extraction of aldosterone from blood passing through the liver is 0.81 in the animals with PCT, corresponding to the level of extraction of aldosterone in the liver of intact dogs [4]. Consequently, under conditions of portocaval transposition of the vessels the enormous metabolic capacity of the liver shows itself.

After portocaval transposition of the vessels there is thus an increase in the metabolic clearance of aldosterone and, at the same time, a balanced increase in secretion of the hormone by the adrenals. This correspondence between the metabolism and secretion of aldosterone in PCT leads to a reduction in the aldosterone concentration in the peripheral blood and, accordingly, to restoration of the normal blood pressure in animals with vasorenal hypertension caused by bilateral CRA. The results of these experiments, in the writers' view, are of considerably clinical importance: Instead of the total adrenalectomy as performed for malignant hypertension, an operation based on the principle of "portalization" of blood from the kidneys and adrenals can be used.

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PHOSPHOINOSITIDE CONTENT IN ERYTHROCYTE MEMBRANES OF RATS WITH SPONTANEOUS HYPERTENSION

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The content of triphosphoinositides (TPI) and monophosphoinositides (MPI) per milligram of membrane protein in ghost erythrocytes of rats with spontaneous hypertension (SHR) is 178 and 74%, respectively, of the content of these lipids in ghost erythrocytes of normotensive rats (NR). The total phosphoinositide content in ghost erythrocytes of NR is more than 120% of the total content of these lipids in ghost erythrocytes of SHR.

KEY WORDS: spontaneous hypertension; erythrocytes; phosphoinositides.

The difference in the permeability of erythrocytes of SHR and normotensive Wistar rats in their permeability to monovalent cations Na^+ and K^+, discovered previously [7], conforms with the hypothesis of spontaneous hypertension as a disease of cell membranes [1]. Investigation of the molecular mechanisms of abnormal permeability of the erythrocyte membranes of rats with spontaneous hypertension was undertaken in order to study the biochemical manifestations of this pathology.

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TABLE 1. Content, as Phosphorus, of Phosphoinositides in Ghost Erythrocytes of SHR and NR Rats (μg P/mg membrane protein; M ± m)

Object	TPI	DPI	MPI
SHR rats (n = 9)	0,380±0,048	0,135±0,018	0,857±0,057
NR rats (n = 6)	0,213±0,023	0,085±0,017	1,146±0,052
p	0,008	0,067	0,002

Legend: n) number of animals.

Phosphoinositide metabolism in the cytoplasmic membranes of animal cells is known to affect the permeability of these membranes to monovalent cations through the participation of intracellular Ca^{2+} [6, 9].

The discovery of polyphosphoinositides in the composition of erythrocyte membranes and demonstration of a change in the Ca^{2+} binding in the course of their metabolism in erythrocytes [3, 8] motivated the study of the phosphoinositide composition of erythrocyte membranes in rats with spontaneous genetic hypertension described below.

EXPERIMENTAL METHOD

Male SHR rats (Kyoto Wistar), aged 12 weeks, with a blood pressure of 170-190 mm Hg, were used in the experiments. The control group of animals consisted of inbred Wistar rats (NR) of the same age with a blood pressure of 90-110 mm Hg.

Erythrocyte membranes were isolated as described in [8]. Blood taken with heparin (20 units/ml) was separated from plasma by centrifugation at 2,500g for 10 min, the plasma and buffy coat were removed and the erythrocytes were washed three times with 0.17 M NaCl by centrifugation under the same conditions, and hemolysed for 10 min in nine volumes of cold (0°C) buffer containing 1.44 mM Tris-HCl and 1.0 mM EDTA (pH 7.5). The ghost erythrocytes were isolated by centrifugation at 13,000g for 15 min and washed three times with 20 mM Tris-HCl buffer (pH 7.4) under the same conditions of centrifugation. Protein was determined by Lowry's method [5].

Lipids were extracted from one volume (V_1) of ghost cells with eight volumes of a mixture of chloroform–methanol–concentrated HCl (100:100:0.6) [10]. After incubation for 30 min, 1.5 volume V_1 of 1 N HCl was added to the mixture, which was centrifuged for 2500g for 5 min, after which the top (liquid) and middle (solid) phases were discarded. The bottom (chloroform) phase was washed with a mixture of chloroform–methanol–0.2 N HCl (3:47:50) and then dried. The lipid film was dissolved in a mixture of chloroform and methanol containing 0.05% HCl and chromatographed for 18 h (ascending chromatography) on F14 paper (East Germany), treated with a mixture of an aqueous solution of formaldehyde with acetic acid and ammonium thiocyanate. Chromatography was carried out in a solvent system consisting of butanol–water–glacial acetic acid–diethyl ether (20:5:25:6) [4]. The chromatograms were developed with a 0.1% solution of Nile Blue dye in 0.1 M H_2SO_4. After washing to remove the excess of dye with distilled water the phospholipids were revealed as dark blue spots against a pale blue background. To determine the lipid phosphorus the spots were cut out and treated with 70% $HClO_4$ (1.2 ml) containing 50-100 μl 5% ammonium molybdate at 200°C for 15 min. Phosphorus was determined by a modified Bartlett's method [4]. The experimental results were subjected to statistical analysis by Student's t test [2].

EXPERIMENTAL RESULTS

Data on the content, as phosphorus, of monophosphoinositides (MPI), diphosphoinositides (DPI), and triphosphoinositides (TPI) in ghost erythrocytes of SHR and NR rats, expressed per milligram membrane protein, are given in Table 1.

Clearly the TPI content in the erythrocyte membranes of SHR rats is 1.8 times but the MPI content only 0.74 times that in the samples from the control group. The difference in the TPI content in the membranes of the experimental and control samples was not statistically significant.

The total content of phosphoinositides calculated with the introduction of stoichiometric coefficients for TPI, DPI, and MPI (1/3, 1/2, and 1, respectively) in the SHR ghost erythrocytes was 0.83 of their content in the control group.

The results thus indicate differences both in the content and in the degree of phosphorylation of phosphoinositides in erythrocyte membranes of the experimental and control rats. This difference may reflect both a difference in the composition of the phosphoinositides of the erythrocyte membranes and also a difference in the activity of the enzymes (mainly phosphokinases and phosphoesterases) responsible for the synthesis and destruction of these compounds during isolation of the ghost erythrocytes. The next stage in the study of the effect of the metabolism of these lipids on permeability of the cell membranes in hypertension will be an examination of this problem and also a study of the relationship between the quantity of erythrocyte-bound Ca^{2+} and the degree of phosphorylation of the membrane phosphoinositides.

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