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EDITORIAL

Na^+ and K^+ Transport Alterations in Hypertension

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It is difficult to enumerate all pathogenetic mechanisms that have been proposed to be involved in the induction and/or maintenance of particular forms of human or experimental hypertension. However, it is evident that increased vascular resistance is the cause of high blood pressure in most if not in all forms of hypertension. Vascular tone which is exerted by exaggerated vascular smooth muscle contraction at the level of arterioles, is a subject of modulation by a rather complicated counterbalance of vasoconstrictor and vasodilating systems. The alterations can be located at different levels of the excitation-contraction process. One of the tentative hypotheses is based upon the changes of cellular ion composition (increased Na^+ and/or Ca^{2+} contents) due to generalized cell membrane defect(s), e.g. increased membrane permeability or reduced active ion transport exerted by some specific carriers. Consequently, basal vascular tone as well as the response to vasoconstrictor agents would be augmented. The hypothesis that altered ion transport across the cell membrane and high blood pressure are somehow related at least in some forms of hypertension is supported by a large body of evidence gathered on cells taken from humans and animals with various forms of hypertension (Jones 1982, Friedman 1983, 1990, Postnov and Orlov 1985, Bing *et al.* 1986, Bohr and Webb 1988, Dominczak and Bohr 1990, Heagerty 1990, Postnov 1990, Swales 1990).

Altered transport of Na^+ and/or K^+ across membranes of various cells in patients with essential hypertension have been reported by several laboratories in the last 20 years (Wessels *et al.* 1970, Edmondson *et al.* 1975, Postnov *et al.* 1977, Garay *et al.* 1980, Canessa *et al.* 1980, Duhm *et al.* 1982). These Na^+ and K^+ transport alterations were considered as important factors in the pathogenesis of hypertension although their exact relationship to hypertension has not yet been clarified.

The attractive idea of Blaustein (1977, 1984) proposed that ion transport defects (increased passive membrane permeability for Na^+ or reduced Na^+-K^+ pump activity) might cause the elevation of intracellular sodium (Na^+_i) in various tissues (including vascular smooth muscle). This would reduce Ca^{2+} extrusion via lowering the rate of $Na^+_o : Ca^{2+}_i$ exchange so that the intracellular ionized calcium (Ca^{2+}_i) would rise and activate smooth muscle contraction. Indeed intracellular Na^+ and Ca^{2+} concentrations were found to be elevated in both essential hypertension (Losse *et al.* 1960, Wessels *et al.* 1970, Edmondson *et al.* 1975, Ambrosioni *et al.* 1981, Erne *et al.* 1984, Bruschi *et al.* 1985, Le Quan Sang and Devynck 1986) and rats with various forms of experimental hypertension (De Mendonca *et al.* 1980b, Duhm *et al.* 1983, Furspan and Bohr 1986, Wauquier *et al.* 1986, Orlov *et al.* 1988a, Wauquier and Devynck 1989, Jelicks and Gupta 1990).

Red cells seem to be an easily accessible model for the study of such ion transport impairments in hypertensive humans and animals. If the alterations of Na^+ and K^+ transport seen in erythrocytes would represent a generalized membrane phenomenon, the results obtained in red cells might indeed guide further research on membranes of cells directly involved in blood pressure regulation.

Red Cell Na^+ and K^+ Transport Alterations in Essential Hypertension

Human essential hypertension is a heterogeneous group of disorders. Discrete effects of major genes and major environmental factors have been identified as determinants of major subtypes of essential hypertension. Large genetic studies carried out in thousands of Utah subjects by Williams and coworkers (1988, 1990) suggested that human hypertension is likely a multifactorial trait resulting from multiple measurable monogenes, blended polygenes and shared family as well as individual environment. Total genetic heritability of about 80% with some recessive major gene effects has been found for several traits associated with hypertension including urinary kallikrein excretion, red cell Na^+ content and Na^+-Li^+ exchange.

Na^+-Li^+ countertransport, K^+-Li^+ cotransport as well as Li^+ leak that are significantly higher in hypertensives (Hunt *et al.* 1985, 1986) are associated with blood pressure mainly via alterations of plasma lipids. There was an especially close relationship with plasma levels of triglycerides and high density lipoproteins. Blood pressure correlated independently with Li^+ leak only (Hunt *et al.* 1986). These data were in good agreement with the previous suggestions on the importance of lipid alterations for the acceleration of Na^+-Li^+ exchange seen in hypertensives (Behr *et al.* 1985, Duhm and Behr 1986) which were later confirmed in other laboratories (Pagnan *et al.* 1989, Hajem *et al.* 1990).

