Pressure-induced variations of K^+-permeability as related to a possible reversible electrical breakdown in human erythrocytes[1]

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Summary. Above a hydrostatic pressure of about 600 b a pronounced reversible increase in the net K^+-efflux from human erythrocytes is observed. The effect is explained in terms of an electro-mechanical compression of the membrane, resulting in a reversible breakdown of the membrane.

Small hydrostatic pressure gradients in plant cells (turgor pressure) can exert pronounced effects on membrane structure and transport processes[2,3]. On the other hand, application of high hydrostatic pressures is known to affect profoundly the structure, physiology and biochemistry of biological systems (for review see[4-6]). Among other things, it has been established that hydrostatic pressure induces significant changes in the transport characteristics of cell membranes and epithelia[7-13].

On the basis of the electromechanical model introduced by Zimmermann et al.[2,14] for the interpretation of the reversible electrical breakdown phenomenon of membranes of animals, plants and bacteria as well as of artificial lipid bilayer membranes, the effects of small pressure gradients (turgor pressure) and those of high hydrostatic pressure on biological membranes can be explained from an integrated viewpoint[2,3]. The electromechanical model postulates that both pressure and electric field compress the membrane or, more likely, parts perpendicular to the membrane plane. The theory predicts that a maximum voltage, can be established, across the membrane, above which reversible electrical breakdown occurs[14,15]. Electrical breakdown of the cell membrane was indeed observed for cell membranes[15] and lipid bilayer membranes[16] when the membrane potential was taken rapidly (nsec to μsec) to a critical value of about 1 V. The breakdown is associated with a reversible increase in membrane conductance and permeability. The breakdown voltage decreases with increasing pressure gradient as shown experimentally[17] and predicted by the theory. From the pressure dependence of the breakdown voltage the value of the elastic compressive modulus perpendicular to the membrane plane can be estimated to be low enough to allow pressure signals of less than 1 b to be transformed into changes in the intrinsic electrical field and, in turn, in membrane processes[2,18]. On the other hand, with the knowledge of the value of the elastic compressive modulus, and with some assumptions concerning the pressure dependence of this parameter, and the interaction of hydrostatic pressure with the membrane structure, it is possible to estimate the pressure at which the breakdown voltage matches the intrinsic membrane potential[2,19]. Thus, the model predicts a critical high hydrostatic pressure above which the electrical breakdown of the membrane leads to a reversible increase in membrane permeability.

The purpose of the present communication was to test the validity of the model at high hydrostatic pressures by using the fact that passive net K^+-efflux from human erythrocytes is dependent on increasing absolute pressure as an indicator for the reversible electrical breakdown of the membrane. With a breakdown voltage of about 1 V at atmospheric pressure[15], and with a value of about 13 mV[20] for the membrane potential, as well as with a value of 35 b for the elastic compressive modulus[2,19] the critical pressure is estimated to be about 600 b.

Material and methods. Human erythrocytes packed by cen-

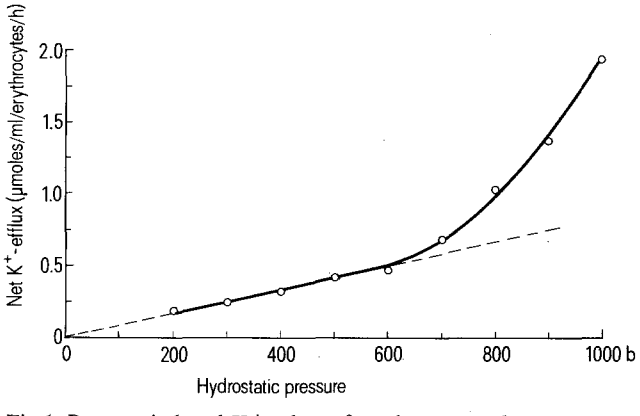

Fig. 1. Pressure-induced K^+-release from human erythrocytes as a function of hydrostatic pressure. Values of the pressure-induced K^+-efflux are given as μmoles K^+ released per ml erythrocytes per h and are corrected for the K^+-loss observed in control experiments under identical conditions, except for the pressure application. Pressure was applied for 20 and 40 min. Compression time varied between 10 and 30 sec depending on the magnitude of the applied pressure. 5 min after decompression the samples are centrifuged at 10,000 × g and the concentration of potassium in the supernatant was determined flamephotometrically. The experiments were carried out on the same blood sample at 22 °C.

