Biochimica et Biophysica Acta, 330 (1973) 351–355

BBA Report

BBA 71190

Modulation of red cell K^+ transport by membrane lipids

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(Received October 17th, 1973)

SUMMARY

Alterations in the state of the membrane lipids affect human red cell K^+ transport. Depletion of membrane cholesterol by 29–34% significantly inhibited both total K^+ influx and ouabain-sensitive K^+ influx. Addition of the hydrophobic anesthetic, chlorpromazine, in concentrations from $2 \cdot 10^{-5}$ to $2 \cdot 10^{-4}$ M increased both total K^+ influx and ouabain-sensitive K^+ influx. In each case the effect on both processes was almost identical which indicates a linkage between K^+ "pump" and "leak". Further, these results demonstrate that red cell K^+ transport can be modulated by local conditions in the micro-environment of the transport system.

K^+ is actively transported up an electrochemical potential gradient into the human red cell by a mechanism whose molecular details remain obscure. The observation that K^+ transport can be modulated by changes in human red cell volume led Poznansky and Solomon[1] to suggest that this volume-dependent response was mediated by a conformational change in a transport site located on the cell surface in conformity with the explanation put forward by Romualdez *et al.*[2] to account for the volume effect on Na^+ transport in dog red cells. Kregenow showed for duck red cells[3], and Poznansky and Solomon[4] for human, that cell volume is regulated by deformation-induced changes in the cell cation transport system. The present study is concerned with the effects of other perturbations of the cell membrane on the K^+ transport system: specifically depletion of the cholesterol content of the cell membrane and the insertion of a hydrophobic anesthetic into the membrane. We have found that each of these perturbations of the membrane array exerts a significant effect on the K^+-transport system.

Red cell cholesterol was depleted by 29–34% using the method of Bruckdorfer *et al.*[5] and cholesterol content was assayed by the method of Parekh and Jung[6]. K^+ fluxes

TABLE I

EFFECT OF CHOLESTEROL DEPLETION ON RED CELL K^+ FLUXES

The ouabain concentration used to inhibit K^+ fluxes was $2 \cdot 10^{-4}$ M in these experiments as well as those in the chlorpromazine series.

Exp.	*Cholesterol depletion*	*ratio* $\frac{depleted}{control}$			
	(%)	*K^+ influx*	*ouabain-inhibitable K^+ influx*	*K^+ efflux*	*ouabain-inhibitable K^+ efflux*
3	29	0.85	0.80	–	–
8	34	0.57	0.56	0.66	0.64
9	31	0.76	0.74	0.76	0.68

were measured as previously described[4] over a 2-h period at 37 °C; the effect of cholesterol depletion on these fluxes is shown in Table I. It is significant that cholesterol depletion exercises an almost identical effect on both total influx and ouabain-sensitive influx. A similar observation may be made about K^+ efflux. Ouabain has often been used to separate the K^+ "pump" from the K^+ "leak" and, largely on this basis, these have been considered as separate and discrete K^+ pathways. The present evidence, on the contrary, suggests a linkage between these processes.

Bruckdorfer *et al.*[5] have shown that cholesterol depletion increases the glycerol permeability of human red cells, and similar results have been obtained on the permeability coefficients of other non-electrolytes in human cells by Kirkwood and Solomon (personal communication). In the guinea pig red cell, however, there is an important difference in the effect of cholesterol. Kroes and Ostwald[7] increased the cholesterol content in these cells and found a decrease in lipophilic solute permeation, consistent with the results in man; however, increased membrane cholesterol caused a decrease in total and ouabain sensitive Na^+ influx, so that in this respect guinea pig red cell Na^+ transport differs from human K^+ transport. This may be related to differences in lipid composition since phosphatidyl ethanolamine comprises 22% of total phospholipid in the guinea pig compared with 3% in man; there is a complementary difference in sphingomyelin content (8% in guinea pig; 22% in man)[8].

