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POPULAR SCIENCE ABSTRACT

1. Research project objectives/ Research hypothesis

The project concerns motor units, the smallest functional elements of the neuromuscular system. The neuronal part of a motor unit is composed of one motoneuron and its axon, whereas the muscular part is formed by a bundle of muscle fibers innervated by this motoneuron. The research will be done in two series of electrophysiological experiments on Wistar rats: (1) investigations of contractile properties on functionally isolated (by splitting ventral roots of spinal nerves) motor units of the medial gastrocnemius muscle, which contains three basic types of motor units – S, slow, FR, fast resistant to fatigue, and FF, fast fatigable; (2) investigations of electrophysiological membrane properties of spinal motoneurons by intracellular recording of electrical potentials by glass micropipettes.
The goal of the project is to determine changes in contractile properties of muscle fibers of motor units as well as changes of electrical parameters of motoneurons alone in a model of the muscle overload, achieved by exclusion from work synergistic muscles (tenotomy), normally operating together during motor tasks. The research is also aimed at broadening knowledge on adaptive capabilities of motoneurons and motor units active under conditions of the functional overload that often can be observed in result of intensive work (overtraining in athletes, poor ergonomic conditions), during aging of an organism, in the post-polio syndrome, following some kinds of injuries of the neuromuscular system (partial denervation, tendon transfer), and in neurodegenerative diseases, as the amyotrophic lateral sclerosis.
In the initial phase of the project the group of synergistic muscles will be cut off their distal attachments (Achilles tendon), with an exception of the medial gastrocnemius muscle that will become the only muscle of the back tibial group acting on the foot. Investigations in the next phase (after 5-12 weeks) will cover procedures of acute experiments, performed under deep anesthesia. The investigated properties of motoneurons and motor units of the functionally overloaded muscle will be compared to properties of neurons and units from the respective muscle of intact, healthy animals of the control group. Two projected tasks of the study are aimed at creating consistent image of adaptive changes after compensatory muscle overload: (1) in muscle fibres of motor units of the medial gastrocnemius muscle; (2) in motoneurons located in lumbar segments of the spinal cord, either these innervating the overloaded muscle or those innervating the synergistic unloaded muscle (lateral head of the gastrocnemius and soleus, cut off from the distal tendon).

The first experimental series will concern influence of the overload on muscle tissue. Investigations on functionally isolated motor units should give answers to questions, whether adaptive response to changed conditions of work of the overloaded muscle leads to altered proportions of FF, FR and S motor units (i.e. if transformational changes of muscle fibers appear), and how long-term overload influences contractile parameters of motor units, effectiveness of summation of twitches into tetanic contractions and mechanisms of force

regulation. Results of pilot experiments suggest changes in the mass of the overloaded muscle and indicate non uniform direction of adaptive changes in individual motor unit types. The second series of experiments is aimed at determining functional changes in spinal motoneurons innervating the overloaded medial gastrocnemius muscle and motoneurons innervating the unloaded lateral gastrocnemius muscle. In electrophysiological studies on motoneurons we consider the observation that not only alterations in activity of motoneurons induce changes in properties of innervated muscle fibers, but the reverse effect also takes place, i.e. muscle fibers can modulate properties of motoneurons by which they are innervated. These experiments should indicate changes in electrophysiological membrane properties and in parameters of rhythmic properties of motoneurons following depolarization (the frequency-current relationship). The results of pilot experiments suggest that adaptive changes will reflect increased excitability of the motoneurons of the overloaded muscle, and opposite changes will be observed in motoneurons innervating the synergistic, but unloaded muscle (lateral head of the gastrocnemius).

The additional research question is, whether potential changes will concern equally fast and slow motoneurons and whether changes in properties of motoneurons will reflect adaptations observed in muscle fibers of motor units? It is worth noticing that contrary to studies on properties of the effector (as muscle fibers), studies on motoneurons in this model are possible with respect either to neurons controlling the overloaded muscle (medial gastrocnemius) or to neurons innervating its synergist (lateral gastrocnemius) that is cut and excluded from motor activity. Comparison of results achieved in both types of motoneurons will enable us to construct more complex image of alterations, what can ease the interpretation of adaptive processes following the functional muscle overload.

