CA^{2+}-binding to erythrocyte membrane of hypertensive men and rats: Effects of acetylcholine and eserine

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Summary. Both acetylcholine and eserine affect Ca^{2+}-binding to mammalian erythrocyte membranes. Differences in Ca^{2+}-binding between normotensive and hypertensive groups (human as well as rats) was found under eserine influence.

Recently it has been pointed out that increase in the degree of phosphorylation of membrane phosphoinositides indicates an alteration of the Ca^{2+}-gating system of erythrocyte membranes in chronic hypertension of rats[2]. According to Michell and coworkers[3] it is possible that the phosphoinositides participate in the functions of the plasma membrane Ca^{2+}-gating system which is controlled in several cases by muscarinic cholinergic receptors.

The observation of the muscarinic cholinoreceptor system in the mammalian erythrocyte membrane[4,5] supported this assumption and gave us grounds for studying the Ca^{2+}-binding to erythrocytes under the influence of some cholinergic substances.

Material and methods. 6-week-old female spontaneously hypertensive rats (SHR, Kyoto Wistar) with systolic pressure of 175–210 mm Hg were used. The control group consisted of inbred normotensive Wistar rats of the same age and sex with systolic pressure of 90–110 mm Hg (NWR). The blood samples were taken from the aorta with a thick-needle syringe, wetted with heparin solution, under deep ether anaesthesia.

Venous blood samples were taken from male patients (age range 35–72 years) with clinically established essential hypertension (HP) using glass tubes wetted with heparin solution; their blood pressure was within a range of 150–230/90–120 mm Hg. The control group consisted of normotensive male patients of approximately the same age range (NP). The samples were centrifuged at 4000 × g for 5 min, the plasma and leucocytes were discarded and erythrocytes were washed twice under the same condition of sedimentation by a 'washing solution' that had the following composition (mM): NaCl 145, KCl 6, $MgCl_2$ 2, $CaCl_2$ 5, glucose 5, Tris HCl 5 (Merck); pH 7.4. The partial volume of erythrocytes in suspension was established by means of a hematocrit centrifuge and was the same as that of whole blood sample. The aliquots of the erythrocyte suspensions (0.5 ml) were mixed with incubation solutions (0.5 ml) in the centrifuge tubes. These solutions were prepared by adding of $^{45}CaCl_2$ (1–2 μCi/ml) and, in several cases, the substances being tested: 10 mM acetylcholine chloride (ACh) or 1 mM eserine sulfate (Calbiochem) to the washing solution. After incubation for 30 min at 37 °C with shaker, the samples were cooled to 0 °C and centrifuged. Each sediment was washed with washing solution 4 times (1 ml, 2 ml, 3 ml, 3 ml) and mixed with 1 ml of washing solution containing 5 mM ethyleneglycol-bis (β-aminoethyl ether) N,N'-tetraacetic acid (EGTA) for rat erythrocytes or 6 mM EGTA for human erythrocytes; pH 7.4 was obtained by means of Tris. Each sample was centrifuged, and 0.5 ml of supernatant was transferred into a vial with 10 ml of

Table 1. The extraction of ^{45}Ca from prelabelled rat erythrocytes (dpm; mean ± SE)

Addition to preincubation medium		Control solution	ACh	p*	Eserine	p*
NWR	A_1	1,014 ± 135	730 ± 153		2,633 ± 368	
(n = 7)	A_2	4,900 ± 306	3,145 ± 185		5,395 ± 385	
	A	7,874 ± 563	5,133 ± 412	< 0.002	10,186 ± 907	NS
	R	7.765 ± 1.174	7.032 ± 1.578	NS	3.869 ± 0.641	< 0.02
SHR	A_1	1,980 ± 118	1,045 ± 182		1,851 ± 87	
(n = 7)	A_2	5,790 ± 24	3,065 ± 210		5,965 ± 108	
	A	10,086 ± 152	5,336 ± 476	< 0.001	10,122 ± 238	NS
	p**	< 0.005	NS		NS	
	R	5.094 ± 0.313	5.106 ± 0.999	NS	5.512 ± 0.289	NS
	p**	< 0.05	NS		< 0.05	

Table 2. The extraction of ^{45}Ca from prelabelled human erythrocytes (dpm; mean ± SE)

Addition to preincubation medium		Control solution	ACh	p*	Eserine	p*
NP	A_1	488 ± 59	181 ± 46		323 ± 38	
(n = 8)	A_2	2,034 ± 211	993 ± 115		1,540 ± 170	
	A	3,335 ± 354	1,571 ± 207	< 0.001	2,479 ± 276	NS
	R	6.834 ± 1.099	8.680 ± 2.485	NS	7.675 ± 1.243	NS
HP	A_1	308 ± 41	110 ± 8		326 ± 46	
(n = 8)	A_2	1,438 ± 106	791 ± 56		810 ± 80	
	A	2,321 ± 189	1,217 ± 86	< 0.001	1,460 ± 158	< 0.005
	p**	NS	NS		< 0.01	
	R	7.536 ± 1.176	11.064 ± 1.122	< 0.05	4.479 ± 0.796	< 0.05
	p**	NS	NS		< 0.05	

* These p-values characterize the significance of the effects of acetylcholine and eserine; ** these p-values characterize the differences between normotensive and hypertensive subjects; NS, not significant.

