that in the course of hindlimb suspension, the decrease of tetanic contractions in Ca-free media fastened in slow, but not in fast muscle. After fatigue development, elicited by tetanic trains, the dramatic fall of tetanic amplitudes, observed in Ca-free solution in control soleus, was abolished after unloading. The similar fall of fatigue resistance under D600 action was not abolished in unloaded muscle. In fast muscle the rapid fall of contractions after fatigue appeared already in control, and did not change in Ca-free media nor after hindlimb suspension. The atrophy was observed only in type 1 (slow) fibres, equally in soleus and in plantaris muscle. The results are analyzed with references to the Ca-requirements of the T-membrane voltage sensors functioning, and the conclusion was made that the regulation of the initial step of ECC is affected by the unloading, at least in slow muscles.

Key words: hindlimb suspension model, muscle fatigue, excitation-contraction coupling, atrophy.

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It is known that real and simulated weightlessness produces in skeletal muscles remarkable structural and functional alterations. These alterations affecting predominantly the slow-twitch muscles, that perform antigravity postural functions, are expressed in general in decrease of muscle strength and degradation of other contractile properties, and as well in fibre atrophy and fibre-type alterations [3, 7, 8, 12, 13, 20, 22]. Among others, the alterations occur in excitation-contraction coupling (ECC) system. It was shown that at least in slow-twitch muscles the Ca^{2+} efflux from sarcoplasmic reticulum (SR) is remarkably fastened, and Ca^{2+} concentration in SR increased under exposure to hindlimb unloading, which results in making the slow muscle to some extent closer to fast one [19]. In addition, under microgravity conditions the constant of Ca^{2+} binding to troponin C decreases [7]. All these findings point out that unloading alters the characteristics of ECC system, which provides modulation of the calcium control over contractile proteins.

In order to obtain more complete understanding of the unloading-induced changes in activation of muscle contraction, it was important to study the initial stage of ECC functioning, i.e. the triggering of SR Ca^{2+}-release activation by extracellular trigger. It is known that slow dihydropyridine (DHP) sensitive Ca-channels, located in the membrane of T-tubules, serve as sensors of transmembrane potential changes, and trigger the SR Ca^{2+} release in response to membrane depolarization [1, 17, 18]. Func-

tioning of these sensors requires the presence of Ca^{2+} in the extracellulare space [2, 10]. Therefore in the present study the Ca-free solution and Ca-channel blocker were used for elucidating their influence upon contractile and fatigue properties of muscle underwent chronic hindlimb suspension.

Methods

For experiments were used Wistar male rats weighting 125-200 g. Animals were hindlimb suspended for 14-16 days by means of stainless steel rings implanted through the skin over the sacrum vertebrae. Implantation was performed under ether anaesthesia. Rats could move around on forelimbs and get food and water ad libitum. The control rats were kept with ring implanted as well but without suspension.

Fast-twitch plantaris and slow-twitch soleus muscles were removed both from suspended and control rats under pentobarbital anesthesia (4 mg per 100 g of weight), and then the animals sacrified by lethal dosage of anesthetics. The contractions of isolated muscles were studied by means of isometric strain gauge (KTD 7B, Russia), with pen recorder and oscilloscope registration. Direct electrical stimulation was performed through two parallel platinum plate electrodes by short (1 ms) stimuli, and tetanization by trains of 50 Hz and 5 s duration. In fatigue studies it was used the stimulation pattern of 0.5 s duration series of 100 Hz stimuli every 3 s. The time which required for force amplitude decline to 50% of the initial level, was used as an index of the fatigue resistance.