2. Research project methodology

The research material will constitute adults (6 months old) Wistar rats. Experiments will be held in three stages: first, the surgical operation evoking the overload of the medial gastrocnemius muscle (tenotomy of synergists, i.e., the lateral head of the gastrocnemius, soleus, and plantaris muscles; the tendons cut will be tied up to the fascia of the thigh muscles). After the operation animals will be kept for 5-12 weeks in wheel equipped cages and will be subjected to treadmill exercise in order to intensify overload-induced changes (and in parallel to avoid sparing of the operated hind limb).

In the second and third stages, acute experiments will be performed under deep pentobarbital anesthesia (or in the case of motoneurons under ketamine-xylasine anesthesia). We will investigate (i) contractile and electromyographic parameters of motor units – recorded with the method of functional isolation by splitting ventral roots of spinal nerves into thin filaments, which will be electrically stimulated; (ii) electrophysiological membrane properties of motoneurons – recorded directly from motoneurons by electrolyte-filled glass micropipettes introduced into the spinal cord.

After recording mechanical and electrical parameters from motor units, the investigated muscle will be removed and taken for further analysis (to determine muscle mass, to make histochemical preparations and to establish heavy-chain myosin content indicating proportions of muscle fibers). After completion of experiments animals will be killed by overdose of an anesthetic.

The applicants have access to the specialist equipment and have experience in performing above described experimental procedures.

3. Expected impact of the research project on the development of science, civilization and society

Results of the project will introduce new elements into basic knowledge on physiology and pathophysiology of muscles, neurophysiology of the spinal cord, and kinesiology. They will enable us to understand mechanisms of adaptive, plastic changes in the neuromuscular system in result of the functional overload caused by a decrease of a number of motor units (muscle fibers), co-operating in realization of motor tasks. Such situation takes place during aging of an organism and numerous diseases associated with injuries or neurodegenerative changes in the neuromuscular system.

Thanks to investigations planned there will be a possibility to explain unknown and previously not described mechanisms of elementary physiological changes evoked by the muscle overload at the cellular level of the spinal cord and at the level of muscle fibers of motor units. As a final result, better understanding of processes taking place in the structures investigated will allow us to verify current methods of therapy and prophylaxis that are commonly used to prevent negative effects of the muscle overload or to extend and improve life quality of aged people. One can also expect that demonstration of consistent elements and mechanisms of adaptation at both levels of a motor unit (neuronal and muscular) will influence creation of new, precisely directed and more effective methods used in physiotherapy. This kind of electrophysiological studies may also be significant in discussion and practical application of conclusions drawn with respect to optimization of training loads, either in hyperactive people (e.g. sportsmen) or in hypoactive convalescents.

4. International cooperation

The project will be realized in co-operation of the two research groups. Joint capabilities, experience and knowledge of both groups ensure accomplishment of original discoveries. Each of the partners included in the project has a distinct scientific identity, and the complete realization of both research tasks requires co-operation of the two teams.
The laboratory of the Polish applicant has an experience in the method single motor unit isolation and recording. The leader of the project has also a long-term experience in electrophysiology of the spinal cord. The Canadian laboratory, led by prof. P. Gardiner has vast experience in intracellular studies on rat motoneurons in vivo – one should especially point on the fact that a substantial part of scientific achievements of his team concerns adaptive changes

following various forms of altered physical activity. Moreover, the international partner has additional laboratory equipment that is not accessible by the Polish group in their laboratory. Therefore, the co-operation gives a chance to apply additional electrophysiological methods, especially with respect to measurement of some properties of motoneurons and to analyses of their evoked rhythmic activity. Modern experimental methods and combination in the two research tasks studies performed at two levels: of spinal neurons and of muscle fibers, will lead to achieve significant progress in neurophysiology and pathophysiology of muscles. It's worth to emphasize that it would be impossible to achieve complex results of the planned investigations (with respect to analysis of adaptive changes at several levels) without co-operation of both research groups.
Methodological aspects and aims of the project were discussed in detail, and pilto experiments were performed during mutual visits members of both teams in 2009-2011.