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RAPID COMMUNICATION

Resting Membrane Potential and Na^+,K^+-ATPase of Rat Fast and Slow Muscles during Modeling of Hypogravity

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Summary

Antiorthostatic hindlimb suspension (unloading) decreased the resting membrane potential (RMP) of skeletal muscle fibers in fast extensor digitorum longus (EDL) and slow soleus (SOL) muscle of the rat by about 10 % within 7 days and more. Inactivation of the membrane Na^+, K^+-pump by ouabain brought about similar depolarization as unloading. The increased sodium permeability of the membrane was excluded as the major cause of this depolarization by experiments in which TRIS was substituted for Na^+ in the medium. On the other hand, the decrease in the electrogenic participation of the Na^+,K^+-pump is apparently one of the causes of RMP decrease during hypogravity, in EDL muscle in particular.

Key words

Skeletal muscle • EDL • Soleus • Resting membrane potential • Hypogravity • Sodium pump

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Changes in activity patterns bring about substantial alterations in the morphological and functional organization of muscle fibers (Zelená *et al.* 1979, Volkov 1994, Flück and Hoppeler 2003). For example, the loss of muscle mass by 37 % in the rat soleus immobilized for 7 days in a shortened position is accompanied by a membrane depolarization of about 5 mV, by decrease in frequency of miniature end-plate potentials and in [^{3}H]ouabain binding (Zemková *et al.* 1990). Profound structural and functional transformation of the muscle were also observed during hypogravity (Thomason *et al.* 1987, Thomason and Booth 1990, Kozlovskaya and Shenkman 1996, Fitts *et al.* 2000, Adams *et al.* 2003). There is one particular parameter of the muscle membrane, the resting membrane potential (RMP) that most importantly, has an immediate effect on the transmission of excitation to contraction (Petrov *et al.* 2009). During modeling of hypogravity, a substantial decrease in RMP was observed together with an enhancement of Na^+ channel expression and Cl^- channel conductance (Desaphy *et al.* 2001, Pierno *et al.* 2002, 2007). However, these reports, while groundbreaking, do not unequivocally identify the mechanism(s) responsible for hypogravity depolarization (Urazaev *et al.* 1995). Here we imitated the hypogravity by hind limb unloading and analyzed the RMP in fast extensor digitorum longus (EDL) and slow soleus (SOL) muscles. Experiments were aimed at verifying the role of Na^+ permeability and the Na^+, K^+ pump in the hypogravity depolarization.

The experiments were done on isolated muscles of male adult Wistar rats (body mass 250-280 g). We followed the international rules and instructions of the Ministry of Health of the USSR and of the European

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Community Directive of 1986 (86/609/ECC). The animals were kept under the conditions ensuring hind limb unloading (Morey-Holton *et al.* 2005) over periods of 7, 14, 21 and 28 days. Animals were suspended individually in special cages. A shoelace was linked at one extremity to the base of the tail by a sticking plaster and at the other extremity to a trolley that can move on horizontal rails at the top of the cage. This allowed the animals to move freely on their forelimbs. Animals were inspected at least three times a day and were sacrificed under ether narcosis.

The isolated muscles were placed in an experimental vessel and superfused with a Ringer-Krebs solution of the following composition (in mmol/l): NaCl 118.0, KCl 5.0, $CaCl_2$ 2.0, $MgCl_2$ 2.0, Na_2HPO_4 1.0, $NaHCO_3$ 24.0, glucose 17.0, aerated with a mixture of 95 % O_2 and 5 % CO_2 pH 7.2-7.4. The RMPs were measured by standard intracellular microelectrode technique (2.5 M KCl, 10 MΩ, Petrov *et al.* 2006) at 20.0±0.2 °C in the extrasynaptic zone of superficially located muscle fibers. When TRIS (or N-methyl-D-glucamine in several experiments with similar results) were substituded for Na^+, their concentrations were selected to maintain the original osmolarity of the solution (308 mOsmol/l). A total of 20-30 measurements of RMP were carried out in each muscle preparation from 20 to 30 min after excision. In experiments with 10^{-4} mol/l ouabain (Serva), the RMP recordings were done 10-15 min after adding the drug. During this relatively stable period of lowered RMP, only electrogenic effect of the pump is inhibited. Further on, the ion gradients are affected and membrane rapidly depolarizes (Vyskočil and Gutmann 1977). The mean values ± S.E.M in each series were obtained from experiments on 6-7 animals and statistical significance of the differences was verified using Student´s t-test, with a significance limit of $p<0.05$ (Volkov *et al.* 2007).

