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Contractions dynamic of "fast" and "slow" rat muscle under spinal shock and modulators of contraction

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Abstract

Aim. To study the dynamics of neuromotor regulation of the contractile function of "fast" and "slow" muscles in rodents during spinal shock by spinal cord transection at the level Th_{11}–Th_{12}.

Methods. The experiments were carried out on laboratory rats weighing 140–180 g. The animals were divided into two groups: "Control" (8 rats) and "Spinal shock" (6 rats). The lower leg muscles, *m. soleus* and *m. extensor digitorum longus* (*m. EDL*), were dissected by partially isolating without disrupting the connection with the body's circulatory system. The sciatic nerve was stimulated with single electrical impulses (10 V, 0.5 ms). Contractions of both muscles caused by electrical stimulation of the sciatic nerve before and after the injection of the substances into the femoral artery — tubocurarine (1 mM) or norepinephrine (10 mM) — were recorded in animals of both groups. After spinalization, muscle contractions were re-recorded during electrical stimulation of the sciatic nerve before and 10 minutes after the injection of tubocurarine or noradrenaline into the femoral artery in the same concentrations.

Results. After spinalization of the animal, the contraction force of the muscle *m. EDL* fibers increased to 0.43±0.03 g (p=0.040), but the temporal parameters remained unchanged. *M. soleus*, on the contrary, showed a decrease in the contraction time to 0.053±0.005 s (p=0.045), and no change in the contraction force was observed under these conditions. Intra-arterial administration of norepinephrine in the control group resulted in an increase of *m. soleus* contractions up to 1.21±0.17 g (p=0.048), and *m. EDL* — up to 0.57±0.07 g (p=0.043). The administration of norepinephrine in spinalized animals caused an increase in the contraction of *m. soleus* up to 1.21±0.09 g (p=0.047), and *m. EDL* up to 0.66±0.05 g (p=0.043). The blocker of postsynaptic cholinergic receptors tubocurarine administration reduced the force of contraction of both muscle types in both control [*m. soleus* up to 0.39±0.03 g (p=0.039), *m. EDL* up to 0.11±0.02 g (p=0.042)] and spinalized [*m. soleus* up to 0.34±0.05 g (p=0.039), *m. EDL* up to 0.15±0.04 g (p=0.040)] animals.

Conclusion. The data obtained demonstrate the presence of significant differences in the mechanisms of control of contractile activity in the "fast" and "slow" skeletal muscles of warm-blooded animals; the persistence of the similar effect of the basic modulators on the contraction of both muscles with such a striking reaction to spinalization highlights the contribution of neurotrophic control to the functioning of "fast" and "slow" motor units.

Keywords: spinal shock, "fast" and "slow" skeletal muscles, isometric contraction, modulators of contractile activity.

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Background. The search for treatment of patients with spinal cord injuries remains relevant for medicine and biology. Moreover, the main attention is paid to the restoration of motor function. However, there are no data on how the parameters of muscle contractions change after spinal cord injury, despite the undoubted presence of these changes [1–4]. These changes should be ensured by the so-called nervous trophism, which is understood as neuronal influences necessary to maintain normal vital activity of innervated structures, namely, neurons and somatic cells [2, 5].

The term "nervous trophism" is not entirely accurate, since substances secreted by nerve endings

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and having a trophic effect are not nutrient substrates and do not provide nutrition for the target cell [5]. To a greater extent, they regulate structural and metabolic processes; therefore, in recent years, the term "neurotrophic control" has been widely used [2]. Neurotrophic control is understood as control associated with special trophic factors formed in the neurons and innervated structures, so-called neurotrophic factors [1–5].

With spinal shock, the influence of neurotrophic factors is eliminated. Spinal shock occurs with various spinal cord injuries. Although the mechanisms of this post-traumatic state have not been fully identified [6–9], the modern concept of the development of spinal shock is based primarily on the works of Sherrington and his followers [9–11]. It is assumed that post-traumatic inhibition of the functions of the spinal structures lying below the injury site is largely the result of the removal of the excitatory effect of neurons in the supraspinal parts of the central nervous system (CNS), which ultimately leads to a change in the efficiency of synaptic transmission in motor units [11].

Based on the heterogeneity of skeletal muscles in terms of morphological and functional characteristics, we can assume that "fast" and "slow" motor units of warm-blooded animals have a different response to spinal cord injury.

The aim of this study is to examine the effect of spinal shock on the amplitude and time contraction parameters of the "fast" *m. extensor digitorum longus (m. EDL)* and "slow" *m. soleus* leg muscles of rats.