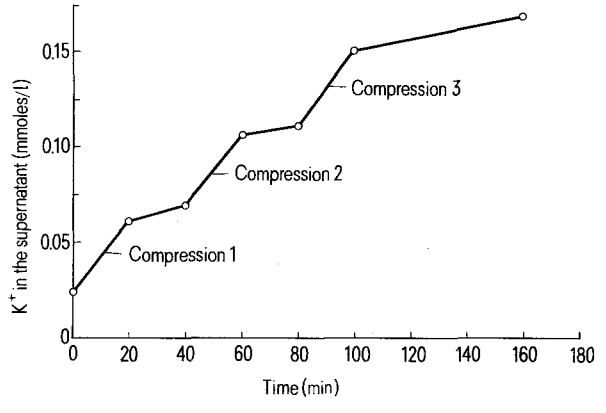

Fig. 2. Release of K^+ from erythrocytes subjected to 3 successive compressions (20 min at 1000 b, 22 °C) of the same suspension. Samples were taken immediately after each decompression step and 20 min later at atmospheric pressure just before the following compression. Note, that after the 3rd compression the K^+ concentration in the supernatant was again determined after 60 min. The suspension density of packed cells to solution was 1:19 (v/v). The K^+-release in response to pressure was not corrected by the potassium loss from the cells observed between 2 compression steps. It is quite obvious that in the limits of accuracy the K^+-release arising from each single pressure application is the same, indicating the reversibility of the pressure-induced effects on membrane permeability.

trifugation at 1000 × g for 10 min were diluted with acid citrate dextrose (ACD) buffer (1:1) and stored at 4 °C for not more than 24 h. Every 4 h, an aliquot was washed and suspended at a ratio of 1:15 (v/v) in a K^+-free saline solution containing 145 mM NaCl, 5 mM $MgCl_2$, 10 mM glucose and 5 mM Tris-HCl (pH 7.4). After temperature equilibration, 4 ml aliquots were exposed at 22 °C to absolute pressures ranging from 200–1000 b in a pressure device described elsewhere[16-20]. Briefly, the hyperbaric chamber consists of a steel cylinder with an inner plexiglass container. The chamber is pressure sealed by means of a steel screw cap. Particular care has been taken to minimize the temperature effects caused by the more or less adiabatic stepwise compression or decompression of the silicone fluid. By means of a pressure vessel specially designed to keep test tubes in an as large as possible water bath, a maximum temperature change of 2 °C was measured in the samples after applying a sudden pressure increase of 1000 b, reached in 30 sec. After decompression, the K^+-content of the erythrocytes and of the incubation medium was determined by flame photometry. The values for the K^+-efflux were corrected for the K^+-release observed in control experiments in which the cell suspensions were kept at 22 °C without applying pressure. The corrected net K^+-efflux should therefore be 0 at atmospheric pressure.

Results and discussion. Results from a typical experiment are shown in figure 1. The pressure induced net K^+-efflux from human erythrocytes increases almost linearly up to pressures of about 600–700 b (0.5 µmoles/ml erythrocytes/h). Above this pressure range a significant increase in the pressure-induced net K^+-efflux is observed. The pressure was applied in this experiment for 20 and 40 min, respectively. The K^+-effluxes have been observed to remain linear for at least 60 min regardless of the applied pressure. The observed permeability change is a reversible event, as shown by 3 consecutive cycles of compression (20 min at 1000 b followed then by 20 min at atmospheric pressure) (figure 2). The release of K^+ from the erythrocytes into the supernatant induced by each single pressure application is indeed the same for 3 consecutive compression steps.

The above results show that a 'narrow' pressure range exists, at which a pronounced increase in the membrane permeability occurs. Experiments conducted at atmospheric pressure by changing the temperature of the incubation medium have definitely allowed us to discard any possibility of a temperature-induced event related to the compression of the fluids. The results presented here thus seem to confirm the predictions of the electro-mechanical model formulated for plant and animal cell membranes. Although the experimental results are consistent with the predictions of the electro-mechanical model, at present other possible mechanisms cannot be excluded with certainty; this will be discussed in detail in a forthcoming paper.

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Octopamine and proctolin mimic spontaneous membrane depolarisations in *Lucilia* larvae*

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Summary. Octopamine and proctolin at concentrations below 10^{-8} M reversibly induce a spontaneous rhythmic depolarization which occurs in body-wall muscles of *Lucilia* larvae. The effect appears to be postsynaptic and mediated by receptors specific for each substance.

Body-wall muscles of cyclorrhaphan dipteran larvae are innervated by a single 'fast' and a single 'slow' axon[2,3]. However, a further type of response can be recorded from these muscles which is not associated with motor axon stimulation.

Materials and methods. Intracellular recording and nerve stimulation of body-wall muscles of *Lucilia sericata* larvae were as previously described[2,4]. Saline used was 172 mM NaCl, 13.3 mM KCl, 4 mM $CaCl_2$, 6 mM $MgCl_2$, 1.0 mM NaH_2PO_4, 1.2 mM $NaHCO_3$, 33 mM sucrose, pH 7.1. Calcium-free saline was prepared by omitting calcium and adjusting the overall osmolarity by increasing the sucrose concentration to 43 mM. D-L Octopamine hydrochloride, tetrodotoxin (Sigma Chemical Co.) and proctolin (Peninsula Labs Inc.) were added to the saline at the concentrations stated.