Control experiments showed that the RMP in EDL had a value of 71.1±0.3 mV (inside minus) and somewhat lower value 68.1±0.2 mV in SOL (Fig. 1, Table 1, cf. Jones and Vyskočil 1975, Zemková *et al.* 1982, 1990). Prolonged unloading brought about a decrease in RMP. After 7 days, it was 65.2±0.5 mV in EDL and 68.1±0.2 mV in SOL. The RMP remained equally decreased (Fig. 1, Table 1) after up to 28 days with little tendency to increase or decrease further. This indicates that the transmembrane potential difference reached a new stationary level which, in turn, assumes the changes in the mechanisms of muscle fiber RMP, as observed under pathophysiolocal conditions such as denervation (Albuquerque and McIsaac 1970, Shabunova and Vyskočil 1982). It is supposed that one of the causes of RMP decrease may be an increase in permeability of the sarcolemma for Na^+ (Desaphy *et al.* 2001, Pierno 2002). To verify the role of Na^+ permeability, RMP measurements were done in solutions where sodium in the Ringer-Krebs solution was replaced with TRIS (or N-methyl-D-glucamine in several experiments with similar results). If the RMP decrease were due to an increase in membrane permeability for Na^+, then the removal of sodium ions from the medium should result in hyperpolarization of the muscle fibers by removing the tetrodotoxin-sensitive Na^+ background current (Vyskočil 1974). However, in control animals, Na^+-free solution decreased the RMP (Fig. 1). After unloading, the absence of Na^+ from the medium also depolarized the muscle fiber membrane, but this change in RMP in muscles after 7-day unloading was significantly less pronounced (Fig. 1). Consequently, the hypothesis of the increased sodium permeability of the membrane as the major cause of RMP decrease under conditions of modeling hypogravity does not appear to be substantiated. This differs from immobilization caused by denervation, where an increase in Na^+ inside the fibers was found (Shabunova and Vyskočil 1982).

Table 1. Mean values of muscle fiber RMP in EDL and soleus in control animals and after unloading (in days).

Animal group	EDL	Soleus
Control	71.1±0.3 mV (*n* = 120)	68.1±0.2 mV (*n* = 120)
7 days	65.2±0.5 mV (*n* = 250) *	62.3±0.6 mV (*n* = 250) *
14 days	66.2±0.3 mV (*n* = 430) *	63.1±0.3 mV (*n* = 314) *
21 days	66.1±0.3 mV (*n* = 250) *	64.2±0.4 mV (*n* = 250) *
28 days	66.3±0.4 mV (*n* = 250) *	62.2±0.5 mV (*n* = 273) *

n = number of fibers; asterisks- significant differences of RMP (inside minus) of muscle fibers after unloading as compared with control ($p<0.05$).

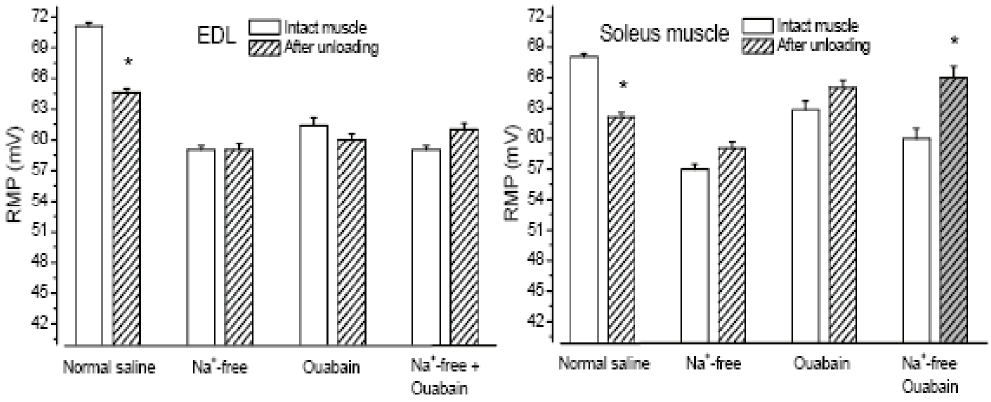

Fig. 1. Effect of 10^{-4} mol/l ouabain and absence of sodium ions (Na$^+$-free medium) on RMP value (inside minus) of muscle fibers of fast EDL and slow soleus recorded in control (intact) rats and in animals after 7 days of unloading, induced by hind limb unloading. Asterisks – statistically significant differences between RMP values of muscle fibers in intact animals and those after unloading.