Ringer solution contained (in mM/l): 126 NaCl, 5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 12 $NaHCO_3$, 1 NaH_2PO_4, 11 glucose (pH 7.3-7.4); it was constantly aerated with carbogen (95% O_2 and 5% CO_2) and kept at 22-24°C. In Ca-free solution $CaCl_2$ was omitted and 2 mM/l $MgCl_2$ added. Verapamil (Sigma) was used in concentration $5 \cdot 10^{-6}$ M. The contractile properties of 24 experimental and 18 control slow soleus muscles, and 16 experimental and 8 control fast plantaris muscles have been evaluated. For histochemical fibre type determination the muscle samples were immediately frozen in liquid nitrogen and transverse sections of 10 mm thick prepared in cryostat. Sections were stained for acid-stable myofibrillar ATPase with preincubation at pH 4.6. Slow type 1 fibres were distinguished as "dark" fibres, and fast type 2 fibres - as "light" (2A) and "intermediate" (2B) ones. Cross-sectional area was measured in 100 fibres of each type by means of image analysis system ASM-68K (Leuca, Germany).

Results

The contractile properties of slow and fast muscles after hindlimb suspension

The amplitude, time of rise, half-time of decay of twitches, and tetanus amplitudes were studied in muscles of control and hindlimb suspended rats. The example of twitches in slow soleus and fast plantaris muscles are presented in Fig. 1. One can see that in soleus muscle after

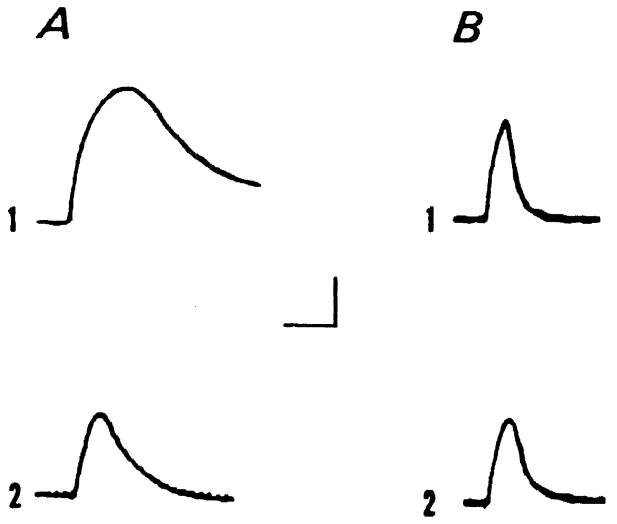

Figure 1. Twitch contractions of soleus (A) and plantaris (B) muscles in control [1] and after hindlimb unloading [2]. Time calibration bar: 0.1 s, force calibration bar: 0.04 N (A) and 0.1 N (B).

suspension the rate of rise of twitch was sufficiently increased and the peak force reduced. No changes can be seen in the contractions of plantaris. Average values of contractile characteristics are shown in Table. 1. Measurements indicated that besides twitches, the amplitude of tetanic contraction dropped in slow muscle after limb suspension as well, but in fast muscle this drop was unreliable.

The effects of Ca-free solution on contraction of control and unweighted muscles

Incubation of slow soleus muscle in Ca free solution resulted in time-depended reducing of the peak force of twitches as well as of maximal tetanic contractions in both control and suspended animals (Table 2). In the Table 1 can be seen that Ca ions removal resulted also in time of rise shortening, and in unweighted soleus - in time of half-decay reducing. Fig. 2A shows that after 15 min exposure in Ca-free medium tetanic force of soleus declined by 45-55%. The tetanic peak tension declined in Ca-free solution more rapidly in suspended than in control muscles (Fig. 2). This effect was reversible when changing the Ca-free to normal Ringer.

The tetanic tension of fast plantaris muscle was also declined in Ca-free solution. But the comparison between fast muscles of control and suspended rats was complicated by the run down of tension occurred in fast muscles spontaneously in all experiments (Fig. 2B). Effect of Ca^{2+} deficiency could be seen after subtraction of curves for normal and Ca-free solution. This subtraction shows that the amplitude of tension did not change in fast muscle. After suspension, it was no considerable decline in tetanic neither in twitch contraction amplitudes in Ca-free medium, as compared with control fast muscles (Fig. 2 and Table 2).

