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MODIFICATION OF ION-TRANSPORTING SYSTEMS OF HUMAN ERYTHROCYTES DURING KEEPING

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The last decade has seen a sharp increase in the number of diagnostic kits, associated with determining the functional state of cell membranes. In one case, these diagnostic kits have been designed for analysis of the efficacy of therapeutic preparations (for example, agonists and antagonists of different classes of receptors, organic calcium antagonists cardiac glycosides, and so on), in another case to detect abnormalities in the functioning of certain membrane-bound systems. In clinical practice, virtually the only object accessible for studies of this kind is the blood cells. The reason why they can be used is that the level of activity of membrane-bound systems in the blood cells and in cells functionally significant for the pathogenesis of the disease of the organs under investigation correlate. In particular, this approach is currently being used for the differential diagnosis of essential hypertension. In an experimental model of essential hypertension, namely spontaneous genetic hypertension of rats, changes detectable in the blood cells in most cases can also be detected in smooth muscle cells, nerve endings, epithelial cells of the renal tubules, i.e., in organs directly related to the functioning of the systems for long-term regulation of the blood pressure [2, 5, 11].

The introduction of these methods into practice on a large scale depends on a number of factors and, in particular, on the stability of the method and the stability of the parameter determined in the case of storage of blood cells.

In this paper we give data showing that if heparinized blood is kept for three days on ice, there is no change in the rate of functioning of Na^+/Li^+ antitransport, Na^+/K^+-cotransport, and Na^+,H^+-exchange in the erythrocytes. Data were obtained previously on the heterogeneity of the kinetic parameters of these carriers in rats with spontaneous hypertension and in humans with essential hypertension [2-4, 7, 8, 10]. The results of the present investigation indicate that the tests may be used to determine the functional state of cell membranes in the investigation of large population groups, for the stability of the parameters determined is such that material can be accumulated, and this greatly simplifies problems related to its collection and transport.

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TABLE 1. Content and Transport of Monovalent Ions in Erythrocytes ($M \pm m$)

Time after taking blood, h	Na	K	Na^+,K^+-ATPase	Na^+/K^+-cotransport	(Ouabain+furosemide)-nonhibited component of K^+ outflow	Na^+/Li^+ antitransport	Velocity of lithium outflow into magnesium medium	Na^+,H^+ antitransport	(Amiloride+SITS)-noninhibited component of H^+ outflow
	meq/liter		μmoles/liter cells·h						
4	18,3±1,64	90,3±2,54	1231±149	346±50	98,8±7,4	256±21	175±11,5	13 200±2640	14 520±4080
28(30)	25,0±0,92	84,2±1,50	1995±858	353±50	86,8±2,7	241±30	207±21,3	12 360±3240	14 220±4680
52	32,0±1,28	82,8±2,52	2146±242	391±50	88,3±2,6	235±31	228±17,5	—	—
72	—	—	—	—	—	—	—	13 320±3000	14 280±3660
p 4,28(30)	0,005	n.s.	0,0001	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
p 4,52(72)	0,0001	n.s.	0,005	n.s.	n.s.	n.s.	0,05	n.s.	n.s.

Legend. n.s.) Not significantly.

EXPERIMENTAL METHOD

Blood from 9 patients (men aged from 27 to 53 years) under investigation in the clinic of the Institute for the Prevention of Noninfectious Diseases, was taken before breakfast from the ulnar vein into test tubes containing heparin solution (20-25 U/ml blood), shaken, and placed on ice, where it was kept for between 4 and 72 h before required for the experiment. The rate of Na^+/Li^+ antitransport (exchange) and the rate of release of lithium into a magnesium medium was determined by the method in [7] with modifications described previously [2]. The method of determining the rate of Na^+,H^+-exchange, induced by the creation of an electrochemical proton gradient (pH_i 6.35 + 6.45, pH_0 7.95 + 8.05), and of the amiloride-noninhibited component of the rate of release of protons under these conditions were described in [3]. Na^+,K^+-ATPase activity, Na^+,K^+-cotransport, and passive permeability of erythrocytes for K^+ were determined as the ouabain-inhibited component of ^{86}Rb inflow, the ouabain-insensitive furosemide-inhibited component of ^{86}Rb inflow, and the (ouabain + furosemide)-noninhibited component of ^{86}Rb inflow, respectively [9]. The method determination of the intracellular sodium and potassium concentration in described in the same citation.

