RESTING MEMBRANE POTENTIAL OF CELLS IN A CULTURE OF THE MYOMETRIUM OF PREGNANT WOMEN

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The value of the resting membrane potential was determined in single cells of a culture of myometrium from pregnant women (in the late stages of pregnancy). The mean value of the membrane potential based on measurements in 26 cells was – 23 mV.

Measurement of the membrane potential (MP) in single cells of smooth muscle fibers, especially in fibers of the myometrium, is carried out without visual control of the insertion of the microelectrode into the cell. Penetration of the microelectrode into the cell is accompanied by the appearance of a potential. By using this method of measuring MP in single myometrial cells several workers have shown that MP in the myometrial cells during pregnancy is negative in animals such as mice [13], rats [18], cats [12], and rabbits [14] and varies between – 30 and – 70 mV.

The membrane potential of smooth muscle fibers is not only an important parameter reflecting the functional state of the cell, but it is also related to the appearance of spontaneous contractile activity of the smooth muscle. A decrease in MP leads to the generation of rhythmic action potentials [10, 11].

It was therefore decided to determine MP of single myometrial cells from pregnant women, cultivated in vitro, because the tissue culture is a widely used model in medical and biological research. It was also reckoned that primary cultures retain the specific features of the original tissues [5, 8]. That this is a fact has been confirmed by cultivation of the myometrium of the nonpregnant human uterus in vitro [3, 9, 16] and by the investigation of human myometrium obtained at the end of pregnancy and during labor in the authors' institute [1].

EXPERIMENTAL METHOD

A culture of human myometrium was used. Biopsy was carried out on the myometrium from the body of the uterus during caesarian section operations performed on two women for various reasons at the 39th-40th week of pregnancy, before the onset of uterine contractions. The method of obtaining cultures of myometrial cells, the morphological characteristics of their growth, the dynamics of their development, and the apparatus used to measure MP were all described previously [2, 6]. Measurements of MP were made 20 days after the beginning of cultivation of the myometrial explant in vitro, when a well-marked zone of growth consisting of conical cells, giving off processes, and fusiform cells had formed from the myometrium (Fig. 1).

Measurements of MP in the cells were carried out on coverslips at a temperature of 37 ± 0.5°C in a special constant-temperature chamber filled with nutrient medium No. 199. A glass microelectrode with a tip less than 0.5 μ in diameter was introduced by means of the MM-1 micromanipulator into cells (fusiform) chosen under the microscope. The microelectrode was inserted into the cell at an angle of 45-60° to the normal of the plane of the coverslip. MPs of cells lying separately were measured in cultures growing in a thin layer of fibrin.

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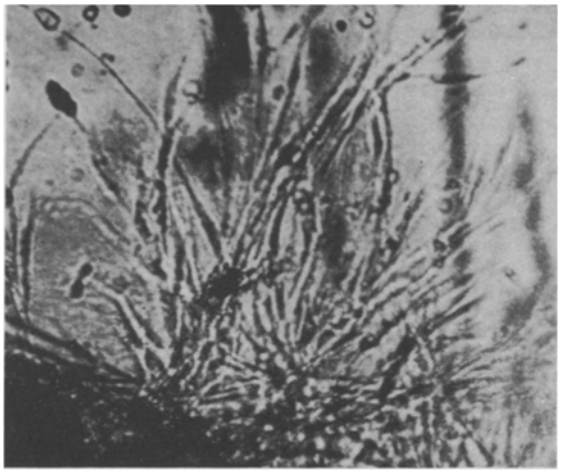

Fig. 1. Explant of myometrium cultivated in vitro for 20 days: conical and fusiform cells in a well-marked zone of growth can be seen. Unstained preparation; photographed in phase-contrast illumination.

EXPERIMENTAL RESULTS AND DISCUSSION

The value of MP became negative within a few seconds after insertion of the microelectrode into the cell and it remained at that level for 2-3 min.

During measurement of the potential both negative and positive values were observed. The mean value (measurements on 26 cells) of the negative membrane potential was − 23 mV.

It was found during measurement of the MP that a positive value of the potential changes into negative under the influence of a sudden shaking of the microelectrode lying in the cell. A series of measurements of MP was accordingly made in 6 cells using the method of shaking the microelectrode. All values of MP recorded under these conditions were negative, and its mean value was the same as that obtained by measurements of spontaneous negative MPs in 20 cells. Analysis of the values of MP by means of Wilcoxon's criterion [7] showed that $\Phi(t) = 0.488$. This suggests that the difference between the series of measurements of the spontaneous negative MP and of the MP arising after shaking of the microelectrode is not significant.

Comparison of the results of these measurements of MP with those described in the literature confirms that the value of MP in cell cultures of myometrium from pregnant women is only half of that found in cells of smooth muscle fibers from the pregnant uterus of the laboratory animals mentioned above. This difference may be due to changes in intercellular relations and also to some aspects of cell metabolism in tissue cultures grown in vitro. However, this problem requires further study, more especially because no reports of work to study MP in single cells of cultures of human myometrium could be found in the accessible literature.

The positive potential discovered in some of the measurements must evidently be regarded as the result of its recording from the cell surface, when the microelectrode tip is indenting the outer membrane into the cell without disturbing its integrity. However, on a sudden shaking of the microelectrode the outer membrane may rupture and the microelectrode penetrate into the cell. As a result the potential changed its polarity and became negative.

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