ANALYSIS OF TEMPERATURE DEPENDENCE OF MUSCLE CELL MEMBRANE REPOLARIZATION IN THE DIAPHRAGM OF RATS OF DIFFERENT AGES

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The writer showed previously with respect to adrenocortical cells [4] that during aging the rate of restoration of the cell membrane potential (MP) within the range of temperature from 7 to 17°C, after preliminary keeping at 0°C, changes in different ways. The temperature coefficient (Q_{10}) of the process, calculated from values of the rate of membrane repolarization at these temperatures, was twice as high in old animals as in young, evidence of a marked increase in the activation energy (E_{act}) of the electrogenic component of active ion transport during aging. Determination of the degree to which this fact applies to properties of the membranes of different cells and reflects their age dynamics is an important problem. It was therefore decided to study age changes in the energy supply for the transport function of membranes on a different object and, in particular, on membranes of muscle cells, the polarization level and pump mechanism of which largely determines their specific function. The investigation described below was devoted to a study of this problem.

EXPERIMENTAL METHOD

Fragments of isolated diaphragm muscle from 82 adult (7-8 months) and old (28-32 months) male albino rats were studied. The animals were decapitated. The muscles were perfused with Tyrode's solution for warm-blooded animals (pH 7.3-7.4), aerated with a mixture of 5% CO_2 and 95% O_2. The technique of determining the cell MP was similar to that described previously [4]. To block mechanisms of active transmembrane transport temporarily and to load the cells with sodium ions, the muscles were incubated in Tyrode's solution at 0°C for 60 min and MP was measured at the end of incubation. When the muscle fragments were subsequently transferred to a solution at 10 or 20°C MP was restored and the dynamics of the change was determined after incubation for 20 min at this temperature. Depending on the gradient of changes in MP over a period of 20 min values of Q_{10} for repolarization and the overall value of E_{act} of the reactions responsible for bringing it about were calculated. Active transport mechanisms controlled by Na,K-ATPase were blocked by treating the isolated muscles for 20 min at 37°C with ouabain, a specific blocker of membrane ATPase, in a concentration of 10^{-4} M in potassium-free Tyrode's solution in which the KCl was replaced by an equivalent amount of choline chloride. To investigate the effect of uncoupling of oxidation and phosphorylation on temperature dependence of repolarization of the cell membranes, 2,4-dinitrophenol (2,4-DNP) was injected intraperitoneally in a dose of 1.5 mg/100 g body weight.

EXPERIMENTAL RESULTS

During incubation of the isolated muscles at 37°C no age differences were found between the values of MP of the muscle cells: in adult animals its value was 70.0 ± 0.79 mV (based on values of MP for 302 cells in 17 rats), whereas in the old rats it was 69.2 ± 0.62 mV (619 cells in 33 rats; $P > 0.05$). These results are in agreement with data in the literature indicating high stability of MP of muscle cells during aging [5, 6].

Cold treatment of the muscles led to depolarization of the cell membranes, which in the adult animals was almost twice as high as in the old rats: in the adults by −15.7 ± 1.64 mV (276 cells in 12 rats) and in the old animals by −8.6 ± 1.24 mV (467 cells in 20 rats).

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TABLE 1. Temperature Dependence of Repolarization of Muscle Cell Membranes in Diaphragm of Rats of Different Ages: Intact, Treated with Ouabain, and Treated with 2,4-DNP

Experimental conditions	Group of animals	Statistical index	Initial value of MP after incubation at 0°C, mV	MP gradient during incubation for 20 min at 10°C, mV	MP gradient during incubation for 20 min at 20°C, mV	Q_{10} of repolarization process	Calculated value of activation energy, kcal/mole
Control	Adult	n_1	10	10	10	10	10
		n_2	252	131	133		
		$M \pm m$	54,5±1,05	+9,1±0,70	+13,4±0,91	1,507±0,066	6,657±0,688
	Old	n_1	18	14	14	14	14
		n_2	447	194	202		
		$M \pm m$	59,5±0,85	+3,4±0,63	+9,9±1,07	3,824±0,440	20,782±1,918
Ouabain	Adult	n_1	7	7	7	7	7
		n_2	191	95	89		
		$M \pm m$	54,2±0,46	+7,7±0,20	+8,7±0,29	1,133±0,016	1,843±0,282
	Old	n_1	7	7	7	7	7
		n_2	169	111	97		
		$M \pm m$	55,9±1,01	+3,3±0,51	+8,8±1,41	2,725±0,245	16,225±1,430
2,4-DNP	Adult	n_1	6	6	6	6	6
		n_2	178	114	121		
		$M \pm m$	65,0±1,16	+6,5±1,11	+6,9±1,09	1,084±0,026	1,317±0,407
	Old	n_1	11	11	11	11	11
		n_2	329	175	202		
		$M \pm m$	60,4±1,07	+3,9±0,71	+10,0±0,90	3,599±0,690	18,317±3,058

Legend. n_1) Number of animals, n_2) number of cells investigated.