The increased activity of Na^+-Li^+ countertransport system (Canessa *et al.* 1980, Adragna *et al.* 1982) represents such ion transport alteration which has been frequently demonstrated in essential hypertensives (Canali *et al.* 1981, Trevisan *et al.* 1983, Brugnara *et al.* 1983, Levy *et al.* 1983, Ibsen *et al.* 1982, Turner *et al.* 1987, Weder *et al.* 1987, Rutherford *et al.* 1990). Aronson (1982) proposed that increased Na^+-Li^+exchange might reflect *in vivo* increased Na^+-H^+ exchange rates. Such concomitant activation of both exchangers was indeed demonstrated in red cells of patients with essential hypertension (Semplicini *et al.* 1989, Orlov *et al.* 1989, Canessa *et al.* 1991). Though some authors favour the view that both transport

phenomena are mediated by the same system (Morgan and Canessa 1990), others present evidence which is not in accordance with such a view (Escobales and Figueroa 1991). The question concerning the identity of these two phenomena in the red cell cannot be therefore decided at present. Nevertheless, Lifton *et al.* (1991) provided evidence that the Na^+-H^+ exchange gene locus does not contribute to quantitative variations in red cell Na^+-Li^+ exchange and to the pathogenesis of essential hypertension, respectively. Hence, the high genetic hereditability of red cell Na^+-Li^+ exchange is possibly not mediated through the gene coding for the putative Na^+-Li^+ exchange protein. An alternative explanation might be a genetic control of plasma lipids modulating the red cell Na^+-Li^+ exchange protein through lipoprotein interactions after insertion into the membrane (Duhm 1989a).

There is much more controversy about the alterations of furosemide-sensitive Na^+-K^+ cotransport system in human hypertension. The original observation of reduced outward Na^+-K^+ cotransport in essential hypertensives (Garay *et al.* 1980) and in normotensives with a positive family history of hypertension (Meyer *et al.* 1981) was only rarely reproduced (Cusi *et al.* 1981, Montanari *et al.* 1984). Several groups observed unchanged (Swarts *et al.* 1981, Duhm *et al.* 1982, Davidson *et al.* 1982, Wiley *et al.* 1984, Delva *et al.* 1985) or even increased red cell Na^+-K^+ cotransport in essential hypertension (Adragna *et al.* 1982, Bin Talib *et al.* 1984, Tuck *et al.* 1984, 1987). It has been proposed (Engelmann *et al.* 1990) that the reduced cotransport activity reported by Garay and coworkers may be the result of a reduction in mean red cell age due to a mild anaemia of chronic disorders associated with a severe essential hypertension.

Some studies on the kinetics of $Na^+-K^+-2Cl^-$ cotransport system disclosed decreased affinity for Na^+_i (Garay *et al.* 1983, De la Sierra *et al.* 1989) whereas others reported increased maximal velocity (Adragna *et al.* 1982, Tuck *et al.* 1987). Delva *et al.* (1985) failed to find any differences in kinetic parameters of the Na^+-K^+ cotransport between normotensive and hypertensive subjects. This was confirmed by Canessa *et al.* (1989) under the conditions of low salt intake whereas at high salt intake there was a tendency to both above mentioned kinetic alterations. There are also substantial racial differences concerning the red cell Na^+-K^+ cotransport system (for review see Canessa 1989).

The presence of certain subgroups among patients with essential hypertension was suggested by Garay (1987). The *Co(−)* patients with a low apparent affinity of the Na^+-K^+ cotransport for Na^+_i (Garay *et al.* 1983) are rarely identical with *Counter(+)* subjects that display elevated Na^+-Li^+ countertransport (Dagher and Canessa 1984). *Leak(+)* hypertensive patients are characterized by an elevated rate constant of the Na^+ leak (Garay and Nazaret 1985) whereas *Pump(−)* patients have a low apparent affinity of the Na^+-K^+ pump for Na^+_i (Diez *et al.* 1987). The existence of such subgroups in Spanish population was confirmed by De la Sierra and coworkers (1988a,b, 1989).

The impairment of cotransport and countertransport systems do not seem to be the consequence of an increased blood pressure *per se* because they were not observed in secondary hypertensive patients without history of hypertension (Canessa *et al.* 1980, Garay *et al.* 1980). Moreover, functional changes of these ion transport systems that are characteristic for essential hypertensives were also found in some of their offspring suggesting genetic inheritance (Meyer *et al.* 1981, Williams *et al.* 1988, 1990). The genetic transmission of such alterations can be

further supported by the evaluation of Na^+ and K^+ carriers in erythrocytes of genetically hypertensive rats. The identification of inherited molecular abnormalities in inbred rat strains with genetic hypertension would give important keys for understanding the pathogenesis of hypertension.