Table 1. Isometric contractile parameters of slow and fast muscles from normal and suspended rats

Solution	Parameter	Soleus		Plantaris	
		Control	Suspended	Control	Suspended
Ringer	Twitch tension mN	113 ± 8 (12)	43 ± 6* (20)	194 ± 14 (12)	196 ± 15 (14)
	Time to peak, ms	85.5 ± 8.0 (12)	58.5 ± 5.5* (17)	19.5 ± 0.9 (10)	23.5 ± 2.5 (14)
	Half relaxation time, ms	104.0 ± 5.5 (12)	94.0 ± 6.0 (15)	14.5 ± 0.5 (11)	15.0 ± 1.5 (14)
	Tetanic tension mN	493 ± 41 (12)	268 ± 21* (18)	478 ± 50 (10)	428 ± 10 (14)
Calcium-free	Time to peak, ms	50.0 ± 3.0$^+$ (11)	44.5 ± 5.8$^+$ (11)	18.0 ± 1.0 (6)	– (1)
	Half relaxation time, ms	105.5 ± 6.0 (11)	72.5 ± 4.5$^+$ (8)	18.0 ± 1.5 (6)	– (1)

Note: Data are means ± SEM with numbers of muscles in parentheses; * significantly different from control value, $p < 0.05$; $^+$ significantly different from Ringer solution, $p < 0.05$.

Table 2. The effect of calcium free solution on twitch tension of slow and fast muscles from normal and suspended rats

Muscle	Soleus			Plantaris		
	Time of incubation, min			Time of incubation, min		
	15	30	45	15	30	45
Control	43 ± 7 (14)	31 ± 6 (12)	19 ± 5 (16)	31 ± 9 (8)	15 ± 4 (8)	5 ± 2 (4)
%						
Suspended	55 ± 7 (18)	37 ± 7 (18)	25 ± 5 (15)	34 ± 7 (12)	21 ± 3 (7)	13 ± 2 (10)
%						

Note: Relative values (tension in Ringer 19 taken as 100%) are means ± SEM with numbers of muscles in parentheses. No significant changes were observed.

Conclusion can be made that hindlimb suspension results in reduction of the resistance to Ca-deficiency in surrounding media in slow but not in fast muscles.

Muscle fatiguability after hindlimb suspension and the action of Ca deficiency

The effects of unweighting on the fatigue resistance of fast and slow muscles have been studied using Ringer and Ca-free solution. Fatigue was induced by repetitive tetani elicited by trains of stimuli applied as described in Methods. Fig. 3 and 4 show the examples of record of the contractions of soleus and of plantaris muscles. In control soleus in Ca-free solution the fatigue appeared more rapidly than in Ringer. However, this fall of fatigue-resistance under Ca^{2+} deficiency conditions was completely

