Structural Changes in the Erythrocyte Plasma Membrane in Dehydration

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Changes in the lipid bilayer and glycocalyx of the erythrocyte plasma membrane are studied by the method of spin labels in dehydrated rats. No appreciable changes are found in the membrane of erythrocytes from rats deprived of water for 3 days. Changes occur in the glycocalyx after a 6-day dehydration, while a 12-day dehydration induces irreversible changes in the plasma membrane lipids and the erythrocyte surface areas.

Key Words: *erythrocytes; membranes; spin labels; dehydration*

Ketoacidosis is a life-threatening complication resulting from absolute or relative insulin insufficiency in diabetes mellitus. It is characterized by accumulation of ketones in the blood due to impaired carbohydrate metabolism. This is accompanied by cell dehydration and the loss of intracellular ions, which leads to total dehydration and electrolyte imbalance. Ketoacidosis always coincides with dehydration; sometimes water deficiency is as high as 10% of body weight [3].

Biological membranes are a finely adjusted system, in which water not only provides spatial organization but is also involved in the processes occurring in it. Functionally active protein molecule should contain water (so-called "bound water" [4]). Water, whose properties differ from those of cytoplasmic water, has been discovered in the plasma membrane glycocalyx by various methods [2]. Thus, normal function of plasma membranes is provided by their specific organization, in which water molecules play an important role.

Our objective was to study the structural state of the plasma membrane of erythrocytes obtained from dehydrated rats, which can be regarded as a model of ketoacidosis.

MATERIALS AND METHODS

Experiments were performed on 35 outbred rats. Control group consisted of 10 animals. Other rats were deprived of water for 3, 6 and 12 days. They were then rehydrated during the same time periods. Each experimental group contained at least 5 rats.

Erythrocytes were isolated from peripheral blood by centrifugation in phosphate buffer at 3000*g* for 20 min two times. The erythrocyte suspension contained 2×10^6 cells/ml Tris-HCl buffer, pH 7.2.

Structural state of the erythrocyte plasma membrane was assessed by the spin label method using an RLS radiospectrometer (Svetlana, Leningrad) at room temperature. Final concentration of the spin label was not higher than 10 M, while that of ethanol (the label solvent) was not higher than 2% v/v.

RESULTS

Previously, it was demonstrated that the presence of water markedly increases the mobility of lipids (primarily of their carbohydrate acyls) in photosynthesizing membranes [8]. Therefore, for detection of structural alterations of the lipid bilayer we employed the following spin labels: analogs of stearic acid with radicals in positions 5 and 16 relative to COOH group. Electron paramagnetic resonance (EPR) spectra of these labels allow for the evaluation of lipid mobility at the distances of 0.6 and 2.0 nm from the plasma membrane surface [5]. The parameter of ordering for label I is proportional to the microviscosity of the microenvironment of spin label. The EPR spectra for label II allow one to

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TABLE 1. Changes in the Parameter of Ordering and Spinning Correlation Time of Labels I and II Depending on the Duration of Dehydration

Duration of dehydration, days	Parameter of ordering	Correlation period, nsec
Control	0.635±0.008	5.3±0.20
3	0.642±0.012	6.1±0.60
6	0.697±0.010	8.3±0.65
12	0.743±0.016	15.8±0.56

calculate the time of spinning correlation which reflects the bilayer "rigidity" at the site of the label localization. The results are listed in Table 1.

Lipid microviscosity did not change considerably in the plasma membrane of erythrocytes of rats deprived of water for 3 days. This was true for the entire lipid bilayer (both at the membrane surface and deep in the bilayer). Presumably, during this period of dehydration water is lost mainly from the cytoplasm and partially from the glycocalyx, while the amount of protein-bound water remains practically unchanged.

After a 6-day dehydration, microviscosity increased in the entire lipid bilayer, the increase being higher deep in the bilayer. This may be associated with changes in native structure of integral proteins which is stabilized by "bound" water [7].

TABLE 2. Changes in *a/b* Parameter of Benzocarboline Spin Label During Dehydration

Duration, days	*a/b* Parameter
Control	0.25±0.080
3	0.38±0.023
6	0.65±0.034
12	1.08±0.043

After a 12-day dehydration, considerable changes occurred in the structural parameters determined from EPR spectra both at the surface and deep in the bilayer. This may reflect substantial transformations of peripheral proteins occupying about a half of the plasma membrane area [1].

