Effect of Ca^{2+} , Mg^{2+} , and K^{+} on the Zeta Potential of Single Heart-Muscle Cells Isolated from Chick Embryos

Isao Inoue, Ken Miyama* and Yonosuke Kobatake*

*Institute for Marine Biology, School of Medicine, Tokushima University, Seto-cho, Naruto, 770 and *Faculty of Pharmaceutical Sciences, Hokkaido University, Sapporo, 060*

ABSTRACT. Single heart-muscle cells were isolated from chick embryos. The zeta potential of these cells was determined from measurements of the cell electrophoretic mobility in accordance with the Helmholtz-Smolukowski equation in a variety of environmental salt compositions. Ca^{2+} , Mg^{2+} and K^{+} shifted the zeta potential to a positive direction.

The plasma membrane of living tissues is negatively charged under physiological conditions, and this generates an electrostatic potential at the surface of the membrane. In excitable membranes, this electrostatic potential is considered to contribute to the ionic process underlying an action potential according to recent electrophysiological data. The role of divalent cations, particularly, is suggested to regulate the electrostatic potential at the outer surface of excitable membranes (5). The aim of the present investigation was to approximate experimentally the electrostatic potential at the outer surface of the excitable membrane.

The zeta potential, an experimental approximation of the electrostatic potential at the surface of charged particles, can be determined from measurements of the electrophoretic mobility of the particles using the Helmholtz-Smolukowski equation (1). By this method, the zeta potentials of various living single cells have been determined (4, 6).

Single heart-muscle cells can easily be enzymatically separated from the embryonic heart while maintaining their electrical excitability (3). These cells are suitable for determining the zeta potential because a large number of similar cells are available at a time. In the present study the electrophoretic mobility of single heart-muscle cells has been measured under a variety of environmental salt conditions. This article describes the experimental results showing that Ca^{2+} , Mg^{2+} , and K^{+} acted to shift the zeta potential to a positive direction. The significance of the results is discussed in connection with the generation mechanisms of the membrane potential.

METHODS

Experiments were performed on single heart-muscle cells isolated from the hearts of 18–20 day-old chick embryos. Cells were isolated by a slightly modified tissue dissection technique with an enzyme developed for tissue culture (8, 12). The ventricles of the hearts were cut into small pieces (approximately 1 mm³ each) with a small pair of scissors and placed in a phosphate buffer solution (PBS). The salt composition of PBS was 137 mM NaCl, 2.7 mM KCl,

8.0 mM Na_2HPO_4 , and 1.5 mM KH_2PO_4 . The pH of the solution was 7.4 at 37°C. After washing off adhering blood with PBS, the heart fragments were transferred to a double flask filled with 20 ml of PBS containing 0.1 mg/ml trypsin (Calbiochem. Co.). The temperature of the enzyme solution was kept at 37°C. A small magnetic stirring bar was inserted into the flask, which was then placed on a magnetic stirrer at a low speed for 7 min. The supernatant was discarded. Trypsinization was repeated with 5 ml of the enzyme solution for another 7 min, and the turbid supernatant that included the single heart-muscle cells was collected. Most of the single heart-muscle cells were beating spontaneously at this stage of preparation. The supernatant was then poured into a chilled, 40 ml centrifuge tube containing 30 ml of PBS. Cells were collected by centrifugation ($200 \times g$ for 10 min), and were suspended in either PBS or a testing solution. The testing solutions were prepared as follows. When the effect of divalent cations was studied, either a 100 mM CaCl_2 solution or a 100 mM MgCl_2 solution was added to a solution buffered with Tris-HCl (pH 7.4 at 37°C). The salt composition of the Tris-buffer solution (TBS) was 137 mM NaCl, 2.7 mM KCl, and 5 mM Tris-HCl. When the effect of K^+ was studied, NaCl in PBS (in the absence of CaCl_2) or in TBS (in the presence of CaCl_2) was iso-osmotically replaced with KCl.

In one series of experiments used to study the effect of enzyme treatments; α -chymotrypsin (Worthington Chem. Co.), Pronase (Calbiochem. Co.), and collagenase (Merck Co.) were used to prepare single heart-muscle cells. The enzyme solutions were dialyzed in PBS at 0°C overnight to eliminate contaminating polyvalent cations. The procedure of cell isolation was basically the same as that described above.

The electrophoretic mobility of the single heart-muscle cells was measured with a cytopherometer (Carl Zeiss) immediately after suspending the cells in the solution. The temperature of the dispersion solution was kept at 37°C throughout the experiment of mobility measurements. The strength of the electric field horizontally applied to the cells inside a mobility measuring chamber of the cytopherometer was 2.2 ± 0.1 V/cm. The polarity of the electric field was reversed in each mobility measurement. The single heart-muscle cells that appeared on the 'front stationary layer', where no movement of the solution was produced by the application of the electric field, were timed at over 98 μm (49 μm in each direction).