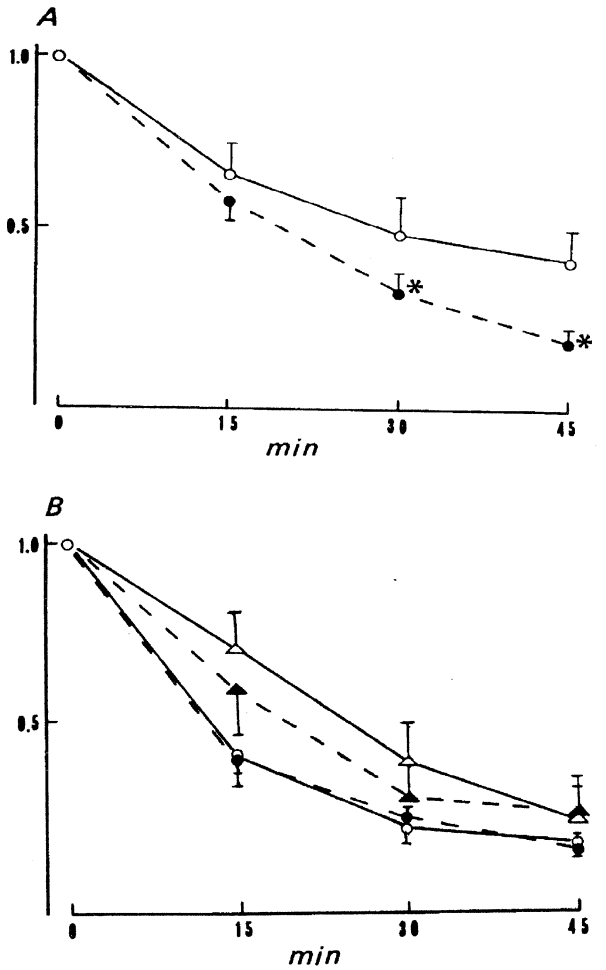

*Figure 2. Tetanic (50 Hz) tension in % to the initial value against the time skale in Ca-free solution of soleus (A) and plantaris (B) muscles in control (○) and after hindlimb suspension (●); in B - as compared with date obtained in Ringer, also in control (△) and after hindlimb suspension (▲). Date presented are the means ± SEM of 4-7 preparations; * - difference reliable, $p < 0.05$.*

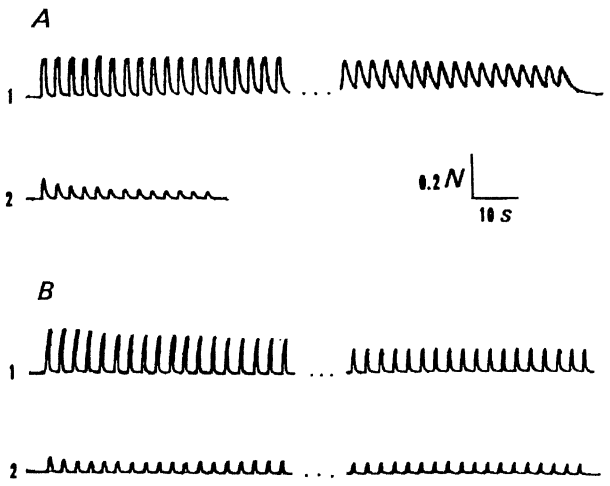

Figure 3. The records of tetanic trains of 0.5 s duration every 3 s, 100 Hz, of control soleus (A) and soleus of unweighted hindlimb (B), in Ringer [1] and in Ca-free solution [2]. The interruptions in records - 100 s.

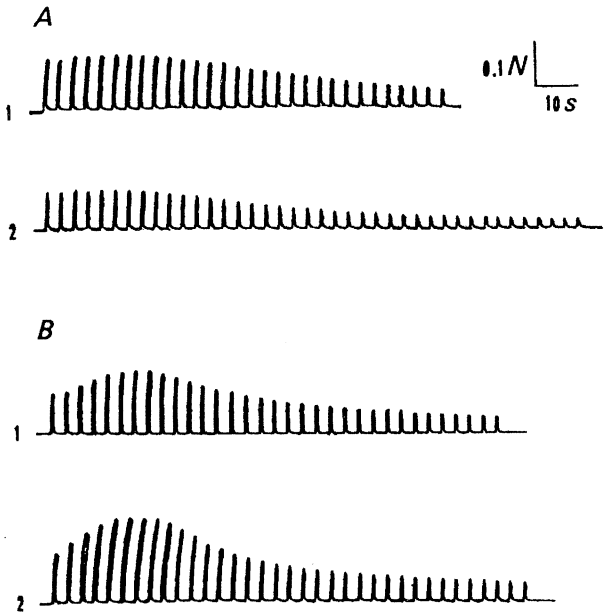

Figure 4. The records of tetanic trains of 0.5 s duration every 3 s, 100 Hz, of control plantaris (A) and plantaris of unweighted hindlimb (B), in Ringer [1] and in Ca-free solution [2].

absent after hindlimb suspension, and the time of fatigue in normal and Ca-free solution became identical (Fig. 5). It can be concluded that unloading made slow muscle much less sensitive to Ca^{2+} removing from external media.

Fast plantaris muscle fatigued more rapidly than slow ones (Fig. 4). The time course of fatigue in fast muscle did not change in Ca-free solution. Neither it was changed in fast muscles after hindlimb suspension both in normal and in Ca-free solutions (Fig. 5). It means that high fatigue ability of fast muscles did not change after unloading.