Another set of four experiments were carried out to measure the effect of the hydrophobic anesthetic, chlorpromazine, on human red cell K^+ fluxes at 37 °C. Chlorpromazine (chlorpromazine · HCl; Smith, Kline and French) in ethanol solution was added to the medium in concentrations from $2 \cdot 10^{-5}$ to $2 \cdot 10^{-4}$ M. The final ethanol concentration was adjusted to 0.09 M under all experimental conditions, including controls. Fig. 1 shows the results of two typical experiments. The top segment of the figure shows that the anesthetic causes total K^+ influx to increase monotonically over the entire concentration range studies. The middle segment shows that the ratio of the chlorpromazine effect on the ouabain inhibitable influx to its effect on the total influx remains close to

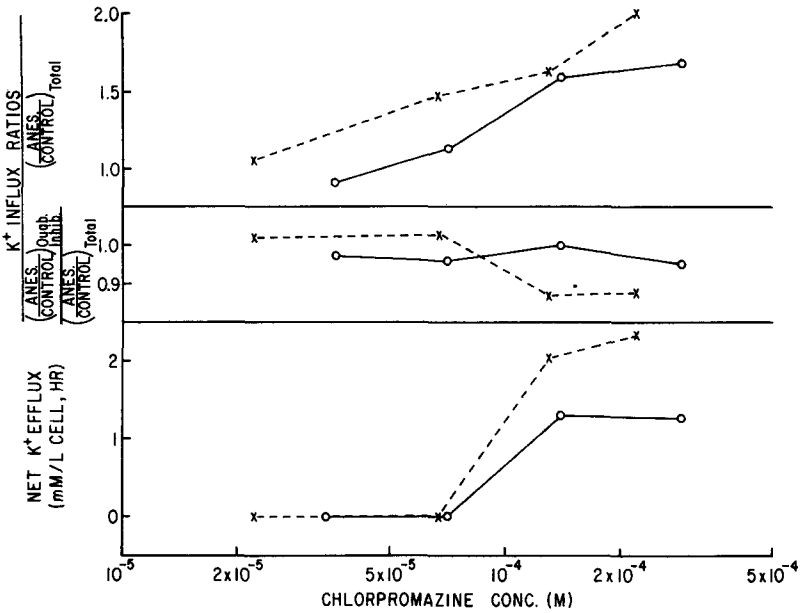

Fig. 1. The top segment shows chlorpromazine stimulation of total K^+ influx as the ratio: anesthetic/control. The middle segment gives a ratio of ratios: (anesthetic/control) for ouabain inhibitable K^+ flux divided by (anesthetic/control) for total K influx. The bottom segment is net K efflux in (mM/l cell, h). The buffer in these experiments, as well as those on the cholesterol depleted cells, had the following composition, in mM: $MgCl_2$ 1.2, $CaCl_2$ 2.4, Na_2HPO_4 1.7, NaH_2PO_4 4.2, KCl 5.0 (+ tracer and carrier), Na_2CO_3 13.5, NaCl 117.8, glucose 10.0. A mixture of 5% CO_2 and 95% air was bubbled through the solution to bring it to pH 7.4.

unity. This ratio shows that the anesthetic exercises virtually the same influence on the "pump" as on the total flux ("pump" + "leak") over the entire concentration range just as in the case with cholesterol depletion. The bottom segment of the figure shows that the K^+-transport system remained essentially in a steady state at the two lower anesthetic concentrations, whereas at the two higher concentrations the cells become grossly leaky to K^+.

