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RAPID COMMUNICATION

Ion Transport Systems in Erythrocyte Membrane of Spontaneously Hypertensive Rats (SHR) as Compared with Normotensive Rats of the Brown Norway (BN.lx) Strain

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Summary

The activity of Na^+, K^+–ATPase in SHR erythrocytes treated with saponin is increased by 30–40 % as compared to the Brown Norway (BN.lx) strain whereas the activity of Ca^{2+}–ATPase is decreased by 20–30 %. Passive permeability of SHR erythrocytes determined by ^{86}Rb influx is increased by 20–30 %. In the presence of orthovanadate erythrocytes of SHR accumulate ^{45}Ca by 80 % more than BN.lx red cells. There was no difference in Na^+/H^+ exchange between erythrocytes of SHR and BN.lx animals.

Key words:
Spontaneous hypertension – Erythrocytes – Ca^{2+}-ATPase – Na^+,K^+-ATPase – Na^+/H^+ exchange – Passive permeability

During last 15 years extensive data have become available on the alterations of ion transport function of the plasma membrane in primary hypertension. The most detailed studies were accomplished using red cells of rats with genetic hypertension. Elevated $Na^+,K^+,2Cl^-$–cotransport was found in Milan hypertensive strain (MHS) whereas increased K^+,Cl^-–cotransport, Na^+/H^+ exchange, ^{86}Rb passive permeability and ^{45}Ca influx (measured in the presence of a Ca^{2+}–ATPase inhibitor – orthovanadate) were observed in spontaneously hypertensive rats (SHR)(Postnov and Orlov 1987). Experiments on F_2 hybrids of normotensive and hypertensive animals are necessary for the estimation of a linkage between ion transport abnormalities and blood pressure (BP) as well as for the identification of genetic loci responsible for these alterations. Recently it was proposed to use a system of recombinant inbred strains derived from SHR and another normotensive strain – BN.lx (Pravenec *et al.* 1989). It became therefore necessary to compare the activity of ionic pumps, carriers and passive diffusion (leakage) in erythrocyte membranes of SHR and BN.lx animals.

Ten male SHR (systolic BP 155–190 mm Hg) and BN.lx rats (115–130 mm Hg) aged 4–5 months were used. Activities of Na^+,K^+–ATPase, Ca^{2+}–ATPase and Mg^{2+}–ATPase were determined in erythrocytes treated with saponin as described by Pokudin *et al.* (1988). Na^+/H^+ exchange was estimated as a value of amiloride-inhibited component of proton efflux rate at pH_i 6.60–6.70 and pH_o 7.95–8.05 (Orlov *et al.* 1989). ^{45}Ca accumulation in the presence of 5 mM orthovanadate was measured according to Orlov *et al.* (1988). The passive permeability of membranes for potassium was estimated as a rate of ^{86}Rb influx in the medium A (140 mM $NaNO_3$, 1 mM KNO_3, 10 mM MOPS–Tris (pH 7.4 at 37°C), 0.2 mM ouabain, 0.5 mM furosemide, 0.1 mM EGTA, 1 μCi ^{86}Rb/ml). In some cases 280 mM sucrose was substituted for $NaNO_3$ (medium B).

Mg^{2+}–ATPase activity in SHR erythrocytes was equal to that of BN.lx (Tab. 1). The activity of Na^+,K^+–ATPase in the presence of 5 μM Ca^{2+} was increased in SHR red cells by 30–40 % while the activity of Ca^{2+}–ATPase was reduced by about 30 % at both 5 and 60 μM Ca^{2+} (Table 1).

Table 1

Adenosine triphosphate activities in saponin–treated erythrocytes of spontaneously hypertensive and normotensive rats

Groups	Free calcium concentration (μM) 0 Mg^{2+}-ATPase	5 Na^+,K^+-ATPase	5 Ca^{2+}-ATPase	60 Ca^{2+}-ATPase
	(mmoles per litre of cells per hour)			
1. BN.lx	13.39±0.39	2.23±0.28	12.63±0.75	10.19±0.96
2. SHR	13.83±0.51	4.19±0.65	8.68±0.86	7.12±0.75
$P_{1,2}$	N.S.	<0.05	<0.05	<0.05

^{45}Ca content in SHR erythrocytes (after 4 h incubation in the presence of orthovanadate) was higher by 75–85 % than that of BN.lx red cells. There was no difference in erythrocyte Na^+/H^+ exchange between the two strains (Table 2).

Table 2

^{45}Ca uptake and Na^+/H^+ exchange in erythrocytes of spontaneously hypertensive and normotensive rats

Groups	^{45}Ca uptake (μmoles per litre of cells per 4 hours)	Na^+/H^+ exchange (mmoles per litre of cells per hour)
1. BN.lx	21.56±1.92	22.69±5.04
2. SHR	36.32±2.12	23.16±8.04
$P_{1,2}$	<0.0005	N.S.

The rate of ^{86}Rb influx to SHR erythrocytes was increased by 20–30 % in the medium A (Table 3), indicating increased passive permeability for potassium. Substitution of sucrose for monovalent cations decreased this parameter substantially. It is interesting to note that the sucrose-inhibited component of ^{86}Rb influx was four times greater in SHR than in BN.lx red cells.

Table 3

Passive permeability of erythrocyte membrane for potassium (^{86}Rb)

Groups	^{86}Rb influx (moles per litre of cells per hour)		
	Medium A	Medium B	$\Delta_{A,B}$
1. BN.lx	0.543±0.014	0.488±0.011	0.056±0.017
2. SHR	0.674±0.014	0.428±0.012	0.243±0.024
$P_{1,2}$	<0.0005	<0.005	<0.0005

Our data demonstrate decreased Ca^{2+}–ATPase activity, increased Na^+,K^+–ATPase activity, higher passive permeability for potassium and greater ^{45}Ca accumulation (in the presence of orthovanadate) in SHR erythrocytes as compared to BN.lx red cells. It was shown earlier that both passive permeability of

plasma membrane for monovalent ions (Friedman *et al.* 1977) and ^{45}Ca accumulation (Orlov *et al.* 1988) were higher in SHR than in WKY erythrocytes. Red cell Ca^{2+}-ATPase activity was equal in SHR and WKY animals (Orlov *et al.* 1989). The data on Na^+,K^+-ATPase activity in saponin-treated erythrocytes of SHR and WKY strains are not available. A comparison of SHR and WKY erythrocytes revealed a 50–60 % elevation of Na^+/H^+ exchange in the hypertensive strain (Orlov *et al.* 1989). This was not confirmed in this study in which SHR were compared with another normotensive strain (BN.lx) possessing a high rate of red cell Na^+/H^+ exchange.

The above mentioned ion transport alterations can be used as quantitative traits in the segregation studies with either F_2 SHR x BN.lx hybrids or with animals of recombinant inbred strains (Pravenec *et al.* 1989). This could help to clarify their role in the pathogenesis of genetic hypertension.

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