Red Cell Na^+ and K^+ Transport in Experimental Hypertension of the Rat

This chapter is focused on red cell Na^+ and K^+ transport phenomena seen in red cells suspended in saline media at nearly physiological ion concentrations. A discussion of Na^+-Li^+ exchange is not included because the existence of this transport system in rat erythrocytes is questionable. The acceleration of Na^+-H^+ exchange in SHR erythrocytes was observed after the establishment of a rather unphysiological pH gradient across the red cell membrane (Orlov *et al.* 1988b, 1989). Moreover, it was recently demonstrated that the altered activity of this exchanger did not cosegregate with blood pressure in F_2 hybrids of SHR and WKY rats (Orlov *et al.* 1991a,b).

a) Red Cell Na^+ Content and Na^+-K^+ Pump in Rat Experimental Hypertension

There are multiple studies concerning the alterations of red cell Na^+ and K^+ transport in various strains of rats with genetic hypertension (Ben-Ishay *et al.* 1975, Postnov *et al.* 1976, De Mendonca *et al.* 1980a, Duhm *et al.* 1983, van de Ven and Bohr 1983, Harris *et al.* 1984, Bianchi *et al.* 1985, Ferrari *et al.* 1987, Duhm *et al.* 1990, Zicha and Duhm 1990). The large variance of results obtained is not only due to the differences among particular hypertensive strains but also due to methodical differences (saline media vs Na^+-free Mg^{2+}-sucrose incubation media, normal vs elevated cell Na^+ content, whole cells vs ghosts or microsomes, maximal velocities vs *in vivo* transport rates, etc.) as well as due to the age-dependent kinetic changes the example of which is the reduction of the maximal velocity of the Na^+-K^+ pump seen in old rats (Sowers *et al.* 1983, Rosati *et al.* 1988, Zicha and Duhm 1990).

The results on ouabain-sensitive (OS) ion transport in various forms of genetic hypertension are rather conflicting. Na^+,K^+-ATPase activity was found to be higher by 25–40% in red cells of spontaneously hypertensive rats (SHR) than in Wistar-Kyoto (WKY) or Brown-Norway (BN.lx) erythrocytes (Orlov *et al.* 1991a,b). There was also a mild elevation of OS Rb^+ uptake (Duhm *et al.* 1983) or OS Na^+ extrusion (De Mendonca *et al.* 1985) in SHR erythrocytes. On the other hand, OS Na^+ efflux from red cells of Milan hypertensive rats (MHS) into Na^+-free media was reduced by 10–20% as compared to their normotensive controls (MNS) due to a lower cell Na^+ content in MHS (Bianchi *et al.* 1985). The intracellular Na^+ concentration in erythrocytes of SHR and stroke-prone SHR rats was found to be elevated (Berglund *et al.* 1981, Feig *et al.* 1985, Chen and Lin-Shiau 1988) or similar to that in WKY rats (Wolowyk and Slosberg 1983, Duhm *et al.* 1983). Unfortunately, we are still lacking the information on OS ion transport rates in BN.lx erythrocytes that have slightly higher cell Na^+ content than those of SHR (Bin Talib *et al.* 1991).

Data on ouabain-sensitive red cell ion transport in salt-dependent forms of experimental hypertension are relatively scarce (Duhm *et al.* 1983, Knorr *et al.* 1985, Wauquier *et al.* 1986, Zicha and Duhm 1990, Zicha *et al.* 1990) and their variance is further increased by different approaches to the induction of particular forms of salt

hypertension (species and strain differences, age of animals, saline drinking vs. high-salt diet, different mineralocorticoid dosage). Nevertheless, the available data did not still exclude the possibility of primary alterations of the Na^+-K^+ pump, namely a reduction of its maximal velocity.

The main drawback of many previous studies was that the activity of the transport systems was measured at a single set of cation concentrations although the full description of the Na^+-K^+ pump requires to determine its kinetic parameters, i.e. both the maximal velocity and the affinity for particular substrates – at least for internal Na^+ (Na^+_i) and external K^+. This information together with the knowledge of *in vivo* substrate concentrations enable to calculate transport rates pertinent to the *in vivo* conditions (Zicha *et al.* 1990, Zicha and Duhm 1990). Combined kinetic alterations such as an increased maximal velocity accompanied by a reduced affinity of the Na^+-K^+ pump for Na^+_i might yield conflicting results depending on the cation concentrations used. In such a case transport rates are smaller than in controls at low (physiological) Na^+_i concentrations whereas elevated transport rates are detected at very high Na^+_i values (Zicha *et al.* 1990) (see below, Fig. 5).

Ouabain-sensitive Na^+ extrusion and $Rb^+(K^+)$ uptake mediated by the Na^+-K^+ pump represents the main active transport pathway opposing passive movements along respective concentration gradients. There are several kinds of method for the evaluation of the Na^+-K^+ pump activity. They include electrophysiological evaluation of electrogenic transport, determination of the changes in cell cation contents (net fluxes), tracer method estimating unidirectional ion fluxes and measurements of the Na^+,K^+-ATPase activity on the basis of inorganic phosphate production using cell membrane preparations (ghosts, inside-outside vesicles, microsomes). Each method gives somewhat different information about this enzyme which also acts as a transport system (Duhm 1989b).