The zeta potential was determined from the electrophoretic mobility in accordance with the Helmholtz-Smolukowski equation,

$$\zeta = \frac{4\pi\eta}{\epsilon} U.$$

Here, ζ represents the zeta potential; U , the electrophoretic mobility; ϵ , η , the dielectric constant and the viscosity of the dispersion fluid, respectively.

RESULTS

1. *Mobility and the zeta potential of single heart-muscle cells dispersed in PBS.* The single heart-muscle cells prepared by trypsinization were dispersed in PBS. During the removal of the enzyme from the bathing medium, the percent of spontaneously beating cells decreased to approximately 10%. However, there was no detectable difference in the electrophoretic mobilities of the beating cells and the still ones. Fig. 1 shows the histogram obtained from 48 individual cells. The lower and the upper abscissae represent the electrophoretic mobility and the corresponding zeta potential, respectively. The mean value of the mobility was 3.51 $\mu\text{m}/\text{sec}/\text{V}/\text{cm}$ with a standard error (S.E.) of 0.11 $\mu\text{m}/\text{sec}/\text{V}/\text{cm}$. The zeta potential was -37.6 ± 1.2 mV (mean $\pm$ S.E.)

Fig. 1. Distribution of the electrophoretic mobility of single heart muscle cells dispersed in PBS. Cells were prepared by 0.1 mg/ml trypsin treatment. For details refer to the text. The upper abscissa represents the zeta potential corresponding to the electrophoretic mobility.

TABLE 1. EFFECT OF ENZYME TREATMENTS ON MOBILITY AND ON ZETA POTENTIAL

Enzyme	Conc. of enzyme (mg/ml)	Treatment time (min)	Number of cells	U ($\mu\text{m/sec/V/cm}$) mean $\pm$ S.E.	Zeta pot. (mV) mean $\pm$ S.E.
Trypsin	0.1	14	48	3.51 $\pm$ 0.11	-37.6 $\pm$ 1.2
α -Chymotrypsin	1.0	20	11	3.12 $\pm$ 0.09	-33.4 $\pm$ 1.1
α -Chymotrypsin	0.1	40	10	3.20 $\pm$ 0.09	-34.3 $\pm$ 1.0
Pronase	1.0	20	10	3.12 $\pm$ 0.18	-33.6 $\pm$ 1.9
Pronase	0.1	40	20	3.31 $\pm$ 0.15	-35.4 $\pm$ 1.6
Collagenase	1.0	120	10	3.05 $\pm$ 0.15	-32.7 $\pm$ 1.6

Similar results were obtained for single heart-muscle cells prepared with other enzymes. Mobility was, however, influenced by enzyme treatment to some extent. Table 1 summarizes the results. Strong enzyme treatment of the heart tended to reduce the value for electrophoretic mobility, and thus to shift the zeta potential to a positive direction. The effect of enzyme treatment was rather small in comparison to the effect of changing the salt composition of the dispersion medium as described below. In these experiments, a short exposure of the cells to a dilute trypsin solution was least effective. Thus the single cells used in the following experiments were prepared with the trypsin solution.

2. *Effect of divalent cations.* Negatively charged groups in the outer layer of the excitable membrane have been suggested to have high affinities for divalent cations, especially calcium ions (5). In this series of experiments, the effects of an addition of CaCl_2 and of MgCl_2 on the zeta potential were studied. In Fig. 2, the zeta potential is plotted against the logarithmic scale of the concentration of CaCl_2 (open circles) or of MgCl_2 (solid circles). Both Ca^{2+} and Mg^{2+} acted to shift the zeta potential to the positive direction. However, Ca^{2+} had a much stronger effect on this shift and thus neutralized the negatively charged groups at the cell surface. Furthermore, the characteristic change in the zeta potential produced by Ca^{2+} was distinct from that caused by Mg^{2+} . At a Ca^{2+} concentration between 0.03 and 0.3 mM, there was a sharp rise in the zeta potential of approximately 8 mV. Another sharp rise in the zeta potential

Fig. 2. Effect of Ca^{2+} (open circles) and of Mg^{2+} (solid circles) on the zeta potential. Bars drawn vertically across the circles indicate the value of S.E.

was observed between 10 and 30 mM Ca^{2+} concentration. With Mg^{2+} , there was only one sharp rise in the zeta potential at a Mg^{2+} concentration between 3 and 30 mM.

3. *Effect of potassium ions.* Potassium ions also acted to shift the zeta potential to the positive direction. However, potassium sensitivity of the zeta potential was observed only when the bathing medium contained no Ca-salt or when the Ca^{2+} concentration was extremely low. In Fig. 3, the zeta potential is plotted against the logarithmic scale of the K^+ concentration. The open circles represent the data obtained by changing the K^+ concentration of PBS. In this case, the zeta potential rose rather

Fig. 3. Effect of K^+ on the zeta potential in the absence of Ca^{2+} (open circles) and in the presence of Ca^{2+} (half-solid circles and solid circles) in the dispersion media. Bars drawn vertically across the circles represent the value of S.E.

linearly with an increase in $\log [K^+]_0$; an elevation of approximately 17 mV was produced by increasing the K^+ concentration from 4.2 to 105 mM. The potassium sensitivity of the zeta potential was somewhat suppressed when 0.1 mM $CaCl_2$ was added to the dispersion media buffered with Tris-HCl (half-solid circles). The suppression effect of Ca^{2+} was stronger in the low K^+ concentration region. When 3.0 mM $CaCl_2$ was present in the bathing media, the zeta potential remained at a constant level of approximately -23 mV over the whole range of K^+ concentrations studied (solid circles).

