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Polyphosphoinositide metabolism in erythrocytes from spontaneously hypertensive rats

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Summary. Incorporation of ^{32}P into di- and triphosphoinositides of erythrocytes from 1-month-old spontaneously hypertensive rats was lower, and diphosphoinositide content higher, than in controls. During development of hypertension these initial differences decreased and were even reversed.

Polyphosphoinositides (PPI) are involved in the regulation of calcium binding[2] and are supposed to control cell membrane permeability for monovalent cations[3].

An increase in passive permeability for monovalent cations and an alteration of calcium distribution in cell membranes from spontaneously hypertensive rats (SHR) and patients with essential hypertension were found in vascular smooth muscles[4], cardiomyocytes[5], erythrocytes[6] and adipose tissue[7]. Hence membrane alterations are considered as a possible cause for the development of essential hypertension[8].

An increase of PPI content in erythrocyte membranes of 2-month-old SHR was reported[9]. We studied erythrocyte PPI content and incorporation of ^{32}P into erythrocyte PPI throughout the development of hypertension in SHR (1-, 2- and 4-month-old rats).

Materials and methods. We used 1-, 2-, and 4-month-old male spontaneously hypertensive rats (SHR, Kyoto Wistar) and inbred normotensive Wistar rats (NWR) of the same age and sex. Both groups of 1-month-old rats had the same systolic blood pressure (115 mm Hg), but the values of 2- and 4-month-old SHR were significantly higher (160 and 205 mm Hg). Blood pressure was measured by tail plethysmography. $Na_2H^{32}PO_4$ was injected i.p. (2 mCi/kg b.wt).

After 90 min the rats were decapitated and the blood collected from each separately in physiological solution with heparin. Haematocrit was determined. From packed erythrocytes the lipids were extracted[10] and the values of specific activity of inorganic phosphate[11] determined. The lipid extracts were chromatographed on formaldehyde-treated paper[12]. Mono-, di- and triphosphoinositide (MPI, DPI and TPI) were analyzed. We measured the radioactivity of the phospholipid-containing paper strips in dioxan scintillation solution with an LKB Wallac 81,000 liquid scintillation counter. The same paper strips were then used for phosphate determination[13] after mineralization of the paper. The content of phospholipids is given in nmoles/ml of intact erythrocytes, and the ^{32}P incorporation rate is expressed as relative specific activity (RSA), that is, as the ratio of specific activity (cpm/μg phosphorus) of each fraction to the specific activity of erythrocyte inorganic phosphate, multiplied by 100.

Results and discussion. We found alterations of phosphoinositide content of erythrocytes between SHR and NWR in accordance with others[9].

Moreover, the alterations of phosphoinositide content as well as differences of ^{32}P incorporation into phosphoinositides were age-dependent. MPI content of erythrocytes was significantly lower in 2-month-old SHR compared with NWR. Furthermore, ^{32}P incorporation into DPI and TPI was lower in 1-month-old SHR (53% and 65% of control groups) and the DPI-content higher (220% of control group) (see table). During development of hypertension, the initial difference between 1-month-old SHR and NWR fell to nearly zero at 2 months, and was even reversed for 4-month-old rats.

The initial differences at 1 month might be explained by a lower activity of the phosphorylation/dephosphorylation processes for erythrocyte phosphoinositides in SHR. This lower activity is presumably connected with the altered

Content (nmole/ml of erythrocytes) and relative specific activity (RSA) of phosphoinositides in erythrocytes of spontaneously hypertensive (SHR) and normotensive Wistar rats (NWR) depending on age (months)

		1 month	2 months	4 months
		nmole/ml of erythrocytes		
TPI	SHR	78.9±2.5	68.4±5.1	90.5 ±4.8
	NWR	69.8±1.9	58.3±4.5	96.8 ±2.0
	p			
DPI	SHR	57.7±3.2	25.6±2.1	38.9 ±3.2
	NWR	26.3±1.6	32.7±5.2	53.7 ±3.4
	p	<0.001		<0.05
MPI	SHR	335±14	222±5	285±13
	NWR	306±14	243±6	313±15
	p		<0.05	
		RSA		
TPI	SHR	29.9±0.7	24.1±1.0	17.3 ±0.8
	NWR	45.6±1.9	21.4±0.7	12.1 ±0.6
	p	<0.001		<0.001
DPI	SHR	17.8±0.8	19.5±2.2	10.22±0.8
	NWR	31.1±1.4	17.8±2.0	7.48±0.9
	p	<0.001		<0.05
MPI	SHR	2.31±0.12	1.40±0.13	0.95±0.04
	NWR	2.30±0.12	1.63±0.16	0.89±0.05

Means± SEM for 5 animals. The significance of the differences between means of SHR and NWR was assessed by Student's t-test.

permeability to monovalent cations[4-6] and with the changes in Ca-binding of cell membranes[8] reported. These initial differences between SHR and NWR might also be understood as a manifestation of compensating mechanisms in SHR, which at this stage are still effective. The alterations in SHR are of interest because they already appear in the prehypertensive stage and may reflect biochemical processes whose failure contributes to the development of hypertension.