The effect of Ca-channel blocker verapamil (Ver) on fatigue of slow soleus muscle was also investigated. Fig. 5 shows that in control soleus Ver evoked the same fall of fatigue resistance as the action of Ca-free solution. But it appeared a difference with the action of Ca-free solution in the muscles of suspended hindlimb. Ver caused in this case the same decrease in resistance to fatigue as in control muscles. In contrast to Ca^{2+} removing, Ver action did not improve the fatigue properties in unloaded soleus.

Muscle fibre size and type ratio after hindlimb suspension

Histochemical estimations of the content of different muscle fibre types have shown that the percentage of slow-twitch fibres (type 1) in soleus muscle in hindlimb

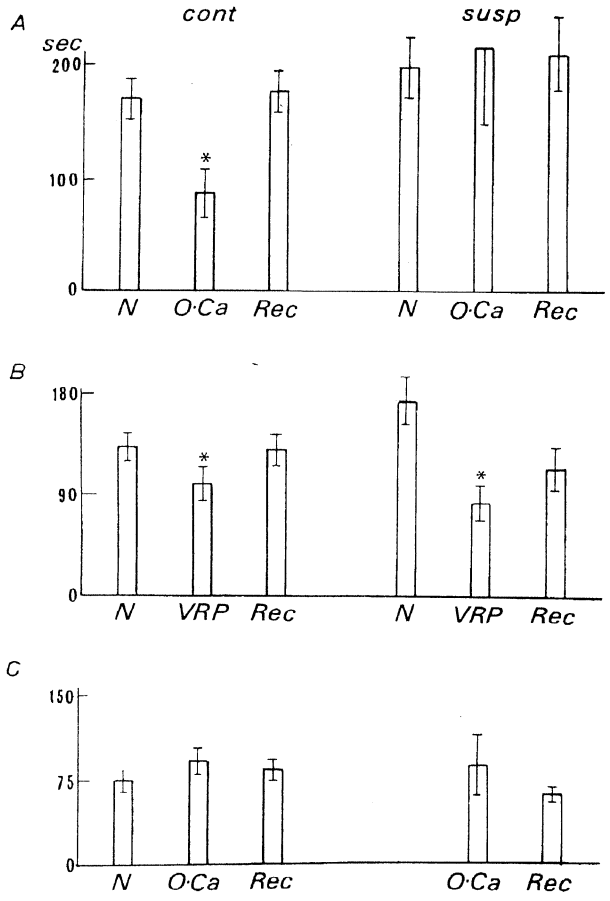

*Figure 5. The effects of Ca-free solution (A and C) and verapamil 5.10-6 M (B) on the fatigueresistance (as time to 0.5 of initial tetanus aplitude) of soleus (A and B) and plantaris (C) in control and after hindlimb suspension. Mean of 5 - 8 records, ± S.D.M., * - difference reliable, p ± 0.05.*

suspended animals was significantly lower (by 15%) than in control group of muscle fibres (Fig. 6). The same trend of decreased percentage of slow-twitch fibres after suspension was observed also in fast plantaris muscle. At the same time the atrophy of muscle fibres was demonstrated after hindlimb suspension in type 1 fibres of soleus as well as of plantaris muscle (Table 3). This Table shows that the mean cross-sectional area (CSA) of type 1 fibres in soleus muscle of hindlimb suspended rats was reduced by 49% as compared to the control values. Atrophy of type 2A fibres (by 37%) in this muscle was also significant but not so profound. Significant decrease (by 26%) of CSA in fast plantaris muscle after hindlimb suspension was found only in type 1 fibres. Values of CSA of 2A and 2B fibres in plantaris muscle of hindlimb suspended rats did not differ from control level (Table 3).