In order to assess structural changes in the glycocalyx containing peripheral proteins and active sites of integral proteins that fulfill various cell functions we employed benzocarboline spin label possessing affinity for hydrophobic groups of proteins. The EPR spectrum of this label allows one to calculate the *a/b* parameter which is proportional to the number of hydrophobic groups reacting with the label. The results are shown in Table 2.

After 3 days of dehydration, the hydrophobic groups of surface proteins become more available for the label (Table 2). This may indicate a decrease in the amount of water bound to the plasma membrane glycocalyx. These changes become more pronounced as the period of dehydration increases, reaching the maximum in rats deprived of water for 12 days, when native structure of integral and most peripheral proteins is changed.

In order to find out whether the structural changes in the erythrocyte plasma membrane are irreversible, rehydration experiments were performed. Structural parameters measured after 3, 6, and 12 days of rehydration are summarized in Table 3.

Judging from these parameters, damage to lipid bilayer and glycocalyx caused by prolonged dehydration (12-days) is irreversible. Neither parameter was normalized by a 12-day dehydration, while a 3-day rehydration eliminated the changes occurring in the plasma membrane after a 3-day dehydration. After 6 days of dehydration and rehydration, structural parameters of the erythrocyte plasma membrane were altered, although no dramatic changes occurred at least in the lipid bilayer.

Thus, our results show that water is an important factor of membrane stability. Presumably, the state of water determines both the dynamic structure of membrane-bound proteins [6] and the microviscosity of membrane lipids. Restoration of structural and functional state of the plasma membranes during rehydration is possible only if conformational changes in membrane proteins are reversible. It can be suggested that interrelated and interdependent conformational transformations leading to irreversible changes start in the plasma membrane after a certain period of dehydration. The mechanisms underlying these transformations will be elucidated in further investigations.

TABLE 3. Changes in Structural Parameters of the Erythrocyte Plasma Membrane Calculated from EPR Spectra of Spin Labels After Rehydration

Duration of rehydration, days	Parameter of ordering	Correlation time	*a/b* Parameter
3	0.638±0.014	5.6±0.4	0.29±0.012
6	0.643±0.012	6.4±0.3	0.46±0.018
12	0.720±0.037	12.4±0.5	0.86±0.042

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Effect of the Opioid Agonist Fenaridin and its Antagonist on the Phosphoinositide Catabolism in Brain Synaptosomes of White Rats *In Vitro*

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The release of 1,2-diacylglycerol and arachidonic acid increases considerably by the 10th sec of incubation of arachidonate-labeled synaptosomes with the opioid antagonist fenetham. The opioid agonist fenaridin elicits an opposite effect.

Key Words: *fenaridin; fenetham; phosphoinositides*

Initiation of the phosphoinositide turnover, a membrane-coupled cascade transducing external signal via the second messenger pathway, is a universal mechanism involved in cell response to hormones, transmitters, and physiologically active compounds [4,5].

Signal transfer to phosphoinositide-specific phospholipase C or phosphoinositide-phosphodiesterase is a key event in the cascade of molecular events initiated by the ligand-receptor interaction. These enzymes cleave phosphoinositide to form the second messengers inositol triphosphate (IP_3) and 1,2-diacylglycerol (1,2-DG) that induce the release of intracellular Ca^{2+} and activation of protein kinase C, respectively [14].

The role of phosphoinositide turnover in the interactions of opioid agonists and antagonists with specific receptors is poorly investigated [13]. Inositol triphosphate may be involved in the release of endogenous opioid peptides [15], while protein kinase C may inhibit the analgesic effect of morphine [1].

Fenaridin (a compound belonging to 4-aminopiperidines) is a potent narcotic analgesic [1]. Its activity is higher than that of morphine, promedol, and fentanyl [2]. Fenetham, a compound with a similar structure, is a "pure" opioid antagonist of the naloxone type [2].

MATERIALS AND METHODS

Experiments were carried out on random-bred male rats weighing 150-180 g. Synaptosomes were isolated as described elsewhere [9]. Incorporation of ^{14}C-arachidonic acid in phosphoinositides was assessed by measuring the radioactivity of the monophosphoinositide fraction [12]. Synaptosomes (6-8 mg protein) were incubated for 45 min at 37°C in a mixture containing 80 mM arachidonic acid, 1 μCi

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