Our experimental approach is based upon a combination of measurements of ouabain-sensitive unidirectional influx of $^{85}Rb^+$ (a non-radioactive congener of K^+) and the determination of changes in cell Na^+ content to estimate ouabain-sensitive Na^+ net movements (Duhm *et al.* 1983, Duhm and Göbel 1984). Using internal Na^+ and external $Rb^+(K^+)$ concentrations varying around the physiological range our method enables a reliable calculation of kinetic parameters (maximal velocity and substrate affinity) of the Na^+-K^+ pump as a function of internal Na^+ and external $K^+(Rb^+)$ (Zicha *et al.* 1990). The experiments were performed in saline media to maintain the physiological *cis*- and *trans*-effects of extracellular Na^+ on the cation binding sites of the Na^+-K^+ pump. This method is resistant to errors in the estimation of Na^+ transport caused by the existence of ouabain-sensitive $1\,Na^+_o : 1\,Na^+_i$ exchange but the measurements of Rb^+ uptake include $1\,K^+_o(Rb^+_o) : 1\,K^+_i$ exchange. These two exchange modes do not represent any true ion net transport but "contaminate" unidirectional fluxes determined by the tracer techniques ($^{22}Na^+$, $^{86}Rb^+$, $^{85}Rb^+$) (Duhm 1989b, Duhm and Zicha 1990). Some of our experiments were carried out in both saline and Mg^{2+}-sucrose media because certain alterations of the Na^+-K^+ pump activity were also disclosed in Mg^{2+}-sucrose media (Bianchi *et al.* 1985, Ferrari *et al.* 1987, De Mendonca *et al.* 1988, Rosati *et al.* 1988, Heller *et al.* 1990). The omission of external Na^+ from the incubation media caused a pronounced increase of maximal velocity associated with a drastic reduction in the affinity of the Na^+-K^+ pump for internal Na^+

(OS Rb^+ uptake at 3.5 mM Rb^+_o: saline medium: V_{max} 16.7±1.1 and $K_{0.5}$ 8.1±.5; choline medium: 26.5±3.8 and 10.0±1.0; Mg^{2+}-sucrose medium: 50.0±2.5 mmol Rb^+ . (l cells . h)$^{-1}$ and 26.5±1.5 mmol Na^+/l cells) (Bin Talib and Zicha – unpublished data). Though it is somewhat difficult to relate the data obtained in Mg^{2+}-sucrose media to physiological conditions, their knowledge might be important for the characterization of respective membrane defects.

The first aim of our studies was to evaluate the relationship between increased red cell sodium, alterations in the kinetics of the Na^+-K^+ pump and the occurrence of various forms of experimental hypertension, namely in rats with salt-dependent and/or genetic hypertension.

Salt-loaded rats with reduced renal mass represent a classical model of hypertension in which suppressed Na^+-K^+ pump activity and elevated red cell Na^+ have been reported (Huot *et al.* 1983, Hannaert *et al.* 1986, De Mendonca *et al.* 1988). Nevertheless, we did not observe any alteration of red cell Na^+ content in young subtotally nephrectomized Sprague-Dawley rats fed a high-salt diet in which salt hypertension developed (Zicha *et al.* 1990). It is important to note that both ouabain-sensitive (OS) and ouabain-resistant (OR) net Na^+ movements were similar as in water drinking controls although OS Rb^+ uptake was moderately reduced in erythrocytes of salt hypertensive rats (Fig. 1). This reduction was due to a decreased OS 1 Rb^+_o : 1 K^+_i exchange which serves no net transport purpose but represents one of the reversible partial reactions exerted by the Na^+-K^+ pump. Such alteration which mimics "sodium pump suppression" has no relation to hypertension because it occurred not only in the young salt hypertensive rats but also in the adult ones that remained normotensive under the same hypertensive regimen (Zicha *et al.* 1990).

Even more striking results were obtained in DOCA-salt treated Brattleboro rats (Bin Talib and Zicha, unpublished data). It is well-known that the development of this form of hypertension is substantially attenuated in vasopressin-deficient homozygous (DI) rats as compared to their vasopressin-secreting heterozygous (non-DI) littermates (Crofton *et al.* 1979, Berecek *et al.* 1982, Zicha *et al.* 1989). Increased red cell Na^+ contents have been observed in DOCA-salt hypertensive Sprague-Dawley rats (Duhm *et al.* 1983, Kawarabayashi *et al.* 1986). Surprisingly, we have found no changes of red cell Na^+ in severely hypertensive non-DI Brattleboro rats but cellular Na^+ content rose progressively in DOCA-salt treated DI rats in which only a mild elevation of systolic pressure occurred (Fig. 2). Thus red cell Na^+ elevation need not be always associated with the development of salt-dependent hypertension.

Red cell Na^+ elevation in DI rats was caused by the augmentation of OR Na^+ uptake in its both components, i.e. furosemide-sensitive (FS) and -resistant (FR) (Fig. 3). Consequently, high cellular Na^+ concentration stimulated OS Na^+ extrusion but we can presume that the number of pump sites did not increase because red cell Na^+ remained elevated. A comparison with the results obtained in K^+-deficient animals (Duhm *et al.* 1983, Duhm and Göbel 1984) indicated that the key to the above mentioned prominent alterations of Na^+ transport might be cellular potassium depletion which was induced by DOCA-salt treatment only in DI but not in non-DI Brattleboro rats (Fig. 3).