Triton X-100-toluene (1:2) scintillation solution. The radioactivity of the mixture was denoted by A_1. Then 0.5 ml of 50 mM EDTA plus 0.2 ml of 50% trichloracetic acid were added to the rest of each sample. This mixture was centrifuged and 1 ml of supernatant was added to 10 ml of the scintillation solution mentioned above. The radioactivity of that mixture was denoted by A_2. The radioactivity was measured with the liquid scintillation counter SL-4200 (Intertechnique). The data are presented as mean ± SE. The statistical significance of the difference between means was assessed by Student's t-test; p-value less than 0.05 was considered significant.

A_2 is a specific radioactivity, i.e. the radioactivity of 1 ml of supernatant of the mixture with EDTA and TCA (the total volume of this mixture is 1.4 ml); it does not differ from the specific radioactivity of the remainder, as we have found, therefore the total radioactivity A of each sample was calculated according to the formula: $A = A_1 + 1.4\,A_2$.

Results and discussion. The ratio A/A_1 (denoted by the parameter R) was chosen as a semiquantitative characteristic feature for cell membrane affinity to Ca^{2+}, because A_1 is the radioactivity of Ca^{2+} extracted under mild conditions (and seems to be proportional to the easily exchangeable Ca^{2+} of the cell surface). The data obtained are specified in tables 1 and 2.

Rat erythrocytes. The erythrocytes of SHR incorporated more ^{45}Ca in the control solution (without ACh and eserine) than the erythrocytes of NWR ($p < 0.005$). This was the result of (at least) an easier isotopic exchange, since this excess of labelling coincides with a smaller value of the parameter R ($p < 0.05$). ACh significantly inhibited the labelling of erythrocytes of SHR and NWR ($p < 0.001$ and $p < 0.002$, respectively) but the difference between these groups is not significant. The parameter R was not considerably altered. Eserine had insignificant influence on the ^{45}Ca-binding to erythrocytes of SHR and NWR; the parameter R was unchanged for SHR but was reduced significantly for NWR ($p < 0.02$). Thus a considerable variation in the parameter R under eserine influence between SHR and NWR was found ($p < 0.05$). It appears reasonable to assume that ^{45}Ca-ions are exchanged with membrane calcium, because the swelling of erythrocytes affected by ACh or eserine was not accompanied by any increase of label incorporation into the cells.

Human erythrocytes. No significant difference, either in ^{45}Ca-binding or in the parameter R between HP ($n = 8$) and NP ($n = 8$) were observed for the control incubation medium. Earlier it was found that HP-erythrocytes have less affinity to Ca^{2+} than the NP-erythrocytes[6]. The different conditions of Ca^{2+}-extraction from erythrocytes may be an explanation of the divergence between the old and new data. ACh reduced the label incorporation into erythrocytes for NP and HP ($p < 0.001$) but there was no difference in this property between these 2 groups. The parameter R was increased only for HP ($p < 0.05$) but the difference between the groups remained insignificant. Eserine reduced the ^{45}Ca-binding to erythrocytes of HP ($p < 0.005$) and did not significantly influence that of NP, but the difference between the groups became significant. The parameter R was decreased for HP and was increased insignificantly for NP; moreover, the difference in this property between these groups was significant ($p < 0.05$).

Thus, we have established that ACh and eserine (the inhibitor of acetylcholinesterase) influence Ca^{2+}-binding to erythrocytes of rats and humans. The variation of the parameter R is not always parallel to that of the incorporation of ^{45}Ca into erythrocytes. This may indicate that not only cell membrane affinity to Ca^{2+} but, in some cases, also the easily exchangeable Ca-pool of cell surface is altered under the action of ACh and eserine. Differences in Ca^{2+}-binding to SHR and HP erythrocytes, on one hand, and erythrocytes of the respective control groups, on the other, were found under eserine influence. It may well be that there is some interrelation between the difference in the influence of eserine on Ca^{2+}-exchange within the 2 rat groups and the different contents of erythrocyte membrane phosphoinositides of SHR and NWR reported earlier[2], because the close interrelation between mammalian erythrocyte membrane acetylcholinesterase and phosphoinositides has been established[7].

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Effect of theophylline and triiodothyronine on some early estrogenic responses in the rat uterus[1]

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Summary. Theophylline increases and triodothyronine decreases uterine edema induced by physiological doses of estradiol-17β. Both of them decrease estrogen-induced uterine eosinophilia and the number of blood eosinophils, suggesting an explanation for the results in the uterus.

Thyroid hormones are known to have antiestrogenic properties[2]. Hyperthyroidism is associated with prolonged diestrus and hypothyroidism with a prolonged estrus cycle[2,3]. There is no agreement as to the mechanism whereby the thyroid influences uterine sensitivity to estrogens[4,5]. Evidence has been published for the mediation of various known metabolic effects of estrogens on the uterus by cyclic AMP[6,7]. Theophylline, when it is administered concurrently with cyclic AMP, also increases the activities of various key glycogenolytic and hexose monophosphate shunt enzymes, as well as uterine glycogen synthesis[6]. It has been proposed that uterine eosinophils mediate estrogen induced uterine edema[8], and it was suggested that this effect could be dependent on the number of eosinophils in the blood[9].