Material and methods. All procedures were carried out with the permission of the local ethical committee of Kazan State Medical University (Protocol No. 10 dated December 23, 2014).

Experiments were carried out on laboratory rats weighing 140–180 g. An oil solution of ether was used for anesthesia [12, 13]. Calf muscles – *m. soleus* and *m. EDL* – were prepared for an in situ experiment: The muscles were partially isolated without disrupting communication with the body's circulatory system, according to our original method [12]. The sciatic nerve was separated without disturbing the innervation of the leg muscles, after which the femoral artery was catheterized.

A stimulating immersion electrode was applied to the nerve (to which single rectangular electrical impulses with a voltage of 10 V and duration of 0.5 ms were applied). Muscle contraction was recorded using the ADInstruments PowerLab mini-laboratory; the analysis of the contraction was carried out according to its strength and duration [13].

All operations were carried out both on the control (8 rats) and spinal shock (6 rats) groups of animals. The number of animals in both groups corresponds to the calculated sample size at the given values of reliability (95%) and accuracy.

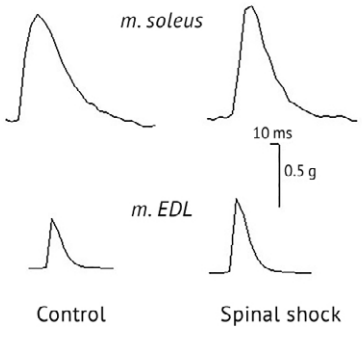

Fig. 1. Effect of spinal shock on the contraction parameters of rat *m. extensor digitorum longus* (m. EDL) and *m. soleus* (selected representative tracks are presented)

Laminectomy and subsequent transection were performed in the spinal shock group at the Th11–Th12 level using the McDowell spinal cord transection method [7]. The animals were placed in a mechanomyographic installation, and the distal tendon ends of the muscles were fixed to isometric sensors of the contraction force using ligatures.

Contractions of both muscles caused by electrical stimulation of the sciatic nerve before and after the introduction of substances into the femoral artery — tubocurarine (1 mM) or norepinephrine (10 mM) (Tocris Cookson and Research Biochemicals International, USA) — were recorded in the control and spinal shock groups [11].

After spinalization, muscle contractions were re-recorded during electrical stimulation of the sciatic nerve before and 10 min after the introduction of tubocurarine or noradrenaline in the same concentrations into the femoral artery.

Mechanomyographic experiments on rat *m. soleus* and *m. EDL* was evaluated using analysis of variance. The level of significance less than 0.05 were taken as reliable. Experimental data are presented as arithmetic mean ± standard error of the mean.

Results. Contraction curves of *m. soleus* and *m. EDL* of the control group are shown in Fig. 1 (left). The contraction force and contraction time of *m. soleus* were 0.80 ± 0.05 g and 0.078 ± 0.005 s, respectively, while those of *m. EDL* were 0.31 ± 0.02 g and 0.032 ± 0.003 s, respectively.

The introduction of norepinephrine to the control group increased the strength, but not the contraction time of *m. soleus,* by up to 1.21 ± 0.17 g ($p = 0.048$) and of *m. EDL* by up to 0.57 ± 0.07 g ($p = 0.043$). The administration of tubocurarine to the control group reduced the contraction force of *m. soleus* by up to 0.39 ± 0.03 g ($p = 0.039$) and that of *m. EDL* by up to 0.11 ± 0.02 g ($p = 0.042$).

The temporal parameters of contraction also did not differ from those before the introduction of the agents.

As shown in Fig. 1 (right), in the spinal shock group (with spinal cord injury), the contraction force of *m. soleus* did not differ from that of the control group (0.830 ± 0.084 g), while the contraction time decreased to 0.053 ± 0.005 s (p = 0.045). The contraction force of *m. EDL* of the spinal shock group (Fig. 1) increased to 0.43 ± 0.03 g (p = 0.040), while the contraction time did not change (0.036 ± 0.005 s).

In the spinal shock group, the dynamics of the strength and duration of contraction of both muscles under the influence of norepinephrine and tubocurarine was preserved. Norepinephrine increased the force of contraction in both muscles, by up to 1.21 ± 0.09 g in *m. soleus* (p = 0.047) and up to 0.66 ± 0.05 g in *m. EDL* (p = 0.043). Tubocurarine reduced the contraction force of both muscles, by up to 0.34 ± 0.05 g in *m. soleus* (p = 0.039) and up to 0.15 ± 0.04 g in *m. EDL* (p = 0.040).