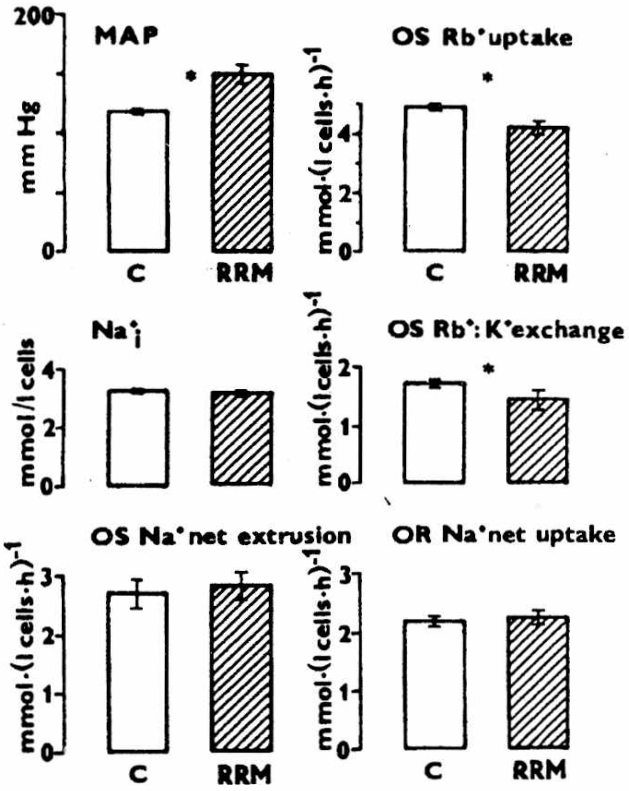


Fig. 1.
Mean arterial pressure (MAP), red cell Na^+ content (Na^+_i) as well as ouabain-sensitive (OS) and ouabain-resistant (OR) Na^+ and Rb^+ transport in erythrocytes of young salt hypertensive Sprague-Dawley rats with reduced renal mass that were fed 8% NaCl diet (RRM, hatched bars) and their normotensive controls fed a low-salt diet (C, open bars). Red cells with native Na^+ content were incubated in saline media containing 137 mM NaCl, 5 mM ouabain and 3.5 mM RbCl. Data are means ± SEM. Asterisks indicate significant differences ($p<0.05$) between hypertensive and normotensive animals. For details see Zicha *et al.* (1990).

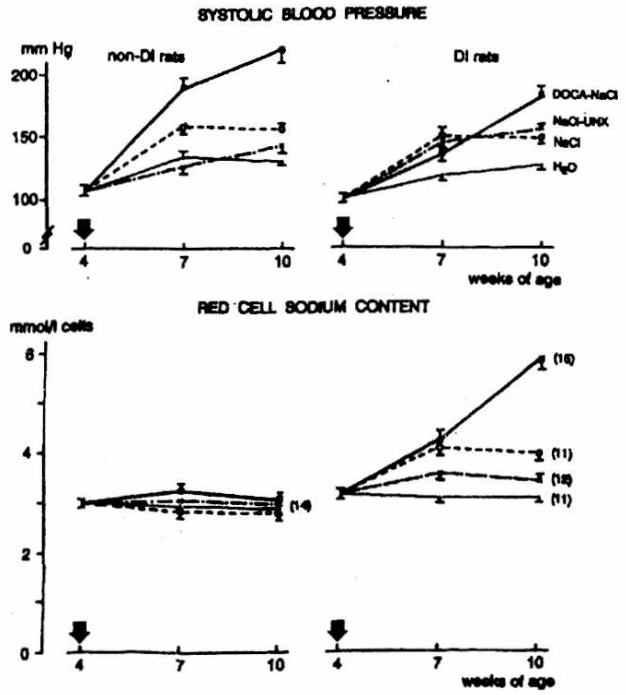


Fig. 2.
Systolic blood pressure development and changes of red cell Na^+ content in young heterozygous (non-DI) and homozygous (DI) Brattleboro female rats subjected to DOCA-NaCl treatment for six weeks which are compared with water drinking (H_2O, full lines), 0.6% saline drinking (NaCl, broken lines) and uninephrectomized saline drinking rats (NaCl-UNX, dashed lines). Data are means ± SEM (n).