Discussion

The results obtained show that slow soleus muscle after hindlimb suspension became more sensitive to external Ca-deficiency. When this muscle was stimulated only once in 10-15 min. and so remained unfatigued, the force output decline in Ca-free solution appeared reliable faster in experimental muscle than in control one (Fig. 2A), although only for tetanic but not for single contractions. It is known that skeletal muscles do not need the external Ca^{2+} for contraction activation, using for this purpose calcium ions stored in SR [5, 11]. But the sensors of membrane voltage changes, which trigger SR Ca^{2+} release, and are connected with slow DHP sensitive Ca-channels on the T-membrane [1, 17, 18], need bound Ca^{2+} for their functioning. These Ca^{2+} must be recycled by repetitive activation during tetanus [4, 10, 14]. This can explain why tetanic tension is more sensitive to Ca-deficiency than single twitches. The suggestion can be made that faster decline of tetanic contractions in Ca-free solution after hindlimb suspension may point to the decrease of Ca-affinity of the voltage sensors on DHP receptors.

The results described here show that in Ca-free solution the fatigue in response to regular tetanization developed much faster than in Ringer. After unloading, in the slow-twitch muscle Ca-deficiency lost its ability to affect the fatigue-resistance (Fig. 3). It is known that the acceleration of fatigue in Ca-free media is the specific property of slow-twitch muscles [16]. In this aspect it is important to note that in our experiments the relative amount of slow fibres in soleus muscle decreased, and the mean CSA of slow-twitch type 1 fibres reduced after hindlimb suspension. These findings are in agreement with the common idea that after hindlimb unweighting the slow muscle acquired contractile and SR properties that were much like those of a fast muscle [7, 13, 19]. The transformation of slow-twitch fibres to some extent into fast-twitch ones, can explain the reduction of sensitivity to Ca-deficiency in suspended animals in our experiments.

Alternatively, an assumption can be made that in our experiments the myoplasmic Ca^{2+} during the active state was much increased, which is due to at least 3 factors: suspension, which increases SR Ca-release [19], fatigue, which decreases SR Ca-uptake by impaired function of SR [21], and decrease of calcium binding to troponin C [7]. Perhaps similar changes develops also in denervated muscles, because the hypertrophy and hyperfunction of SR were described after denervation [6, 9, 15].

If the activation induced myoplasmic Ca^{2+} increase is really enhanced in slow muscle fibres after chronic muscle unloading, this can explain some date described here. Under Ca-deficiency the voltage sensors on the T-membrane decline their activity during tetanus, because they need, as mentioned above, free Ca^{2+} recycling for repetitive activation, and the fatigue accelerates. After hindlimb unweightening, in the case of sarcoplasmic Ca^{2+} elevation, evoked by above mentioned reasons, and in the absence of Ca outside, calcium ions can penetrate the slow Ca-chan-

