DYNAMICS OF THE CELL MEMBRANE POTENTIAL OF MOUSE EMBRYOS DURING THE PREIMPLANTATION PERIOD OF DEVELOPMENT

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The dynamics of the membrane potential (MP) of single mouse embryos from the zygote stage to the middle blastocyst stage is described. The MP begins to rise significantly from the zygote stage (MP≃15 mV) to the stage of eight blastomeres inclusive (MP ≃ 10 mV). Later, the MP remains on a plateau until the middle blastocyst stage. The MP is always negative in sign.

In accordance with the membrane theory of its origin, the membrane potential (MP) reflects the distribution of various ions on both sides of the membrane and the state of their passive and active transport through it [11, 14]. Meanwhile it must be emphasized that the transport of ions through the membrane is connected with the penetration of compounds such as amino acids [7], carbohydrates [6], etc., into the cell.

In some somatic cells, and also in embryos in the earliest stages of development (the cleavage period) definite changes in MP depending on the phases of mitosis are found [1]. Studies of the dynamics of MP in the period of early embryogenesis of various types of animals have shown that MP rises after fertilization. This has been shown, in particular, for echinoderms (starfish and sea urchins) [10, 15, 16] and vertebrates (amphibians and fishes) [1, 12]. The writers have recently shown that MP in zygotes of the sea urchin Strongylocentratus intermedius begins to rise from the moment of fertilization and reaches a plateau by the time of the first cleavage division [3]. A similar pattern has been described for other species of sea urchins [10, 15].

Investigation of the dynamics of a phenomenon in the period of early embryogenesis of mammals is interesting not only for the biology of development, but also as a promising tool for studying the mechanisms of the embryotoxic effects of various physical and chemical factors.

No investigations into the dynamics of the membrane potentials in mammals could be found in the literature.

The object of this investigation was to study the dynamics of the MP of mouse embryonic cells in the period of preimplantation development.

EXPERIMENTAL METHOD

MP was measured in unfertilized oocytes and also at various times after fertilization (from the zygote to the middle blastocyst stage). The embryos were washed out of the oviducts or uterine cornua by the method described by the writers earlier [2]. The unfertilized and fertilized oocytes thus washed out were placed in a constant-temperature cell containing Brinster's nutrient medium [8]. The temperature of the nutrient medium was kept at 37 ± 0.5°C and its pH between 6.8 and 7.4 (by passing CO_2 gas through the nutrient medium). The oocytes were fixed by microsection under visual control by means of the MBR-3 microscope. A microelectrode filled with 3 N KCl solution as described previously [4] was then introduced into

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the ovum. The diameter of the tip of the microelectrode was less than 0.5 μ. The intrinsic potential of the microelectrode did not exceed 10 mV. The connection to ground was through an Ag-AgCl electrode immersed in the nutrient medium. The signal from the microelectrode was led to a pH-340 millivoltmeter. The output of this instrument was connected to a type ÉPP-09 automatic writer, which recorded the membrane potential.

Wilcoxon's criterion was used to determine the statistical significance of the differences [5].

EXPERIMENTAL RESULTS AND DISCUSSION

In mouse embryonic cells MP was found to begin to rise significantly after the zygote stage and until the stage of eight blastomeres inclusive, during which period it approximately doubled. Later, the values of MP remained on a plateau until the middle blastocyst stage. The decrease in MP observed in the modular stage was not significant. The mean values of MP in the unfertilized oocytes and also in the stages of zygote, 2,4, and 8 blastomeres, morula, and early and middle blastocysts were as follows: 15 ± 4.3, 18 ± 6.0, 22 ± 7.0, 28 ± 11.4, 42 ± 9.5, 31 ± 9.6, and 40 ± 7.7 ($M \pm \sigma$) respectively.

A significant increase in MP from the zygote stage to the stage of eight blastomeres could be the result of an increase in the utilization of pyruvate and oxygen by the embryos [13, 17]. Pyruvate is known to be one of the main sources of energy required by the mouse embryos during this period of development [16]. If the existence of coupling between the transport of pyruvate molecules, oxygen, and K^+ and Na^+ ions in the membranes of mouse embryos can be accepted, the increase in MP thus demonstrated can be understood.

It is unlikely that the increase in MP which was found is connected with the transport of amino acids and nucleic acid bases, for during this period mouse embryos can develop normally without any source of nitrogen [9]. Experiments to study various aspects of the metabolism of mammalian embryos would shed some light on the mechanism of the increase in MP during the period of early embryogenesis. The possibility cannot be ruled out that MP reflects the dynamics of the intracellular concentration of ions required to provide optimal conditions for the changing metabolism of the embryos. The study of the role of MP in the mechanisms of certain embryotoxic effects will be the subject of future investigations.

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