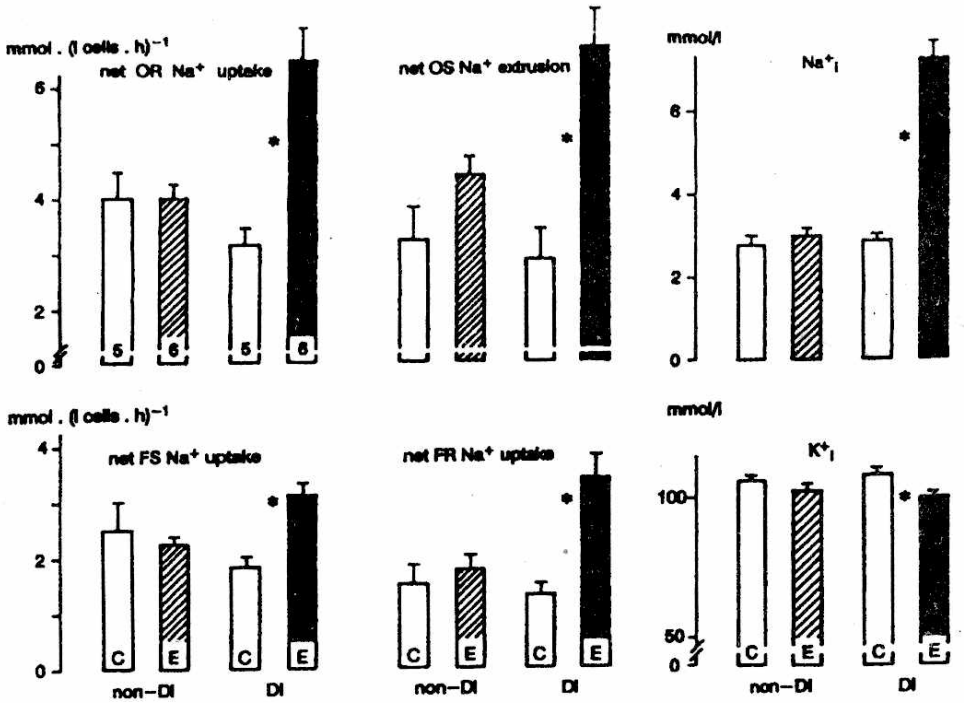


Fig. 3.
Red cell Na^+ and K^+ contents, ouabain-sensitive (OS) and ouabain-resistant (OR) as well as furosemide-sensitive (FS) and -resistant (FR) Na^+ transport in erythrocytes of water drinking (C) and DOCA-NaCl treated (E) non-DI and DI Brattleboro rats after six weeks of the experiment. Erythrocytes with native Na^+ content were incubated in saline media with 5 mM ouabain, 1 mM furosemide and 3.5 mM RbCl. Asterisks indicate significant differences ($p<0.05$) from controls.

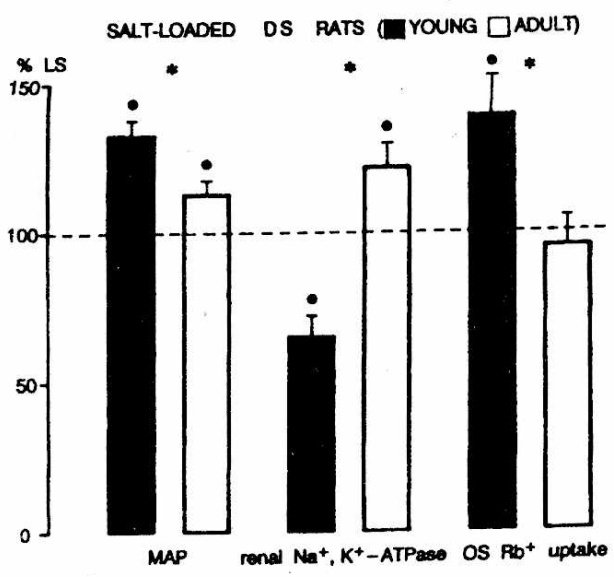
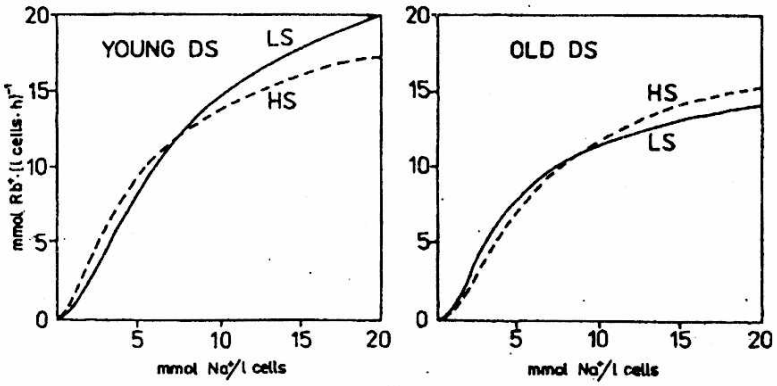


