EFFECT OF HYPOTHERMIA ON DEVELOPMENT OF ISCHEMIC DAMAGE TO RAT SKELETAL MUSCLE

R. R. Islamov, A. P. Kiyasov, and V. V. Valiullin

UDC 616.74-005.4-036-092:612.592]-07-092.9

KEY WORDS: ischemia; hypothermia; allografting; anterior chamber of the eye; lumbrical muscle; immunohistochemistry; myosins

Morphological and functional characteristics of skeletal muscle are modified by ischemia [3, 4]. The situation may arise when prolonged interruption of the blood flow in a muscle leads to the onset of irreversible ischemic damage in it, preventing recovery of the muscle as an organ [1]. Meanwhile, an important problem in clinical practice is the longest possible maintenance of the viability of organs when their blood supply is disturbed. However, the times of appearance of these critical ischemic changes in a muscle and the effect of tissue cooling on their development have not yet been adequately studied. A traditional method of prolonging the viability of tissues, and one most frequently used, is cooling [2].

In this investigation we undertook a morphological and immunohistochemical study of a complete lumbrical muscle of a rat, subjected to ischemia of varied duration associated with hypothermia, and subsequently transplanted into the anterior chamber of the eye (ACE) of a rat.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred albino rats weighing 160-180 g. Under deep ether anesthesia the animals were decapitated and the hind limbs amputated; in the first series of experiments (n = 5) these limbs were kept at room temperature (21-23°C), and in the second series they were kept in a refrigerator at 2-4°C. The second or third lumbrical muscles were isolated from rats of series I 3, 6, 7, 8, 9, 10, and 11 h after amputation and from the rats of series II 6, 9, 10, 11, 12, 13, 14, 15, and 16 h after amputation, and transplanted into ACE of a rat [6]. On the 7th day after transplantation the recipient animals were decapitated under deep ether anesthesia and the graft was removed, and placed in freshly isolated liver together with a normal lumbrical muscle. By histochemical methods on frozen sections 8 μm thick, succinate dehydrogenase (SDH) activity was determined and immunohistochemical staining (by the PAP method) was carried out [5] with monoclonal antibodies (AB) to fast myosin heavy chains (Sigma). Sections for morphological study of the graft were stained with hematoxylin and eosin.

EXPERIMENTAL RESULTS

Immunohistochemical staining revealed fast and slow muscle fibers (MF) in normal lumbrical muscle, the majority being fast (Fig. 1a). In its SDH activity the lumbrical muscle behaves as a "red" muscle, with MF of B and C types in it (Fig. 1b).

Department of Histology, S. V. Kurashov Kazan' Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR A. P. Avtsyn.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 113, No. 1, pp. 92-94, January, 1992. Original article submitted March 19, 1991.