Discussion. This study compared the responses of fast *m. EDL* and slow *m. soleus* muscles of rodents. Skeletal muscles constitute a heterogeneous population, for example, muscles can be "fast" and "slow," differing in strength, contraction rate, and qualitative composition of contractile proteins [1, 2].

Various characteristics of skeletal muscles are controlled by the nervous system, that is, the phenomenon of neurotrophic control [3, 4]. This control is carried out by motor neurons using low-molecular-weight factors synthesized in α-motor neurons and delivered to the muscle by axonal transport and is realized due to a pattern of impulse activity specific for each type of muscle.

However, the possible role of the higher divisions of the CNS in the implementation of neurotrophic control is not unclear. The unclear one is the role of associative neurons in such regulation, despite the extreme importance of knowledge of the possible contribution of such neurons to the implementation of neurotrophic control in clinic practice, especially its association with spinal cord injuries [1–4]. Insufficient research into the consequences of spinal cord injury is associated with inadequate information on the interrelated changes in the parameters of muscle contractions following a spinal shock.

Spinal shock is a trauma-caused state of temporary inhibition of the reflex activity of the spinal cord, which is described according to the degree of injury. In our studies, when choosing an experimental model of spinal shock, guided by the need for complete destruction of the spinal cord, we stopped at its anatomical transection [7, 11]. This model provides the onset of the most complete and lasting spinal shock, which is characterized, among other things, by a sharp change in the muscle tone of the paralyzed limbs [5, 14].

The results of our studies indicate that damage to the spinal cord changes the nature of muscle activity. Thus, spinalization contributed to an increase in the speed of the "slow" *m. soleus* and increased the contraction force of the "fast" *m. EDL*. The "fast" and "slow" muscles of intact rats, which respond in the same way to norepinephrine and tubocurarine, respond differently to spinal cord injury. This allows us to reveal the deeper than previously assumed differences between them, which consist not only in the differences in the qualitative composition of contractile proteins but also in the sensitivity to impaired neurotrophic control.

Based on the results of this study, we relied on the following provisions. Generally, neurotrophic control of various skeletal muscles is carried out by motor neurons using trophic factors delivered to muscle fibers by axonal transport and is realized through impulse activity. The contribution of each of the above components to the implementation of neurotrophic control of skeletal muscle has been studied in sufficient detail [10, 15]. However, neurotrophic control of skeletal muscles, not limited to the contribution of motor neurons, also includes nerve cells of the overlying parts of the CNS [2, 6]. The contribution of the above cells to the neurotrophic control of various skeletal muscles is ambiguous [1–4] and still remains poorly understood.

Moreover, significant differences were found in the contractile activity of "fast" and "slow" skeletal muscles of warm-blooded animals during spinalization. Why did spinalization increase the speed of *m. soleus* and the contraction force of the "fast" *m. EDL*? Normally, slow muscles are inferior to fast ones and fast muscles have high endurance. The results of this study suggest that neurotrophic control is primarily responsible for this specificity. Indeed, a similar finding was noted earlier even in "tonic" muscles, which differ more specifically from the phase muscles analyzed [16].

The denervation from the "tonic" nerves and the subsequent initial reinnervation by the more rapidly growing "phasic" axons lead to the idea that the tonic muscle loses its specific properties and contracts as a phasic one [17], which is not accompanied by the formation of a typical morphological "phasic" nervous terminals [18]. In addition, when the nerve from the phasic muscle, which did not contain "tonic" axons, was sutured to the tonic muscle, the tonic muscle lost its specific component of contraction for the entire observation period (up to 15 months) [17]. Thus, the severity of fast and

slow functioning during spinalization "smoothes out" and the nature of contractile activity becomes more average. Nevertheless, it appears that further research is needed to answer these issues.

CONCLUSIONS

1. Obtained data demonstrate significant differences in the mechanisms of control of contractile activity in "fast" and "slow" skeletal muscles of warm-blooded animals.

2. The preservation of a similar effect of basic modulators on the contraction of both muscles with such a striking reaction to spinalization sets off the contribution of neurotrophic control to the functioning of "fast" and "slow" motor units.

Author contributions. A.E.Kh. and A.R.Sh. conducted the research. A.A.E. and N.M.K. were responsible for collecting and analyzing the results. A.Yu.T. was responsible for the maintenance and pre-experimental operations with animals. V.V.V. and S.N.G. supervised the study work.

Conflict of interest. The authors declare no conflict of interest.

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