Short Communication

Stomatocytosis of Latex Particles (0.26 μm) by Rat Erythrocytes by the Electrical Breakdown Technique

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Summary. The uptake of macromolecules by erythrocytes can be achieved with the electrical breakdown technique [2, 4]. In this technique the erythrocyte membranes are subjected to a high external electrical field pulse for a short period. Local, reversible breakdowns of the cell membrane occur above a critical field strength which lead to a time-dependent increase in the permeability of the membrane. By this means, human erythrocyte membranes can be made permeable to DNA, pharmaceutical compounds, and latex particles following an electrical field pulse [1, 3, 5].

Larger particles should also be taken up by erythrocytes using this method. Vienken et al. [5] demonstrated the entrapment of latex particles with a diameter of 0.091 μm in human erythrocyte ghosts, although this was shown with only a single electron micrograph which does not prove that the ghost membrane was intact.

In our experiments in order to entrap latex particles with a diameter of 0.26 μm rat erythrocytes were subjected to an electrical field pulse of 12 kV/cm with a decay time of 60 μs.

Experiments using the electron microscope show that after such an electrical field pulse the uptake of latex particles by rat erythrocytes follows the stomatocytic pathway. We show further that using electron microscopic techniques, a single section cannot demonstrate the completed uptake of a latex particle by the erythrocyte.

Key words: Electrical breakdown technique – Stomatocytosis of latex particles – Rat erythrocytes

Materials and Methods

Blood taken from Wistar rats (Max-Planck-Institut für Biochemie, Martinsried, FRG) weighing 200 g was washed several times in solution A (138.6 mM/l NaCl, 13.3 mM/l Na2HPO4, 2.7 mM/l NaH2PO4, pH 7.4) and the erythrocytes were pelleted at 1500xg. The erythrocytes

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Fig. 1. Schematic diagram of the discharge apparatus. It consists of a Plexiglas chamber and two stainless steel electrodes, between which the cell suspension was placed. The cell suspension was incubated at a temperature of 4°C and an electric field pulse of 12 kV/cm with a decaying constant of 60 μs was applied. Following the pulse the cells were held for 10 min at a temperature of between 0°C and 4°C in order to increase the probability that the latex particles were taken up [6]. Finally, the cell temperature was increased to 37°C and the cells were normally prepared for electron microscopy after 30 min.

were resuspended at a ratio of 1:10 in solution B (105 mM/l KCl, 20 mM/l NaCl, 4 mM/l MgCl₂, 7.6 mM/l Na₂HPO₄, 2.4 mM/l NaH₂PO₄, 10 mM/l glucose, pH 7.3).

To this suspension, with a cell concentration of 8 x 10⁶ erythrocytes/ml, latex particles of 0.26 μm diameter were added. The latex particles adhere to the erythrocyte cell membranes due to their carboxylated surface. For the entrapment experiments this erythrocyte and latex particle suspension was introduced into a discharge chamber and an electrical field pulse was applied.

The high voltage necessary for the electrical field pulse was produced by a capacitor which was charged from a high voltage generator. The capacitor was discharged by a spark-gap switch, the cell suspension acting as the resistance for the discharge. Figure 1 shows a schematic diagram of our pulse apparatus.

For electron microscopy specimens were prefixed with glutaraldehyde and postfixed with osmium tetroxid using routine procedures. After dehydration in an ethanol series specimens were embedded in Epon 812 resin. Ultrathin sections were double-stained with lead citrate and uranyl acetate and viewed in a JEOL 100B microscope at 80 kV.

Results and Discussion

The section series A (Fig. 2a–f) shows the uptake of several latex particles with a diameter of 0.26 μm by a rat erythrocyte to which an electric field pulse of 12 kV/cm with a decay time of 60 μs had been applied. The breakdown of the membrane produced by the pulse enabled the uptake of latex particles via the stomacytotic pathway.

However, the completed uptake of latex particles by an erythrocyte cannot be demonstrated with a single section. Figure 3a and b shows the section series B, which seem to demonstrate the entrapment of latex particles, but Fig. 3c shows that the latex particles had not been completely taken up by the cell.

In erythrocyte ghosts, which no longer contain haemoglobin, it is even more difficult to demonstrate the uptake of particles using electron microscopical methods. Therefore, in order to prove that the ghost membranes are intact, a series of sections