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Trace metal requirements in total parenteral nutrition: A theoretical approach by mathematical modeling

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Summary. A new computer-based approach has been developed to assess the optimal doses of essential trace metal ions which should be included in nutritive mixtures, used in human total parenteral nutrition, to compensate for the ligand-induced losses of these metal ions. An example of application is given for zinc, copper and manganese.

Although the need for supplementation of trace elements, especially zinc, during total parenteral nutrition (TPN) is well recognized, the quantitative requirements are not precisely known. They are difficult to estimate because they depend on the clinical setting and because urinary excretion of transition metals in promoted by the administration of naturally occurring ligands[1-4]. Accordingly, the doses of trace elements which have thus far been included in TPN fluids range widely between limits established by experimental trial and error[5-7]. We thus decided to investigate the effect of TPN on the metal-ligand equilibria in human blood plasma and to attempt to estimate ideal quantities of zinc, copper and manganese ions which ought to be routinely added to TPN solutions. However, there are no experimental methods available which could accomplish this; the very small amounts of transition metals that occur in biofluids and the labile nature of the equilibria involved would thwart most known analytical techniques. So a theoretical approach, based on computer simulation of the relevant chemical interactions was adopted. Computer models of 9000 complexing species formed by about 60 components have been developed for blood plasma[8-10]. These were used directly to look at the effect of infused ligands in vivo and were adapted to provide information about metal binding in the infusate[11].

The concentrations of aminoacids in the blood plasma of 4 patients on prolonged TPN were measured using column liquid chromatography. The subjects were all suffering from severe digestive disorders: 2 had Crohn's disease of the small bowel, and 2 had chronic radiation enteropathy. The concentrations of the main ligands in the nutritive solution (aminoacids were from Bellon Laboratories, Neuilly, France) commonly used in the TPN unit of one of us (C.M.) were (mmole/l): histidine 6.25, cysteine 1.67, glycine 23.23, valine 20.68, leucine 20.53, proline 13.47, lysine 18.32, phenylalanine 14.66. Our computer simulation studies revealed that cysteine and histidine are the predominant low-molecular-weight ligands bound to zinc in blood plasma[11]. Furthermore, the computer models predicted that although the size of the low-molecular-weight zinc fraction would be directly related to the concentrations of both ligands, it would be most sensitive to the level of cysteine because of an important biscysteinato zinc complex. As the concentrations of cysteine are, on average, more than doubled by the TPN infusions, it seems very likely that this TPN component is the chief cause of the zinc losses. On the assumption that TPN solutions should contain metal ion supplements which cause the smallest possible effect on the metal binding in normal blood plasma, we have also calculated the amounts of calcium, magnesium, zinc, copper and manganese which need to be included in the nutritive mixture most commonly used in the TPN unit of one of us (C.M.) These values, shown in the table, all lie

Total concentrations and predicted doses for zinc, copper, manganese, calcium and magnesium to be added in the TPN aminoacid solution[a] used at $-\log[H^+]=7.4$

Metal	Total concentration[b] (mole · dl^{-1})	Predicted dose[b] (mg/day)
Zn(II)	1.914×10^{-5}	32[c]
Cu(II)	7.857×10^{-7}	1.3
Mn(II)	5.537×10^{-13}	10^{-6}
Ca(II)	4.903×10^{-4}	507
Mg(II)	4.070×10^{-4}	257

[a] Identical to that indicated in the text. [b] The calculations are based on a mean value of the calculated free metal ion concentrations[8] and on the measured aminoacid ligand concentrations in the nutritive mixture. The predicted dose is the calculated amount of each metal in 2.5 l of nutritive mixture, this being the usual volume administered daily. [c] Partly calculated from statistically estimated equilibrium constants; the experimental determination of these constants (to be performed by one of us (GB.)) should result in a lower dose.