Phosphoinositide content in the erythrocyte membrane of rats with spontaneous and renal hypertension

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Summary. The increase of the content of triphosphoinositide in the erythrocyte membrane in rats with spontaneous and renal hypertension and decrease of phosphatidylinositol at spontaneous hypertension were revealed.

The erythrocyte membrane of rats with spontaneous hypertension has a higher passive permeability for certain monovalent cations, as compared with normotensive rats 2-4. The higher passive permeability of the erythrocyte membrane for Na^+ and K^+, and the change in binding of calcium on it, were found also in patients with essential hypertension[5,6].

The data concerning the influence of phosphoinositide metabolism on the calcium-dependent permeability of cell membrane for monovalent cations[7,8], and the close relationship between binding of calcium at erythrocytes and metabolism of phosphoinositides[9,10], led us to study their content in the erythrocyte membrane in 2 types of experimental hypertension in rats: spontaneous and renal.

Material and methods. 12-week-old male spontaneously hypertensive rats (SHR, Kyoto Wistar) with arterial pressure of 170-190 mm Hg were used. The control group consisted of inbred normotensive Wistar rats of the same age and sex with arterial pressure of 90-110 mm Hg (NR). Renal hypertension in female Wistar rats weighing 180±20 g was produced by constriction of the left renal artery (2-kidney Goldblatt hypertension)[11]. The group of animals with stable hypertension (145-180 mm Hg) was selected 8 weeks after the operation. The control group (70-80 mm Hg) consisted of intact rats. Arterial pressure was measured by tail plethysmography.

Acid lipids were extracted from suspension of erythrocyte ghosts obtained by the Schneider and Kirschner technique[9] by using 8 vol. of chloroform-methanol - concentrated hydrochloric acid mixture (100:100:0.6;v:v:v).

Content of phosphorus of monophosphoinositides, diphosphoinositides and triphosphoinositides (% of phosphorus of acid lipids)

Group	Number of rats	Phosphoinositides MPI	DPI	TPI
Rats with spontaneous hypertension	9	4.01 ± 0.26	0.65 ± 0.09	1.74 ± 0.21
Normotensive rats	6	5.10 ± 0.11	0.39 ± 0.08	0.99 ± 0.13
p		< 0.005	NS	< 0.001
Rats with renal hypertension	9	4.85 ± 0.36	1.70 ± 0.15	1.59 ± 0.19
Normotensive rats	7	5.35 ± 0.37	1.30 ± 0.11	1.05 ± 0.09
p		NS	NS	< 0.05

NS, not significant.

The chloroform phase was obtained through phase separating after addition of 1.5 vol. of 1 N HCl (in relation to the volume of suspension) and centrifuging at $2.5 \cdot 10^{3\times}$g for 5 min. This phase was washed by shaking with the mixture of chloroform-methanol-0.2 N HCl (3:47:50;v:v:v), separated under the same conditions of centrifugation and dried by the rotor evaporator. Lipid film was dissolved in chloroform-methanol mixture (I:I;v:v) which contained 0.05% concentrated HCl.

The paper chromatography was performed in the system of solvents - butanol-water-glacial acetic acid-diethyl either (20:5:25:6;v:v:v:v) with paper of the type FI4 (Filtrak, GDR) treated by acetic acid, formaldehyde and ammonium thiocyanate. The chromatograms were developed by 0.1% Nile blue solution in 0.1 M H_2SO_4. Identified spots of phosphoinositides were cut out and mineralized by 70% $HClO_4$ and 5% ammonium molibdate (29:1;v:v) within 15 min at 200 °C. Phosphor was determined by modified technique of Bartlett[12]. The results of the experiment are presented as mean values ±SE. The significance of the difference between general mean values was assessed by Student's t-test. The difference was considered significant at $p < 0.05$.

Results and discussion. The table contains the data on the content of phosphorus of monophosphoinositides (MPI), diphosphoinositides (DPI) and triphosphoinositides (TPI) (in percentage to phosphorus of acid lipids) of the erythrocyte membranes of rats with spontaneous and renal hypertension and also of rats of the control groups.

By comparison of the data of these experiments, it was found that in both cases of hypertension in rats the relative TPI content is significantly higher than in the controls. The increase of DPI content in both cases is not significant (NS), while reduction of MPI content is significant only in a case of spontaneous hypertension. Nevertheless, the increase of the degree of phosphorylation of phosphoinositides of the erythrocyte membranes may be considered as a general manifestation for these types of hypertension in rats. According to Michell et al.[13], it is possible that the phosphoinositides participate in the fuction of the Ca^{2+}-gating system of plasma membrane that controls the transport of surface-bound Ca^{2+} ions into the cell.

It is tempting to supppose that the increase of the degree of phosphorylation of membrane phosphoinositides may indicate the alteration of the Ca^{2+}-gating system of the erythrocyte membrane in both types of chronic hypertension in rats.

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