LACTATE DEHYDROGENASE ISOZYME ACTIVITY OF A SOLEUS MUSCLE TRANSPLANT IN THE ANTERIOR CHAMBER OF THE EYE

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To understand the principles governing skeletal muscle regeneration knowledge of the character of tissue interaction is essential [1, 3]. The presence of motor innervation is an important factor controlling regeration of skeletal muscle [2]. Most studies of skeletal muscle regeneration have been undertaken on free muscle grafts [3, 8, 10]. In particular, it has been shown that lactate dehydrogenase (LDH) activity in such grafts declines rapidly, but within the course of a week returns to its original level. Glucose-6-phosphate dehydrogenase activity in the graft also falls initially, but increases sevenfold on the 8th day, and later falls to normal [14].

On transplantation of a minced fragment of rat and cat skeletal muscle into the anterior chamber of the eye, the graft survived for 36 days [4, 15]. However, metabolism of the muscle has not been studied under these conditions and, in particular, it is not known whether innervation of the graft has any effect on the character of its oxidative metabolism.

The aim of this investigation was to study the LDH isozyme spectrum of the soleus muscle transplanted into the anterior chamber of the eye, and the effect of motoneurons of this parameter, in order to determine the character of oxidative metabolism in the transplanted muscle and the role of the neurogenic factor, in order to establish this characteristic of the graft.

EXPERIMENTAL METHOD

Experiments were carried out on mature albino rats of both sexes. The soleus muscle was isolated, cut up into pieces measuring 2 x 2 mm, and placed in Hanks' medium at room temperature. The cornea of the recipient rats, which were deeply anesthetized with ether, was divided under sterile conditions, after instillation of 0.1% atropine solution, along the edge of the limbus, and a fragment of muscle was introduced into the incision. Besides a piece of muscle, a segment of the spinal cord of newborn rats containing motoneurons was introduced into the anterior chamber of animals of another group. Four animals were deeply anesthetized with ether 2, 5, 7, 11, 15, and 21 days after transplantation, decapitated, and the eyes were enucleated and the graft together with the cornea studied. Samples of tissues frozen in liquid nitrogen were mounted on the block holder of a cryostat, sections were cut to a thickness of 8 μ, and activity of succinate dehydrogenase (SDH) [6] and myosin ATPase [11] was determined. Under the MBS-2 microscope some of the grafts were freed from connective tissue and spinal cord fraoments, homogenized with the addition of 0.15 ml of distilled water, and centrifuged at 8000g for 30 min. Next, 0.05 ml of supernatant was applied to concentrating gel and fractionated by electrophoresis in polyacrylamide gel. Gels with activity of LDH isozymes thus revealed were photographed and the negatives subjected to densitometry on an IFO-451 dual-beam recording microphotometer. The results were subjected to statistical analysis by the t test [7].

EXPERIMENTAL RESULTS

The rat soleus muscle consists of muscle fibers which differ in their SDH and myosin ATPase activity. Different types of muscle fibers could be identified in the transplanted muscle fragment before the 11th-15th day by their SDH activity. On the 21st day the muscle fibers had lost their characteristic features of differentiation, with simultaneous formation of new muscle fibers at the periphery of the graft. Combined transplantation of skeletal muscle and segments of the spinal cord did not lead to loss of signs of differentiation of the muscle fibers at this stage, and muscle regeneration (with many muscle tubes and immature

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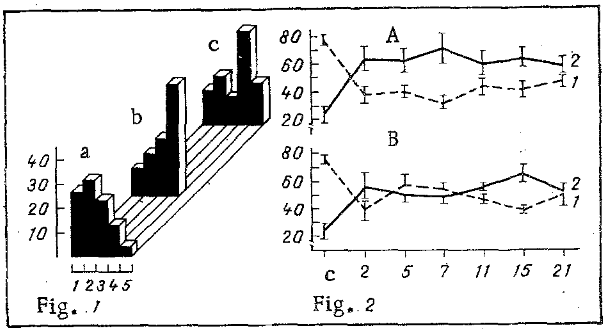

Fig. 1. Fig. 2.

Fig. 1. LDH isozyme spectrum of soleus muscle on 21st day after transplantation into anterior chamber of the eye. Abscissa, LDH isozymes ordinate, relative content of isozymes (in %); a) intact muscle, b) graft of muscle, c) graft of muscle + spinal cord.

Fig. 2. Content of H-subunits (1) and M-subunits (2) of LDH in soleus muscle transplanted into anterior chamber of eye. Abscissa, time of observation (in days); ordinate, fractions of H- and M-subunits (in %). A) Graft of muscle + spinal cord, B) graft of muscle alone. C) Control.

muscle fibers present) was more strongly in evidence.

The soleus muscle *in situ* is characterized by a H-type of LDH isozyme spectrum, i.e., predominance of LDH_1 and LDH_2 (Fig. 1a). Starting with the 5th day an increase was observed in LDH_4 and LDH_5 activity in the graft, i.e., the isozyme spectrum changed to the M type (Fig. 1b). Identical changes were observed in the LDH spectrum of muscle transplanted together with motoneurons, but after the 11th day LDH_1 activity was higher than in the graft of muscle alone (Fig. 1c). In the intact muscle 76.5% of LDH activity was accounted for by the H-subunit, whereas the contribution of the H-subunit to the transplanted muscle by the 2nd day was only 38.7%, from the 5th through the 7th day some predominance of the H-subunit was observed in the spectrum, but on the 21st day the contribution of the H- and M-subunits became equal (Fig. 2B). The M-subunit predominated at all times in muscle transplanted together with motoneurons (Fig. 2A).

The results of investigation of the skeletal muscle graft show that muscle fibers undergo degenerative changes, manifested as disappearance of differences in their level of SDH activity. A characteristic feature of muscle fibers of the soleus muscle is that 70 to 80% of the muscle is composed of slow muscle fibers, functioning under conditions of aerobic metabolism [5]. Transplantation of skeletal muscle is accompanied by ischemia of the graft [12] which, in turn, should lead to predominance of anaerobic metabolism. The test chosen for the investigation, namely activity of LDH isozymes, enables the state of oxidative metabolism to be adequately assessed, for the M-spectrum is characteristic of organs functioning under anaerobic conditions [9, 13]. By the 2nd day metabolism of the graft had shifted already toward the anaerobic type. It will be noted that starting with the 5th day after transplantation differences between the content of H- and M-subunits were minimal, wheras after combined transplantation of muscle and spinal cord activity of the M-subunit predominated at all times. We know that differential synthesis of H- and M-subunits is controlled by two genes. The differences in relative content of H- and M-subunits which we found under these experimental conditions suggests that motoneurons control differential expression of the H- and M-genes of LDH subunits in the transplanted muscle. In other words, the character of oxidative metabolism with respect to this feature is under the controlling influence of the nervous system.

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