0007-4888/92/0001-0133$12.50

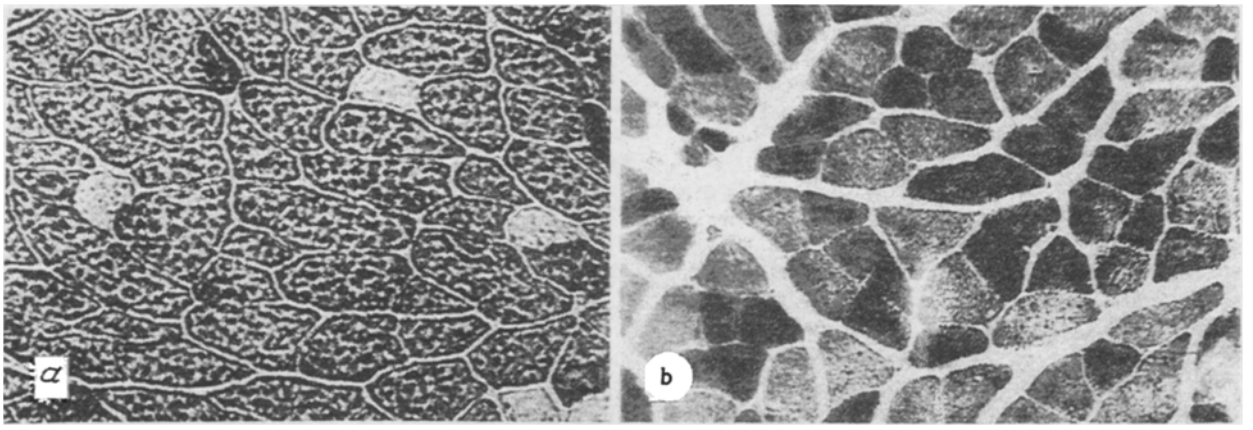

Fig. 1. Rat lumbrical muscle: a) immunohistochemical staining with monoclonal AB to fast myosin heavy chains. Pale MF – slow, dark MF – fast; b) SDH activity. Dark MF – C type, pale – B type.

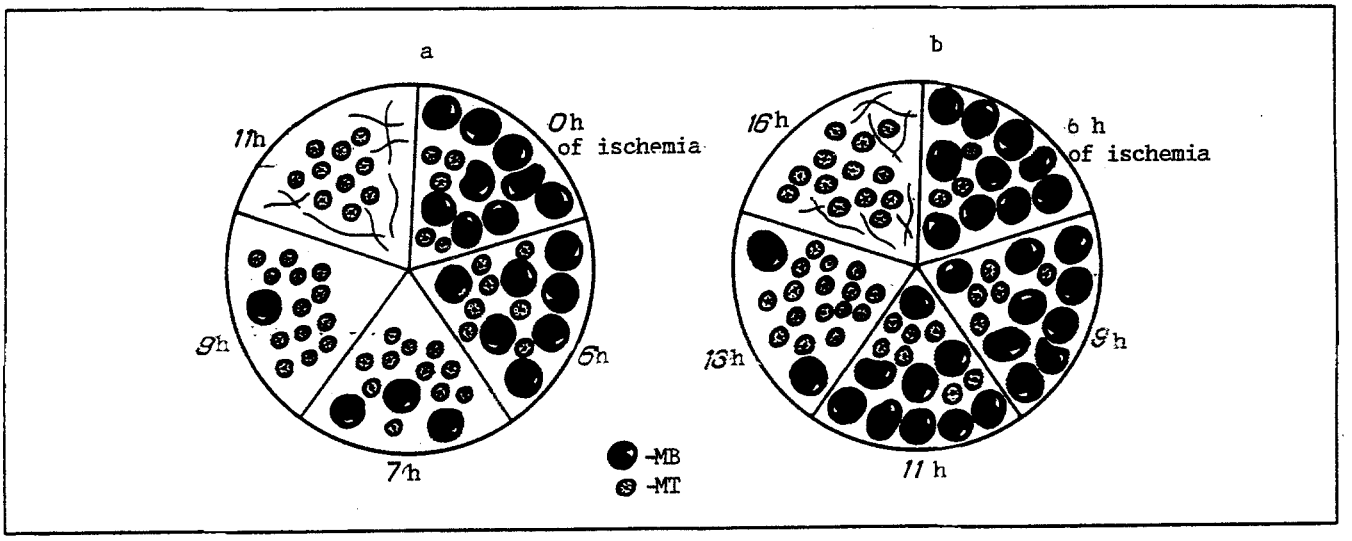

Fig. 2. Morphological characteristics of graft of rat lumbrical muscle on 7th day of culture in ACE after ischemia of varied duration: a) at 21-23°C; b) at 2-4°C. 1) Surviving MF; 2) MT.

In the experiments of series I at all times of ischemia of the muscle up to 6 h (Fig. 2a) both surviving MF and newly formed muscle tubes (MT) were found in the graft. Immunohistochemically, fast and slow MF were distinguished, and with respect to SDH activity, they belonged to the B type. High SDH activity characteristic of MF of the C type was found in MT, and staining with AB revealed fast myosin in them. Incidentally, these histochemical and immunohistochemical characteristics of MT were observed in all series of experiments.

After ischemia of the muscle for 7-9 h, only solitary remaining MF were found among MT in the graft. They did not stain with AB, and responded to the B type in their SDH activity. After 10 and 11 h of ischemia only MT were found in the graft.

