AGE DIFFERENCES IN REPOLARIZATION OF ADRENOCORTICAL CELL MEMBRANES

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Reversible depression of the function of the sodium pump of cell membranes by application of cold (from 0 to 5°C) is accompanied by accumulation of Na ions within the cell and by the outflow of K ions from the cell [4]. This redistribution of ions is one of the most important factors activating membrane Na,K-ATPase at optimal temperatures [9].

There is abundant experimental evidence that active ion transport through the membrane is accompanied as a rule by the development of a potential difference on it, i.e., it rests on an electrogenic basis [11, 12].

The mechanisms of maintenance of the cell membrane potential (MP) undergo definite changes during aging [6].

It was shown previously in experiments on adrenocortical cells (ADC) [3] that after preliminary cold preincubation, the rate of membrane repolarization of cells of the zona fasciculata of the adrenal cortex changes in different ways within the temperature range from 7 to 17°C. The temperature coefficient Q_{10} for this process, calculated from the rate of membrane repolarization at 7 and 17°C was found to be almost twice as high in old as in mature animals.

Analysis of this phenomenon would seem to be best carried out by studying the possible role of free-radical reactions (FRR) in the phenomena described above. This conclusion was based on data in the literature indicating that during aging the concentration of free radicals (FR), capable of reacting with different cell components, in the body tissues increases [7, 8, 10].

The object of this investigation was to study the possible contribution of FRR to membrane repolarization of ACC during activation of mechanisms of transmembrane transport.

EXPERIMENTAL METHOD

Experiments were carried out on the isolated adrenals of 80 young (5 months) and old (28-29 months) albino rats. The animals were decapitated. The conditions of incubation of the adrenals and the method of determination of MP of cells of the zona fasciculata of the cortex were described previously [3]. Restoration of MP of cells depolarized beforehand by "loading" with sodium ions, by keeping the adrenals at 0°C in Krebs–Henseleit solution for 1 h, was studied. The value of Q_{10} for repolarization (RP Q_{10}) and the activation energy (E_{act}) of the reactions maintaining it were determined from the gradients of rise of MP during 20 min of subsequent incubation in solutions at temperatures of 7 and 17°C. The synthetic FRR inhibitor ionol (4-methyl-2,6-di-tert-butylphenol), dissolved in a 25% aqueous solution of the solubilizer Tween-80, was injected intraperitoneally in a dose of 100 mg/kg body weight 3 or 24 h before decapitation of the animal. These times were chosen on the basis of data in the literature [1] showing that the antioxidant activity of the tissues reaches a maximum 3-6 h after injection of ionol, whereas its fall to the initial level or below, on account of a sharp decrease in the content of natural oxidants, and also of parallel excretion of the synthetic FRR inhibitor, takes place toward the end of the first day after injection. An artificial increase in the tissue FR level in young animals was produced by whole-body x-ray irradiation in a dose of 700 R on the RUM-17 apparatus (tube voltage 180 kV, current 15 mA, focus-skin distance 0.4 m, dose rate 100 R/min, duration of irradiation 7 min, filter 0.5 mm copper + 1.0 mm aluminum).

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TABLE 1. Effect of Ionol (100 mg/kg intraperitoneally) on Temperature Dependence of Membrane Repolarization of ACC of Zona Fasciculata in Rats of Different Ages

Group of animals	Statistical index	Initial MP after incubation at 0°C, mV	MP gradient during incubation for 20 min, mV		Temperature coefficient RP Q_{10}	Calculated value of E_{act}, kcal/mole
			at 7°C	at 17°C		
Intact:						
young	n_1	10	7	7	7	7
	n_2	217	70	70		
	$M\pm m$	17,7±0,26	12,2±0,46	18,1±1,12	1,481±0,051	6,311±0,558
old	n_1	23	9	9	9	9
	n_2	435	89	97		
	M±	22,9±0,27	9,3±0,72	25,2±2,10	2,732±0,191	16,040±0,985
Treated with ionol:						
young	n_1	7	7	7	7	7
	n_2	148	70	70		
	$M\pm m$	18,1±0,20	12,7±0,73	18,2±1,15	1,438±0,027	5,875±0,305
	P				< 0,05	< 0,05
old	n_1	8	8	8	8	8
	n_2	166	80	80		
	$M\pm m$	25,0±0,73	13,7±0,97	20,1±1,49	1,467±0,028	6,194±0,309
	P				<0,001	<0,001

Legend. Here and in Table 2: n_1) number of animals; n_2) number of cells tested. P) Significance of differences between values of index in intact animals and those treated with ionol.

