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AGE DIFFERENCES IN TEMPERATURE DEPENDENCE OF REPOLARIZATION OF THE ADRENOCORTICAL CELL MEMBRANE

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In experiments on isolated adrenals of male rats of two age groups (5 and 28-29 months) no age differences were found in the membrane potential of cells in the zona fasciculata of the cortex. The temperature dependence of repolarization of the cell membrane in the zona fasciculata of the adrenal cortex was investigated in rats of different ages within the temperature range from 7 to 17°C after preliminary cooling of the adrenals. The temperature coefficient of repolarization, calculated in old animals (Q_{10} = 2.732) was significantly higher than in the young animals (Q_{10} = 1.481). With age, the contribution of reactions with high activation energy increases in total balance of processes determining repolarization of the adrenocortical cell membrane.

KEY WORDS: Aging of the endocrine system; adrenal cortex; membrane potential; temperature dependence.

An important place in the study of the mechanisms of age changes in the functions of cells is occupied by the study of their biophysical properties, which are mainly determined by the state of the membrane: its polarization, excitability, and transport function. The level of polarization of the cell membranes plays an important role in the control of cell metabolism also [1, 5, 14].

Much factual evidence has now been gathered on age changes in the glands of internal secretion [2, 4, 6, 7], yet there is no information on the electrical properties of their cell membranes: the membrane potential (MP), details of active transport, and the supply of energy for it.

MP of the cells of certain organs and tissues (the liver, various structures of the nervous system, muscles, some epithelial cells) preserves its relative constancy during aging, despite significant changes in the concentrations of electrolytes in the tissues, and a decrease in the intensity of formation and in the concentration of high-energy phosphorus compounds, which provide for the work of the sodium pump [3, 8, 9, 15]. It can therefore be postulated that during aging changes arise in the mechanism of maintenance of MP of the cells.

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TABLE 1. Temperature Dependence of MP (in mV) of Adrenocortical Cells from Rats of Different Ages

Group of animals	Statistical index	Initial value of MP after incubation at 0°C	MP after incubation for 20 min at 7°C	MP after incubation for 20 min at 17°C	MP gradient after incubation for 20 min at 7°C	MP gradient after incubation for 20 min at 17°C	Temperature coefficient, Q_{10}	Calculated value of activation energy, kcal/mole
Young (5 months)	n_1	10	7	7	7	7	7	7
	n_2	217	70	70				
	M	17,7	30,3	35,7	12,2	18,1	1,481	6,311
	$\pm m$	0,26	0,64	1,27	0,46	1,12	0,051	0,558
Old (28-29 months)	n_1	23	9	9	9	9	9	9
	n_2	435	89	97				
	M	22,9	31,8	47,7	9,3	25,2	2,732	16,040
	$\pm m$	0,27	1,06	2,23	0,72	2,1	0,191	0,985

Legend. n_1) number of animals; n_2) number of adrenocortical cells investigated.

It was therefore decided to investigate age differences in the temperature dependence of repolarization of the adrenocortical cell membrane and to determine its temperature coefficient and activation energy.

EXPERIMENTAL METHOD

Experiments were carried out on the isolated adrenals of noninbred albino rats of the age groups: young (5 months) and old (28-29 months). The value of MP was determined for cells of the zona fasciculata of the adrenal cortex. After decapitation of the animal the adrenals were dissected, cut into halves, and fixed to a plastic substrate. Perfusion was carried out with Krebs–Henseleit solution with bicarbonate buffer to stabilize the pH between 7.2 and 7.4. For aeration, a gas mixture containing 95% O_2 and 5% CO_2 was passed continuously through the solution. Bioelectrical potentials were measured by a standard microelectrode technique. Glass microelectrodes with a tip under 1 μ in diameter and a resistance of between 15 and 30 MΩ, filled with 2.5M KCl solution, were used. The values of MP of cortical cells of intact adrenals were measured during incubation of the glands at 37°C. The temperature dependence of the repolarization process was investigated after preliminary incubation of the adrenal tissue in cold Krebs-Henseleit solution (0°C) for 60 min, ensuring a uniform level of depolarization of the cell membrane, as confirmed by the value of MP at 0°C; measurements were made immediately before the adrenals were placed into chambers containing the warm solution. On subsequent transfer of the adrenals to solution at temperatures of 7 or 17°C the values of MP were restored; they were measured every 20 min during incubation at that temperature. The temperature coefficient of the repolarization process and the activation energy of the reactions supporting it were calculated from the gradients of changes in MP of the adrenocortical cells over a period of 20 min.

EXPERIMENTAL RESULTS AND DISCUSSION

After incubation of isolated adrenal glands from rats of different ages at 37°C no age differences were found in the level of polarization of the adrenocortical cell membranes: MP in rats aged 5 months was 53.4 ± 0.41 mV (from data for MP of 117 adrenocortical cells from 10 rats), and in rats aged 28-29 months it was 52.7 ± 0.35 mV (357 adrenocortical cells from 23 rats; $P > 0.05$). The results of these experiments showed the high constancy of the level of polarization of the adrenocortical cell membrane during aging.

Recent investigations have shown that an important role in the maintenance of the cell MP is played by active electrogenic transport of ions, utilizing energy of high-energy phosphorus compounds [10-13]. With a reduction in the intensity of formation and in the concentration of high-energy compounds with age, increased importance becomes attached to the analysis of the energy supply for the transport function of the membrane. One possible method of approach to the investigation of age differences in the mechanisms of maintenance of the level of cell membrane polarization is to study the temperature dependence of the process of membrane repolarization of cells loaded with sodium ions as a result of preliminary cold incubation, and the temperature coefficients calculated on the basis of these data can characterize in the general form the nature of the reactions responsible for the repolarization process.

Investigation of the temperature dependence of membrane repolarization of adrenocortical cells from rats of different ages showed that the dynamics of restoration of MP at 7 and 17°C after preliminary cold incubation of the adrenals undergoes significant changes with age (Table 1).

As these results show, the increase in MP of the adrenocortical cells during incubation for 20 min at 7°C was greater for young rats than for old, whereas at 17°C the increase in MP was considerably greater in the old animals.

We know that the higher the activation energy of the reaction, the faster the reaction velocity rises with an increase in temperature. If, therefore, the process takes place through competitive reactions, whose velocity depends in different ways on temperature, at low temperatures reactions with a low activation energy will dominate, and when the temperature rises the role of reactions with a high activation energy will increase.

The value of the temperature coefficient of repolarization of the adrenocortical cell membrane, calculated from the experimental results, was significantly greater in the old animals than in the young, and the activation energy of the reactions determining the rate of rise of MP within the temperature range studied was correspondingly much higher in the old animals.

Differences discovered in the MP gradients in the young and old animals are evidence that with age the ratio between reactions having different activation energies in the general balance of processes responsible for membrane repolarization changes; the contribution of reactions with a high activation energy increases.

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