Resting Membrane Potential of Diaphragm Muscle Fibers in Rats under Conditions of Total Ischemia

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Ischemic damage of skeletal muscle occurs in cases of trauma, acute thrombosis, embolies, and some other vascular disturbances. Skeletal muscle tissue is one of the least sensitive to ischemia [5]. After some time, however, pathological changes assuming an irreversible character are caused by ischemia even there. The importance of determining these time parameters is governed by the specific clinical situation. However, the critical times of the appearance of such irreversible changes have not yet been elucidated for different muscles, this being connected with the absence of reliable indicators of ischemic damage to muscle.

Changes of the resting membrane potential (RMP) in the muscle fiber (MF) have been shown to be one of the earliest manifestations of ischemic disorders of the functional properties of muscle [3, 4, 7]. It is known that the character of the changes in RMP reflects the work of the ion-transporting system and, consequently, the functional state of the cell membrane [1].

The diaphragm muscle is a suitable model for physiological investigations *in vitro*. During muscle incubation in experimental ischemia of the diaphragm all the superficial MF are approximately under equal conditions, preventing the appearance of a "reperfusion defect" [5], and the use of Ringer solution for incubation prevents the development of damage caused by granulocyte activation, as is observed for reperfusion of ischemized muscle with blood [6].

RMP monitoring in diaphragm muscle fibers of rats was performed in order to establish the critical times of the appearance of irreversible changes in MF membranes under conditions of total ischemia.

MATERIALS AND METHODS

The experiments were carried out on 50 noninbred albino male rats weighing 160-180 g. The chloroform-narcotized animals were decapitated and held at 21-23°C during 1-8 hours. After that a fragment of diaphragm muscle with a width of 4-5 mm was isolated and placed in a bath for electrophysiological investigations [2]. Ringer solution (36°C) for warmblooded animals with the following composition (in mM): NaCl 136, KCl 3, $CaCl_2$ 2, $MgCl_2$ 1, glucose 11 in tris-maleate buffer, pH 7.2-7.4, was pumped through the bath at a constant flow rate (5 ml/min). The Ringer solution was preliminarily saturated by a gas mixture of 95% O_2 and 5% CO_2. RMP was measured in the extrasynaptic region of the superficial MF using standard microelectrode techniques. RMP was monitored in the muscle tested throughout its incubation in Ringer solution during 10-40 min. At each time of incubation 10-15 measurements were performed. The results were subjected to statistical analysis by Student's t test with the use of STATGRAPHICS software (IBM PC).

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TABLE 1. RMP of MF in Rat Diaphragm at Different Times of Ischemia ($M \pm m$)

Time of ischemia, h	RMP (mV), incubation time			
	10 min	20 min	30 min	40 min
Control	80.3±1.0	77.4±0.8	75.6±0.7	73.0±0.7*
1	65.9±0.9	64.6±1.3	65.0±0.9	65.0±1.2
2	53.8±1.1	54.9±1.1	57.1±1.3	54.3±0.9
3	49.2±1.0	54.7±0.9	52.3±0.9	54.0±0.9*
4	37.1±2.2	46.0±2.1	50.4±1.9	49.1±1.9*
5	33.5±1.5	38.0±1.4	40.2±1.0	43.0±1.1*
6	26.0±1.0	29.2±1.2	30.1±1.4	28.7±1.4
6.5	25.9±1.1	18.2±1.0	15.8±1.0	12.7±0.9*
7	20.1±1.5	18.9±1.6	17.9±1.6	13.3±1.4*
8	11.1±1.2	7.2±0.9	7.5±0.7	6.7±0.7*

Note: *) indicates reliable differences ($p<0.01$) between RMP values at the 10th and the 40th minute of muscle incubation in Ringer solution.

Each experimental series consisted of 5 animals. A diaphragmatic preparation isolated immediately after decapitation served as the control.

RESULTS

The control value of the RMP in the diaphragm was 80.3 mV. In all the experimental series ischemia caused a depolarization of the MF membrane, i.e., it lowered the RMP value (Table 1). Thus, within an hour of ischemia the RMP fell by 16 mV, and in the course of ischemia development a further RMP drop occurred, while 8 hours later its value was 11.1 mV.

The changes of RMP values during ischemized muscle perfusion depended on the times of ischemia development (see Table 1). No RMP alterations in the 1st and 2nd hour of ischemia were registered during incubation. Hyperpolarization of the MF membrane, leading to an RMP increase by 5, 12, and 10 mV, was noted during perfusion of the muscle subjected, respectively, to 3, 4, and 5 hours of ischemia. Investigation of the muscle in the 6th hour of ischemia showed that the RMP value practically did not change throughout an incubation period of 40 min. Later, a decrease in RMP was observed during muscle perfusion, so that a conclusion may be drawn of a further MF membrane depolarization under these conditions.

The data obtained reflect changes of the functional state of the MF membrane during ischemia development. A tendency toward RMP restoration was noted during perfusion at times up to 6 hours of ischemia, and so it may be assumed that the changes occurring in the MF membrane under the given conditions are reversible. At the same time, a more prolonged ischemia in the muscle leads to the development of irreversible disturbances in the MF membrane, causing RMP disappearance.

Thus, judging from the nature of the RMP changes in diaphragm MF under conditions of total acute ischemia at 21-23°C, an ischemia lasting more than 6 hours must probably be considered the critical time for the beginning of irreversible changes in the membrane.

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