TABLE 2. Effect of Total X-Ray Irradiation (700 R) on Temperature Dependence of Membrane Repolarization of ACC of Zona Fasciculata in Young Rats

Experimental conditions	Statistical index	Initial MP after incubation at 0°C, mV	MP gradient during incubation for 20 min, mV		Temperature coefficient RP Q_{10}	Calculated value of E_{act}, kcal/mole
			at 7°C	at 17°C		
Intact rats	n_1	10	7	7	7	7
	n_2	217	70	70		
	M±	17,7±0,26	12,2±0,46	18,1±1,12	1,481±0,051	6,311±0,558
2—8 h after irradiation	n_1	9	9	9	9	9
	n_2	202	99	106		
	M±	19,9±0,48	8,5±0,55	21,2±1,26	2,515±0,099	14,868±0,599
24 h after irradiation	n_1	8	8	8	8	8
	n_2	181	96	104		
	M±	21,1±0,98	11,8±0,56	19,6±1,02	1,666±0,032	8,255±0,319
48 h after irradiation	n_1	8	8	8	8	8
	n_2	181	91	99		
	M±	20,7±0,63	11,2±0,41	17,4±0,46	1,558±0,050	7,137±0,518

EXPERIMENTAL RESULTS AND DISCUSSION

The results of investigation of the temperature dependence of repolarization of ACC membranes after administration of ionol show that 3 h after injection, RP Q_{10} and E_{act} of the reactions determining this process were significantly reduced. As the results in Table 1 show, against the background of ionol the dynamics of membrane repolarization of ACC of the old animals closely resembled that in intact young animals: the rise in MP during incubation for 20 min at 7°C was increased, but at 17°C it was reduced. The calculated value of RP Q_{10} for the cell membranes was reduced, with a corresponding fall in E_{act} of the reactions determining this process. In the control experiments on young animals the dynamics of membrane repolarization of ACC was unchanged by ionol (Table 1).

The higher the value of E_{act} of a reaction, the faster the increase in the velocity of that reaction with a rise of temperature [5]. If, therefore, the process takes place by competing reactions, the velocities of which depend differently on temperature, at low temperatures reactions with a low E_{act} will dominate, and when the temperature is raised, the role of reaction with a higher E_{act} will increase. Accordingly, the decrease in the value of RP Q_{10} of ACC membranes of old animals was reduced by ionol and that E_{act} of the reactions determining it also was reduced can be regarded as evidence of the existence of a definite contribution of FRR in old animals to the general balance of processes determining the mechanisms of active electrogenic transmembrane ion transport.

This hypothesis was confirmed in experiments on old animals in which the temperature dependence of membrane repolarization of ACC was investigated 24 h after injection of ionol. The results showed that RP Q_{10} and E_{act} of the reactions determining it rose sharply 24 h after injection of ionol in the old rats: Q_{10} from 2.732 ± 0.191 (n = 9) to 4.059 ± 0.885 (n = 7), and E_{act} from 16.040 ± 0.985 to 20.537 ± 3.385 kcal/mole.

Further analysis of the hypothesis that FRR make a contribution to membrane repolarization of ACC in old animals was undertaken by artificially raising the FR level in the body tissues of young animals by whole-body x-ray irradiation, which is known to lead to an increase in the content of FR in the tissues in the early stages after irradiation (the first few hours), and to a decrease in their content with effect from the second day after irradiation [2].

The results showed that during the first few hours (2-8 h) after x-ray irradiation the dynamics of membrane repolarization of ACC in the young rats was similar to that in intact old rats (Table 2): The increase in MP during incubation for 20 min at 7°C was reduced, whereas its increase at 17°C was increased. The values of RP Q_{10} and E_{act} of the reactions determining it were increased up to the characteristic values for intact old animals. RP Q_{10} and E_{act} were significantly reduced ($P < 0.001$) 24 h after irradiation, but after 48 h their values corresponded to those characteristic of young intact animals.

The various factors capable of changing the FR content in the tissues can thus significantly affect the temperature coefficient of membrane repolarization and E_{act} of the reactions determining it. This suggests that the mechanism of electrogenic active transport, under certain conditions, can be coupled with reactions of free-radical character. During aging, just as in the early stages after x-ray irradiation in young animals, the phenomena taking place are evidently of a general biological character, the essence of which is an increase in the role of FRR in the general balance of reactions determining active transmembrane transport which, in young animals, takes place entirely (or predominantly) on account of energy of ATP hydrolysis.

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