4. *Effect of caffeine.* An addition of K-salt to the bathing medium produces contraction of heart-muscle cells (11). To check the effect of this contraction on the zeta potential the electrophoretic mobility of the single heart-muscle cells prepared by trypsinization was measured in PBS containing 5 mM caffeine, an agent known to contract heart-muscle cells (10). The mobility obtained from 10 individual cells was $3.57 \pm 0.12 \mu\text{m}/\text{sec}/\text{V}/\text{cm}$ (mean $\pm$ S.E.). The zeta potential was calculated to be -38.1 ± 1.3 mV (mean $\pm$ S.E.). The results show that treatment of the heart-muscle cells with caffeine did not produce a significant change in the zeta potential. Therefore, the electrochemical properties of the cell surface is not altered by changes in the configuration of the cells, e.g. contraction.

DISCUSSION

Calcium, magnesium, and potassium ions acted to neutralize the negatively charged groups at the cell surface. The density of the negative-charges at the surface of cells dispersed in PBS can be estimated by assuming that the electrolytes in PBS are the 1:1 type using the Gouy-Chapman relationship for a flat double layer in the form

$$\sigma = (\epsilon RTI/500\pi)^{1/2} \sinh(F\zeta/2RT)$$

where σ is the charge density; I , the ionic strength of the dispersion medium; R , the gas constant; T , absolute temperature; F , the Faraday constant. The charge density calculated from the value of the zeta potential obtained in Fig. 1 is $-3.5 \pm 0.1 \mu\text{C}/\text{cm}^2$ (mean $\pm$ S.E.).

The results in Fig. 2 suggest that the outer surface of the cell possesses at least two kinds of binding groups for Ca^{2+} . One is highly specific to Ca^{2+} with a binding constant estimated to be 6.2×10^3 l/M. The other is non-specific to divalent cations with a binding constant estimated to be 6.5×10 l/M. Fig. 3 can be understood by assuming that K^+ acts to neutralize the Ca^{2+} specific charged groups antagonistically to Ca^{2+} .

The electrophysiological properties of single heart-muscle cells from chick embryos have been studied extensively by DeHaan and Gottlieb (2), and by Lehmkuhl and Sperelakis (7). Thus we compared the characteristics of the zeta potential determined in the present investigation with potentials of the membrane reported previously in order to determine the role of the zeta potential in the generation of the membrane potential. According to Lehmkuhl and Sperelakis, changes in the external Ca^{2+} and Mg^{2+} concentrations in a range of 0 to 15 mM did not produce a significant change in the membrane potential. They also reported that an increase in the K^+ concentration (above 10 mM) of the bathing medium which contained 1.8 mM $CaCl_2$ caused a rise in the membrane potential with a slope of 60 mV per ten-fold change of K^+ concentration. These findings indicate that K^+ is the only ion species among Na^+ ,

K^+ , Ca^{2+} , Mg^{2+} to alter the membrane potential, and that the membrane behaves as an ideal potassium electrode over a wide range of K^+ concentrations. These characteristics of the membrane potential are quite different from those of the zeta potential given in Figs. 2 and 3 in the present article. In other words, there seems no direct relationship between the behavior of the membrane potential and that of the zeta potential.

According to Teorell (13), Meyer and Sievers (9), the potential difference across a homogeneous, charged membrane can be expressed as the sum of the phase-boundary potential at the two membrane-solution interfaces and the intramembrane diffusion potential. The phase boundary potential is approximated by the surface potential or by the zeta potential. In such a homogeneous membrane, alterations in the zeta potential directly contribute to alterations in the membrane potential. [This direct contribution of the zeta potential to the membrane potential has been observed in a biological system, the true slime mold, *Physarum polycephalum* (4).] On the other hand, in a spatially heterogeneous membrane, especially in a non-conductive membrane studded with highly conductive sites or channels, the zeta potential can behave differently from the membrane potential. [Note that the membrane potential in a heterogeneous membrane is the function of the e.m.f., conductivity, and the fraction of the area of each component of the membrane, whereas the zeta potential simply exhibits the averaged quantity over the whole membrane surface.] Therefore, the behavior of the zeta potential independent of that of the membrane potential in single heart-muscle cells suggests that only a small fraction of the area highly permeable to K^+ (K-channels) is responsible for determining the membrane potential and that charged groups sensitive to both Ca^{2+} and K^+ are not involved in the K-channels.

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