The dynamics of repolarization of cell membranes during subsequent transfer of the muscles from a solution at 0°C to a solution at 10 or 20°C showed significant age differences (Table 1). The essence of the differences was that both at 10°C and at 20°C the rate of repolarization of the muscle cell membranes of adult animals was significantly higher than in old animals. Values of Q_{10} for repolarization and the overall value of E_{act} of reactions determining the dynamics of restoration of MP within the temperature range tested, calculated on the basis of these results, were significantly higher for old than for adult animals.

Analysis of age differences in the mechanisms of maintenance of stability of MP in muscle cells and of the differences discovered in the temperature dependence of repolarization of cell membranes was undertaken with the aid of ouabain and 2,4-DNP.

When the muscle was incubated at 37°C treatment with ouabain led to depolarization of the cell membrane, which was significantly greater in the adult animals than in the old rats: in adults MP fell from 70.4 ± 1.24 to 55.6 ± 1.09 mV, on average by 14.8 ± 0.75 mV (201 cells in 7 rats), whereas in the old animals it fell from 70.9 ± 1.08 to 62.7 ± 0.68 mV, on average by 8.2 ± 0.86 mV (200 cells in 7 rats).

The fact was noted that the degree of membrane depolarization after treatment of the muscle with ouabain agreed almost completely with the character of membrane depolarization of the muscle cells after their exposure to cold. These data can be interpreted as confirmation of a reduction in the contribution of enzymatic (determined by an ATPase mechanism) reactions to the total balance of processes maintaining age constancy of MP during aging. At the same time, the possible role of structural changes in the lipid phase of the membrane, determining sensitivity of enzyme proteins, and changes in permeability of the membrane for inward and outward sodium and potassium ion currents cannot be ruled out.

After oubain treatment followed by exposure to cold the dynamics of cell membrane repolarization in muscles of animals of both ages showed definite changes (Table 1). In the adult rats a fall in Q_{10} of repolarization was observed, whereas in old animals preliminary treatment of the muscles with oubain also led to a decrease in Q_{10} of repolarization, but by contrast with adult rats, its value as before exceeded 2.

The decrease in Q_{10} of repolarization observed in old animals after receiving ouabain is evidence of a definite contribution of enzymic processes controlled by membrane Na,K-ATPase to the general balance of the action determining repolarization of the cell membrane. However, the fact that after treatment with ouabain Q_{10} of repolarization did not fall to the characteristic values for physical processes (close to unity), as was the case in the adult rats, from the writer's point of view is evidence of significant age changes in the mechanisms of active transmembrane ion transport.

Aging is accompanied by changes in the relationship between different pathways of energy generation in the cell: strengthening of the role of glycolysis and weakening of the role of oxidative phosphorylation [1, 6, 7]. Analysis of the possible influence of changes in the contribution of different pathways of energy generation to the total balance of energy-providing reactions of the cell on the temperature dependence of repolarization was undertaken on animals treated with 2,4-DNP (Table 1). In adult animals the rate of repolarization of the cell membrane was significantly slowed both at 10°C and at 20°C ($P < 0.001$). The value of Q_{10} of repolarization fell close to unity. It will be noted that treatment of the muscles with 2,4-DNP led to almost the same change in the character of the dynamics of cell membrane repolarization in the adult animals as was observed during blocking of membrane ATPase by treatment of the muscles with ouabain (Table 1). In old animals uncoupling of oxidation and phosphorylation caused virtually no change in the character of repolarization.

These findings point to a decrease in the role of processes of oxidative phosphorylation in the provision of energy for repolarization of cell membranes in old animals, and by their character they confirm data obtained during treatment of the muscles with ouabain.

Consequently, both when membrane Na,K-ATPase is inhibited and when oxidation and phosphorylation are uncoupled, Q_{10} of repolarization in adult animals falls to values close to unity, characteristic of physical processes.

The results of these experiments are evidence of considerable age changes in the energy supply for mechanisms of active transmembrane transport against the background of constancy of the initial muscle resting potential during aging.

Together with data published previously [4], these observations indicate that the phenomenon of age differences in the temperature dependence of repolarization of cell membranes examined in this paper is of general biological nature, and its significance lies in an increase in the energy of activation of the electrogenic component of active transmembrane ion transport during aging.

It has recently been established that activity of membrane Na,K-ATPase may vary within wide limits depending on changes in the relations of the lipid components of the cell membrane [2, 3]. At the same time, during aging synthesis of different phospholipid fractions, which maintain the liquid phase-state of the membrane, is depressed [8]. This suggests that during aging significant changes may take place in the phase state of the lipid part of the membrane, which evidently determines the changes in the properties of the active transport mechanism established by the present investigation.

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