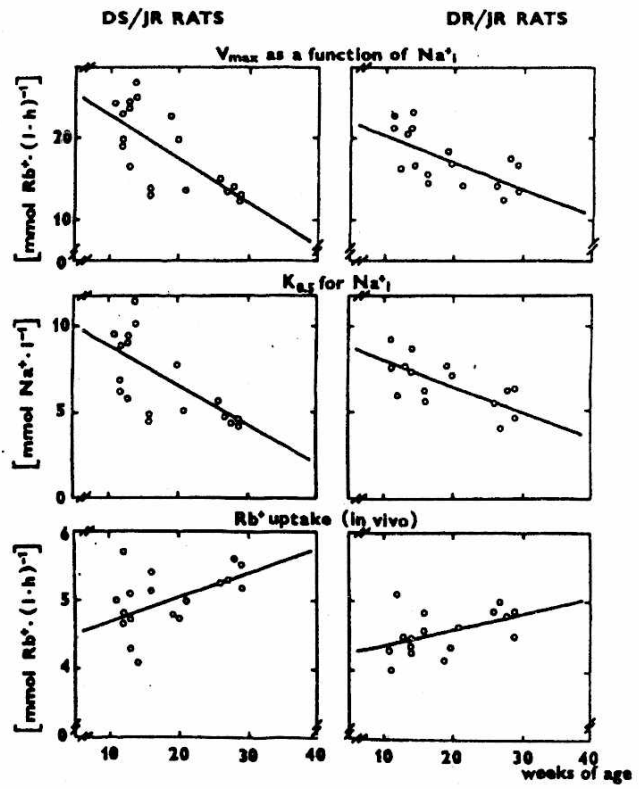
Fig. 4.
Mean arterial pressure (MAP), renal microsomal Na^+,K^+-ATPase activity and ouabain-sensitive (OS) Rb^+ uptake in red cells with native Na^+ content of young and adult salt-loaded Dahl salt-sensitive (DS) rats fed 8% NaCl diet from the age of 4 weeks (young) or 12 weeks (adult). Data are expressed in percentages of values found in normotensive DS rats fed a low-salt diet (LS). Significant difference ($p<0.05$): full dots – high-salt vs low-salt animals, asterisks – young vs adult animals. For details see Zicha *et al.* (1987).



	LS	HS	LS	HS
V_{max}	27	23	18	20
$K_{0.5}$	9.0	7.3	5.7	7.2

Fig. 5.
The dependence of ouabain-sensitive Rb^+ uptake on intracellular Na^+ content in young (left) and old (right) Dahl salt-sensitive rats fed a low-salt diet (LS, full lines) or high-salt diet (HS, broken lines). The corresponding kinetic parameters are under respective panels. V_{max} in mmol Rb^+ . (l cells . h)$^{-1}$, $K_{0.5}$ in mmol Na^+/l cells. For details see Zicha and Duhm (1990).

Fig. 6.
Age-dependent changes in the maximal velocity (V_{max}) of the $Na^+ - K^+$ pump, in the affinity ($K_{0.5}$) of the pump for internal Na^+ and in ouabain-sensitive Rb^+ uptake relevant to *in vivo* concentrations of internal Na^+ and external K^+. Data were obtained in red cells of Dahl salt-sensitive (DS/JR) and salt-resistant (DR/JR) rats fed a low-salt diet. Erythrocytes were incubated in saline media containing 5 mM ouabain and 3.5 mM RbCl.



The second question concerned the consequences of kinetic alterations found in erythrocytes of hypertensive animals for Na^+ and K^+ transport under the *in vivo* and *in vitro* conditions. We have previously reported a suppressed Na^+,K^+-ATPase activity in renal homogenates but increased OS Rb^+ uptake in red cells of young salt-sensitive Dahl rats with a severe salt hypertension (Zicha *et al.* 1987). In contrast, augmented renal Na^+,K^+-ATPase activity without significant changes in red cell ion transport were found in adult DS rats with a moderate salt hypertension (Fig. 4). Later data enabled to explain this apparent contradiction (Zicha and Duhm 1990). It should be noted that certain combinations of kinetic alterations might result in decreased, normal or increased transport rates according to cation concentrations used. Fig. 5 demonstrates a lowered maximal velocity of the Na^+-K^+ pump combined with an increased affinity for internal Na^+ in young salt hypertensive Dahl salt-sensitive (DS) rats whereas the reverse changes were seen in erythrocytes of old salt-loaded DS animals which failed to rise blood pressure. Consequently, red cell OS ion transport which is measured at physiological cell Na^+ concentrations, is indeed accelerated in young salt-loaded Dahl rats whereas their microsomal Na^+,K^+-ATPase activity (measured at saturating Na^+ concentrations) is reduced. There is a good agreement between changes of maximal velocity of the Na^+-K^+ pump in erythrocytes of Dahl rats and alterations of microsomal Na^+,K^+-ATPase activity in particular organs (kidney, heart, brain) (Zicha *et al.* 1987, Zicha and Duhm 1990).

It may be rather dangerous to draw detailed conclusions from incomplete kinetic data. The maximal velocity of the Na^+-K^+ pump is known to decrease with the age of rats. This decline is faster in hypertensive rats (SHR, Dahl S) than in respective normotensive strains (WKY, Dahl R) (Rosati *et al.* 1988, Zicha and Duhm 1990). At least in Dahl erythrocytes incubated in saline media this kinetic change is overcompensated by a concomitant age-dependent increase in the affinity of the Na^+-K^+ pump for internal Na^+. This combination of kinetic changes results in a trend of increasing OS ion transport rates estimated at *in vivo* concentrations of internal Na^+ and external K^+ (Fig. 6).

b) Ouabain-Resistant Na^+ and K^+ Transport in Rat Experimental Hypertension

Tosteson and Hoffman (1960) stated in their "pump-leak concept" of cell volume regulation that each living cell must maintain the balance between sodium penetrating into the cell (through different pathways) and sodium pumped by the Na^+-K^+ pump out of the cell. It means that the rise of ouabain-resistant (OR) Na^+ influx, namely passive membrane permeability for sodium, is followed by cell Na^+ elevation which in turn stimulates Na^+-K^+ pump activity by occupying more internal binding sites. It would be therefore logical to focus the attention on particular components of OR transport although in the past more effort has been paid to the Na^+-K^+ pump activity.