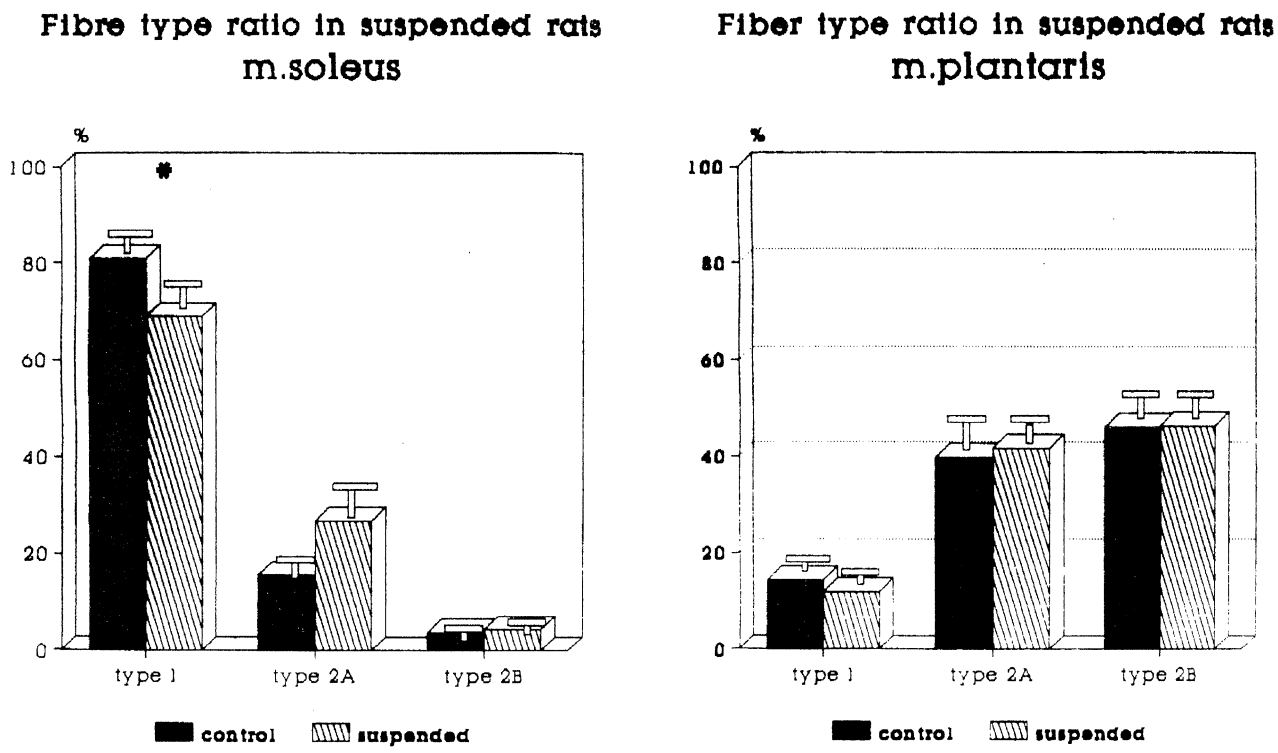

*Figure 6. Muscle fibre type ratio in control and suspended soleus (A) and plantaris (B) muscles. Mean of * - difference reliable, $p < 0.05$.*

Table 3. The effect of suspension on transversal section area of different types of rat muscle fibres ($m\mu^2$)

		Soleus		Plantaris		
Fibre type		I	2A	I	2A	2B
Control,	mean	2234	1397	914	1074	181
	± SEM	189	145	70	123	163
Suspension,	mean	1143	887	674	962	1845
	± SEM	86	35	49	65	98
Difference,	%	-49	-37	-26	-10	–

nels from inside by gradient and bound voltage sensors on the outside of T-membrane. This will restore the fatigue resistance, as was observed on suspended animals in experiments. This assumption was supported by D600 action. In control its action was similar to those of Ca-free solution, reducing the fatigue resistance. But in unloaded muscles the increased myoplasmic Ca^{2+} can not penetrate Ca-channels, because the channels are blocked by D600, and in addition there is no Ca^{2+} gradient (outside is normal Ca^{2+} concentration). As a result, in unweighted muscles D600 did not improve the fatigue resistance as did the Ca-free solution.

In fast plantaris muscle any obvious changes in Ca-sensitivity after hindlimb suspension were not registered. Very small and unreliable changes were observed in fibre atrophy. These results are consistent with the published works suggesting that the microgravity affects predominantly the slow, antigravity muscles, and very little if ever influences the fast ones [7, 13, 22].

The cause of the peak contraction force fall after suspension can be assumed to be the muscle fibre atrophy (decrease of fibre CSA) and/or decrease of type 1 percentage in slow muscle [7]. In our study, the twitch contraction peak in unloaded soleus decreased by 62% and tetanic contraction peak fell by 46%, while the percentage of slow-twitch fibres in this muscle decreased by 15% and their CSA fell by 49%.

The conclusion can be made that the response of at least soleus muscle to fatigue and to Ca-free solution was sufficiently altered after exposure to gravitational unloading. This muscle becomes resistant to the influence of Ca-free medium in the course of fatigue. The results suggest that the unloading can modulate the functioning of the initial part of the ECC system, presumably the voltage sensors on the T-membrane.

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