Chlorpromazine, with a membrane:buffer partition coefficient of 1600, is among the most hydrophobic of the anesthetics studied by Seeman and his collaborators[9]. At an external concentration of $6 \cdot 10^{-5}$ M, which is in the middle of our concentration range, chlorpromazine increases the area of human red cell membrane ghosts by about 2%. At this concentration there is approximately one chlorpromazine molecule per 200 $Å^2$ of membrane, which is the equivalent of a chlorpromazine concentration of about one molecule for every three of four phospholipids. Chlorpromazine affects human red cell permeability in many ways including complex interactions with the Na^+ and glucose transport systems[9–14]. Other lipophilic anesthetics such as procaine, cocaine and ethanol have also been shown to affect red cell cation transport[15,16]; like chlorpromazine, these drugs cause a net K^+ efflux, but they also inhibit K^+ influx, whereas chlorpromazine stimulates this process.

Two conclusions may be drawn from the present study. The close connection between "pump" and "leak" provides additional evidence for the linkage previously shown when human red cells were perturbed by reversible changes in cell volume. Thus, there must be either a common step, possibly protein in nature, in the pathway for "pump" or "leak" or both systems must respond in a similar fashion to these three functionally separable perturbations of the cell membrane. This implies that the separation between "pump" and "leak" produced by ouabain cannot be accepted as proof that "pump" and "leak" proceed by two independent, separate and distinct pathways. Rather ouabain could be considered to alter the conformation of its receptor so that the ion-transport characteristics of the ouabain–receptor complex became different from normal.

The second conclusion is that the active transport of K^+ can be modulated by altering the state of the membrane, either by insertion of chlorpromazine or the partial removal of membrane cholesterol. The requirement of soybean phospholipid for the action of the reconstituted Ca^{2+} pump has been shown by Racker[17]. Rothfield and Romeo[18] have shown a specific requirement for phosphatidylethanolamine to interact with a lipopolysaccharide in the first step of the galactosyltransferase reaction. This reaction is sensitive both to the nature of the polar group and the conformation of the fatty acid chains in the phospholipid. Overath and collaborators[19,20] have found a sharp break in the Arrhenius plot of β-galactoside transport into *Escherichia coli* which is correlated with the melting point of the lipid in the membrane.

The present study shows that the active K^+ transport in human red cell membranes can be modified by relatively smaller perturbations affecting the membrane lipid. Insertion of anesthetic in the membrane is thought by Seeman[9] to introduce disorder in the structure, a conclusion primarily based on the studies of Metcalfe *et al.*[21] on nuclear magnetic resonance relaxation of benzyl alcohol in red cell membranes. Papahadjopoulos[22] suggests that the effect of local anesthetics, such as chlorpromazine, may be ascribed to interference of the ionic interactions of the phospholipid head groups. Furthermore, one cannot exclude a direct action of chlorpromazine on the K^+-transport system since chlorpromazine has been shown to bind strongly to albumin[23] and oxyhemoglobin A[24]. However, it is probable that the effect is mediated through an alteration of the state of the membrane either in the packing of its components, or surface charge, or both. This is the more likely because of the high concentration of chlorpromazine in the membrane and its diverse effects on so many red cell transport processes.

Whereas chlorpromazine increases K^+ influx in human red cells, cholesterol depletion inhibits it. Cogan *et al.*[25] have shown that introduction of cholesterol into phosphatidylcholine liposomes increases the microviscosity, an observation which fits with the increased permeability to nonelectrolytes[4,6] caused by cholesterol depletion in human red cells. De Gier *et al.*[26] agree that the primary effect of cholesterol on the red cell is a condensing one, but point out that the high dipalmitoyllecithin content of the membrane would permit a local liquefying effect of cholesterol. In either case, the present evidence indicates that the red cell K^+ transport system is sensitive to the condition of its microenvironment. In this sense it is hardly surprising that the introduction of an anesthetic

affects the K^+ transport system in an opposite sense from that produced by removal of membrane cholesterol.

This study has been supported in part by the National Institutes of Health (grant GM HL 15692-05) and by the Atomic Energy Commission (contract AT(11-1)-3010). We should like to express our thanks to B. Hille for suggesting the use of anesthetics to perturb the lipids and to E.A. Dawidowicz and J. Rothman for general discussions of the properties of lipids.

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