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Physiological triggers involved in reduced slow myosin expression in disused postural muscle

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Objective It is well known, that the reduced contractile activity of the postural soleus muscle under bedrest, immobilization or a space flight leads to decrease of slow myosin heavy chain (MyHC) expression rate and increase of the fast myosin isoforms expression [Pette, 2003; Stevens, 1999, 2000 et al]. The significant decline of the slow myosin mRNA content was found as early as after 24 hours of rat hindlimb unloading [Giger et al., 2009]. However, in the meantime, the mechanisms of this process had been substantially unexplored. At the same time, the main pathways involved in the control of transcription of *Myh7* gene (MyHCIβ) are well known. These mechanisms are based upon traffic of messenger molecules (NFATc1 and Class IIA histone deacetylases) transducing positive and negative signals for *Myh7* gene expression in muscle fiber nuclei. This traffic is known to be triggered by myoplasmic calcium content. Almost nothing is known about the roles of other physiological regulators (nitric oxide and high-energy phosphates) in *Myh7* transcription control [Martins et al., 2012; Putman et al., 2015].
Our study was aimed to disclose the physiological triggers involved in the decline of *Myh7* expression in postural muscle at the early stages of disuse state. We supposed that at the early stage of unloading (24 hours) it was the shift of the ATP/ADP/AMP balance (ATP accumulation due to muscle inactivation) to drive the *Myh7* gene expression decline via AMP-activated protein kinase (AMPK) dephosphorylation and HDAC4 myonuclear import. Then we supposed that the mechanisms involved in the reduction of *Myh7* expression during the first week of disuse are implemented via the decrease of NO muscle content [Lomonosova et al., 2011] and subsequent NFATc1 nuclear export in the GSK3β-dependent manner [Lomonosova et al, 2017].
Methods Three experimental series were performed in order to testify the hypotheses. Unloading of the hindlimbs was induced by using a standard rodent hindlimb suspension/unloading (HU) model (Morey-Holton & Globus, 2002). During the first series, using the selective AMPK activator AICAR we evaluated the roles of the AMPK dephosphorylation during the first days of unloading which we found earlier [Vilchinskaya et al., 2015; Mirzoev et al, 2016]. Animals were daily treated with AICAR (400 mg kg^{-1}) or saline for 6 days before HU as well as during 24 h of HU.
The second series was designed to investigate the impact of high-energy phosphates ratio changes on AMPK activity and slow-type MyHC isoform expression in rat soleus muscle at the early stages of unloading. It is known that administration of β-guanidin-propionic acid (βGPA) allows shifting ATP/ADP/AMP balance to the enhanced ATP breakdown. We used administration of β-guanidinopropionic acid (βGPA), before (6 day) and during 24-h HU.
The third series was aimed to identify the functional relationship between the decrease of the nitric oxide (NO) content, the GSK-3β phosphorylation (leading to the GSK-3β activation), the NFATc1 amount in the muscle nuclei, and the MyHC I(β) isoform expression in the rat soleus muscle under gravitational unloading. Male Wistar rats were divided into five groups: the vivarium control group; the group of animals with a 7-day hind limb suspension receiving placebo; the group of HU animals receiving a NO donor (L-arginine); the group of HU animals receiving a NO donor and a NO-synthase inhi bitor (L-NAME) and the group of HU animals receiving a GSK-3β inhibitor.
Results In the 1st experimental series we discovered that AICAR treatment prevented a decrease in content of phospho-AMPK and pre-mRNA and mRNA expression of MyHC I as well as MyHC IIa mRNA

expression. Twenty-four hours of HU resulted in HDAC4 accumulation in the nuclei of rat soleus but AICAR pretreatment prevented this accumulation. The results of the study indicate that AMPK dephosphorylation after 24 h of HU had a significant impact on the MyHC I and MyHC IIa mRNA expression in rat soleus. AMPK dephosphorylation also contributed to HDAC4 translocation to the nuclei of soleus muscle fibers, suggesting an important role of HDAC4 as an epigenetic regulator in the process of myosin phenotype transformation.

In the 2nd experimental series after 24-h HS we observed a decrease ($p<0.05$) in phospho-AMPK content vs. control group, but in HS+ βGPA group didn't differ from the control. After 24-h unloading we found a significant increase in the content of nuclear HDAC4 in the HS group, but in the HS+ βGPA group the content of nuclear HDAC4 didn't differ from the control group. 24-h unloading resulted in a decrease in MyHCI(β) pre-mRNA and mRNA expression vs. the control group. The expression level of MyHCI(β) pre-mRNA and mRNA in HS+ βGPA group didn't differ from the control. Thus, βGPA administration prevents a decline in AMPK phosphorylation. Therefore, we can conclude that at the early stage of gravitational unloading an accumulation of high-energy phosphates (ATP, ADP and creatine phosphate) may lead to reduced AMPK activity and a slow to fast myosin fiber type transition.

The third experimental series dealt with the fate of the nuclear NFATc1 transcription factor which triggers the myh7 expression but can be easily exported from myonuclei being phosphorylated. It is supposed that it is NO-dependent GSK3β that phosphorylates NFATc1 and promotes its nuclear export. We have shown that a 7-day unloading leads to a NO content decrease in the soleus muscle, and this effect is prevented by L-arginine administration. In addition, administration of L-arginine blocks the GSK-3β phosphorylation decrease, NFATc1 export from the muscle nuclei, and MyHC I(β) expression decrease caused by unloading. The NO-synthase inhibitor can block the L-arginine effect in each case. Administration of the GSK-3β inhibitor prevents the unloading-induced NFATc1 export from the muscle nuclei and a decrease of the MyHC I(β) expression.

Conclusions The data obtained in the described experimental series give evidence for the novel view on the well-known phenomenon of slow-to-fast fiber type transition during unloading/disuse. It is obvious that the signaling pathways involved in the slow myosin gene expression control during unloading are time-dependent and consecutive in the course of the exposure to unloading. The earliest triggering factor is supposedly the shift of the balance of high-energy mononucleotide phosphates leading to decrease of AMP and accumulation of ATP content. This signal is accepted by the AMPK as a universal energy sensor and transduced to the transcription level by the altered HDAC4 traffic. It seems possible that at the next stages of the exposure to unloading the alteration of the calcineurin/NFATc1 signaling pathway takes place due to the activated calcineurin inhibitors [Lomonosova et al, 2017] and enhanced NFATc1 phosphorylation and myonuclear export. We obtained the novel evidence that at these stages the decline of *Myh7* expression might be provided by the GSK3β activation and NFATc1 phosphorylation due to the decrease of NO content in the soleus muscle.

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