In the experiments of series II, during ischemia of the cooled limb for up to 11 h the graft resembled that in series I, but after 6 h of ischemia it consisted of surviving MF and MT (Fig. 2a, b). Among MF staining with AB demonstrated both fast and slow fibers (Fig. 3a), but in their SDH activity they corresponded to the B type (Fig. 3b). The morphological and immunohistochemical characteristics of the graft after ischemia for 12-14 and 15-16 h at a low temperature were similar to those of the grafts in the experiments of series I, but after ischemia for 7-9 and 10-11 h respectively (see Fig. 2a, b).

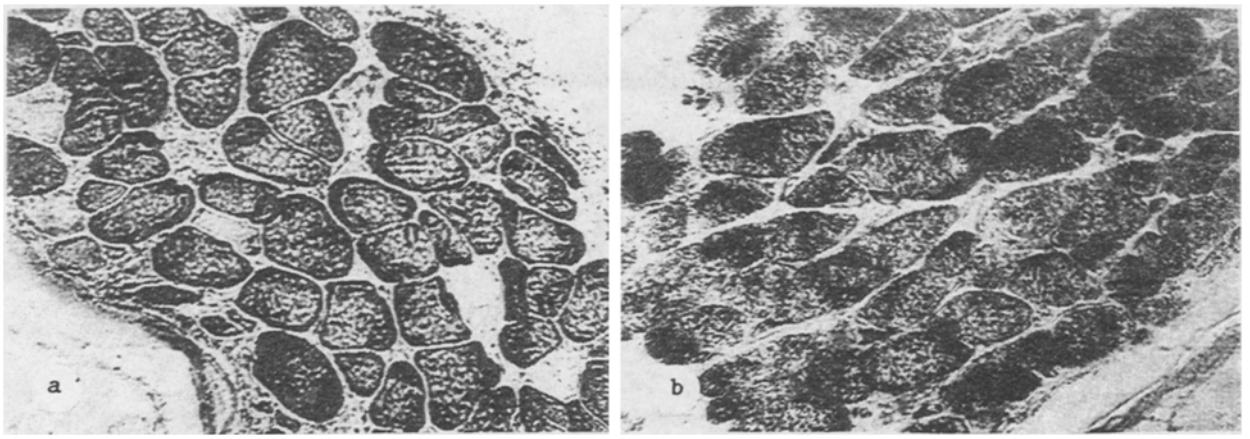

Fig. 3. Lumbrical muscle of rat after 11 h of ischemia on 7th day of culture: a) immunohistochemical staining by monoclonal AB to fast myosin heavy chains. Pale MF – slow, dark MF – fast; b) SDH activity, fibers of B type.

The results demonstrate that after 6 h of ischemia at room temperature a 7-day-old graft of the lumbrical muscle remains intact morphologically, but after ischemia lasting more than 9 h, no definitive MF, but only MT, can be found in the graft. If the amputated limb is cooled to 2-4°C, it remains morphologically intact even after ischemia lasting up to 11 h, but after 16 h of ischemia it consists of MT only.

Thus cooling of the limb to 2-4°C shifts the time of onset of irreversible ischemic damage in the rat lumbrical muscle by 5 h (from 6 to 11 h) compared with ischemia of the limb developing at room temperature.

LITERATURE CITED

1. M. G. Gonchar' and E. P. Mel'man, Arkh. Anat., No. 6, 13 (1990).
2. A. Kenji, K. Yasunori, I. Kihoshi, et al., J. Jpn. Ass. Thorac. Surg., **37**, No. 9, 78 (1989).
3. J. P. Roberts, M. O. Perry, R. J. Hariri, and G. T. Shires, Circulat. Shock, **17**, 253 (19??).
4. J. M. Rocko, J. Tikellis, D. Barillo, et al., J. Surg. Res., **41**, 574 (1986).
5. L. A. Strenberger, Immunochemistry, New York (1979).
6. H. Yellin, Exp. Neurol., **51**, 579 (1976).