Another cause of an increase in the depolarization of muscle fibers during hypogravity modeling might be due to a change in the efficiency of the sarcolemma Na$^+$, K$^+$-pump. It is known that the overall size of the RMP includes a potential arising from the electrogenic activity of the Na$^+$, K$^+$-ATPase during an asymmetric transfer of Na$^+$ and K$^+$ ions in particular (Akaike 1979).

In an intact EDL, the inactivation of the Na$^+$, K$^+$-pump by ouabain decreased the RMP of control and suspended muscles within 10 min in similar way as removal of Na$^+$ from the solution and the values of RMP were similar in both a sodium-free solution and in the presence of ouabain (Fig. 1 left, cf. Novotný *et al.* 1962, Vyskočil and Gutmann 1977). This decrease was less pronounced in control SOL. Consequently, the overall size of the RMP includes the electrogenic pump component, but the contribution is smaller in slow muscle. What is important, addition of ouabain to EDL in Na$^+$-free solution did not further enhance the depolarization. It follows that the decrease in the RMP under the influence of ouabain or sodium deficiency appears to result from Na$^+$, K$^+$-pump inhibition. Because this decrease is relatively smaller after unloading (cf. Fig. 1, white columns vs. hatched ones in normal and Na$^+$-free solutions without and with ouabain), it indicates less participation of the Na$^+$, K$^+$-pump in the RMP during unloading. Inasmuch as inactivation of the ionic pump as well as the suspension brought about muscle membrane depolarization (Fig. 1), one of the causes of RMP decrease and transition to a new, lower level is apparently the decrease in the electrogenic participation of the Na$^+$, K$^+$-pump in the RMP, rather than an increase in membrane permeability for Na$^+$ ions.

In the slow SOL muscle, in contrast to the fast EDL one, we found smaller RMP decrease in the presence of ouabain after seven days of unloading in comparison with control values. It may imply the changed ratio of different Na$^+$, K$^+$-ATPase isoforms with different sensitivity to ouabain (Dostanic-Larson *et al.* 2006). It is of interest that significant increase in RMP was found upon addition of ouabain to the SOL in Na$^+$-free medium after unloading (Fig. 1, hatched column). Similar hyperpolarization by ouabain was observed earlier in the muscles of hibernating hamsters (Teisinger *et al.* 1981) and in some neurons (Matsumoto *et al.* 2008). It probably reflects Ca^{2+} influx (Shenkman and Nemirovskaya 2009) and subsequent increase of the Ca^{2+}-dependent K$^+$ conductance, which can result in hyperpolarization. Still, this interesting finding is at present difficult to interpret unequivocally and further specific investigation will be required, as well as the role of Cl ions.

Unloading brings about a decrease in RMP and transition to a lower stationary level in the fibers of both fast and slow mammalian muscles. The reason for such a decrease is most probably caused by the lowering of the electrogenic component of the Na$^+$, K$^+$-pump. A similar drop in the Na$^+$, K$^+$-ATPase expression in the membrane (lowering of ouabain-binding sites) was observed in SOL muscle immobilized in a shortened position (flexion) by a cast of plastic material by Zemková *et al.* (1990). As the muscles of suspended hind limbs are free to move, this state cannot be rightfully called immobilization; rather muscles are virtually unloaded and can shorten only isotonically. Because this model of hypogravity simulates to some extent the conditions during spaceflight (Caiozzo *et al.* 1996), the decrease in RMP could be a significant link in the chain of events leading to the destructive and eventually atrophic changes in the muscle fibers. Hovewer, not all functional changes could be apparently linked to RMP changes; there are several studies (even in man, Flück and Hoppeler 2003) demonstrating that the unloading affects – contrary to present results – more significantly the slow antigravity SOL muscle that looses its tonic activity; the intermitently contracting fast EDL muscle is mostly unaffected (Adams *et al.* 2003). Interestingly, the long-term unloading due to six-week tail suspension hypokinesia did not induce any deleterious morphological signs on mATPase stained SOL and EDL muscle sections. (Asmussen and Soukup 1991).

It is known that an accumulation of Ca^{2+} ions in the fibers plays important role in the pathogenesis of these processes (Ingalls *et al.* 1999, Shenkman *et al.* 2004, Mukhina *et al.* 2006). It was recently shown that the basic path of movement of Ca^{2+} into the suspended fiber appears to be the activation of voltage- and dihydropyridine-sensitive Ca^{2+}-channels of the sarcolemma (Mukhina *et al.* 2006). It is quite possible that the decrease in RMP may increase the probability of opening of voltage-activated Ca^{2+} channels, which in turn increase the level of free Ca^{2+} in the myoplasm and trigger unloading atrophy (Shenkman and Nemirovskaya 2009).

Conflict of Interest

There is no conflict of interest.

Acknowledgements

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