EXPERIMENTAL RESULTS

The data given in Table 1 show that none of the carriers of monovalent ions studied in the work changed its kinetic parameters during storage of heparinized blood samples on ice for one day. On the second day there was a very small (13%) increase in the velocity of Na^+,K^+-cotransport whereas the velocity of Na^+/Li^+- and Na^+,H^+-antitransport remained unchanged until the 2nd and 3rd days of storage of the blood, respectively.

In the presence of ouabain and furosemide, of ouabain in a sodium-free (containing magnesium) medium and, finally, amiloride and a stilbene-disulfonic derivative (SITS), the principal of the presently known systems responsible for inflow of K^+ (Na,K^+-ATPase and Na^+K^+-cotransport) and Na^+ outflow (Na,K^+-ATPase: Na^+,Na^+-exchange) and H^+ outflow (Na,H^+-exchange and anionic HCO_3^-/Cl^--exchanger), are inhibited, respectively [6]. It can be postulated that under these conditions passive (simple) diffusion of ions through the membrane is recorded. It follows from Table 1 that passive membrane permeability for K^+ (^{86}Rb) and Na (Li^+) does not differ significantly, whereas passive permeability for protons is one to two orders of magnitude higher. During keeping the passive membrane permeability for sodium increases by 30% whereas passive permeability for potassium and protons is unchanged.

During keeping on ice, Na^+,K^+-ATPase was virtually inactivated, leading to an increase in the Na_i^+/K_i^+ ratio; however, as will be clear from Table 1, preincubation for 30 min at 37°C before the commencement of recording of the ionic flow could not completely restore this ratio. Evidently the 70-80% increase in Na_i^+ observed by the second day of keeping was the immediate cause of activation of Na^+,K^+-ATPase.

Thus on the basis of these results it can be concluded that keeping blood for 2-3 days on ice with the addition of heparin does not affect the kinetic parameters of the ion-carriers of the erythrocytes, responsible for the facilitated diffusion of Na^+, K^+, and H^+. This conclusion, based on a statistical analysis of the results of the study of blood from 9 individuals, is confirmed also by the data in Fig. 1, which gives individual curves for four patients. It is also clear that, unlike Na^+,K^+-ATPase, the range of variations of the velocities of translocation of the ion carriers has marked individual differences. As was shown previously, the range of variation of the velocity of work of the ion carriers varies in a given individual in the course of 10

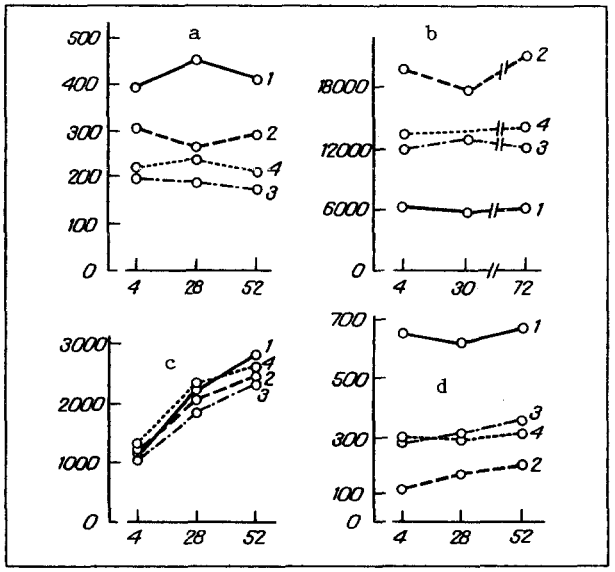

Fig. 1. Na^+/Li^+-antitransport (a), Na,K-ATPase (b), Na^+/H^+-antitransport (c), and Na^+/K^+-cotransport (d) in erythrocytes from four blood donors. Abscissa, time of keeping erythrocytes (in h); ordinate, velocity of ion transport by corresponding systems (in μmoles/liter of cells/h). 1-4) Individual curves.

months of observation by not more than 5-10%, which lies within the limits of experimental error and demonstrates the strong genetic control of these parameters [1]. This last conclusion is extremely important for population studies.

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