As far as ouabain-resistant Na^+ and K^+ transport is concerned, most studies revealed an increased cation leak in red cells of SHR (Postnov *et al.* 1976, Friedman *et al.* 1977, Wiley *et al.* 1980, De Mendonca *et al.* 1982, Harris *et al.* 1984). A resulting mild red cell Na^+ elevation is accompanied by an increased or normal Na^+-K^+ pump activity in SHR erythrocytes (Berglund *et al.* 1981, Wolowyk and Slosberg 1983, De Mendonca *et al.* 1984, 1985). The role of altered activity of

$Na^+-K^+-2Cl^-$ and K^+-Cl^- cotransport systems (susceptible to the inhibition by loop diuretics) remains still open because these systems, contributing to the regulation of cell volume, can mediate net transport in both directions depending on the driving forces given by the concentrations of respective ions on both sides of the cell membrane (Duhm 1987). Under physiological conditions the $Na^+-K^+-2Cl^-$ cotransport system of rat erythrocytes mediates net transport in the inward direction whereas in Na^+-free media the opposite operation mode takes place. Moreover, particular loop diuretics as furosemide or bumetanide differ in their inhibitory action on these two cotransport systems (Duhm *et al.* 1990) what might complicate the interpretation of the results obtained. Cotransport rates in SHR erythrocytes were thus reported to be increased (Duhm *et al.* 1983, Feig *et al.* 1985, Saitta *et al.* 1987), unchanged (Wolowyk and Slosberg 1983) or decreased (De Mendonca *et al.* 1981, 1982, Rosati *et al.* 1988, Heller *et al.* 1990).

It would be also desirable to pay more attention to the kinetics of particular cotransport systems ($Na^+-K^+-2Cl^-$ and K^+-Cl^-) in relation to Na^+_i and K^+_o concentrations which can vary under the *in vivo* conditions. Kinetic studies carried out in red cells of SHR (Saitta *et al.* 1987, Rosati *et al.* 1988) indicated a higher maximal rate of bumetanide-sensitive Rb^+ influx, a reduced affinity of bumetanide-sensitive outward Na^+-K^+ cotransport system for Na^+_i and a decreased maximal transport rate of ouabain-sensitive Na^+ extrusion. The former finding was disclosed in saline media whereas the latter two alterations were observed in erythrocytes incubated in Mg^{2+}-sucrose media. It should be mentioned that furosemide-sensitive (FS) and bumetanide-sensitive (BS) Na^+ effluxes measured in Na^+-free incubation media refer to an outward Na^+-K^+ cotransport whereas FS and BS Na^+ net uptakes determined in cells suspended in saline media must be considered as a sum of inward and outward Na^+ movement mediated by the Na^+-K^+ cotransport system. Moreover, high furosemide doses exert an inhibitory action on the Na^+ leak (Scholz and Hropot 1987).

The last topic of our studies was the passive membrane permeability for Na^+ and $K^+(Rb^+)$ ions in genetic hypertension. It is evident in young salt hypertensive Dahl rats (Zicha and Duhm 1990) that red cell Na^+ content was not elevated due to Na^+-K^+ pump suppression but due to augmented furosemide-resistant (FR) Na^+ leak (Fig. 7). The Na^+ leak might have some relations to this form of hypertension because it was greater in salt-sensitive rats as well as in young animals. Moreover, Na^+ leak was augmented by high salt intake only in salt-sensitive but not in salt-resistant Dahl rats (Fig. 8). A certain caution is, however, necessary because red cell Na^+ elevation was present not only in young and adult salt hypertensive Dahl rats but also in the old ones which remained normotensive when kept on a high-salt diet for about 12 weeks (Fig. 9). The reason why old rats are quite resistant to hypertensive effects of high salt intake remains unclear. Not only Na^+ leak but also Rb^+ leak were greater in salt-sensitive than in salt-resistant Dahl rats although the latter was independent of salt intake level (Fig. 10).

Just this observation brought us to examine whether the increased passive permeability for monovalent cations that was repeatedly described in rats with genetic hypertension (Friedman *et al.* 1976, Postnov *et al.* 1976, De Mendonca *et al.* 1985, Feig *et al.* 1985), cosegregates with blood pressure. A simple comparison of an inbred hypertensive strain with a "respective" normotensive control strain is insufficient to reveal whether the differences in genes or quantitative traits are

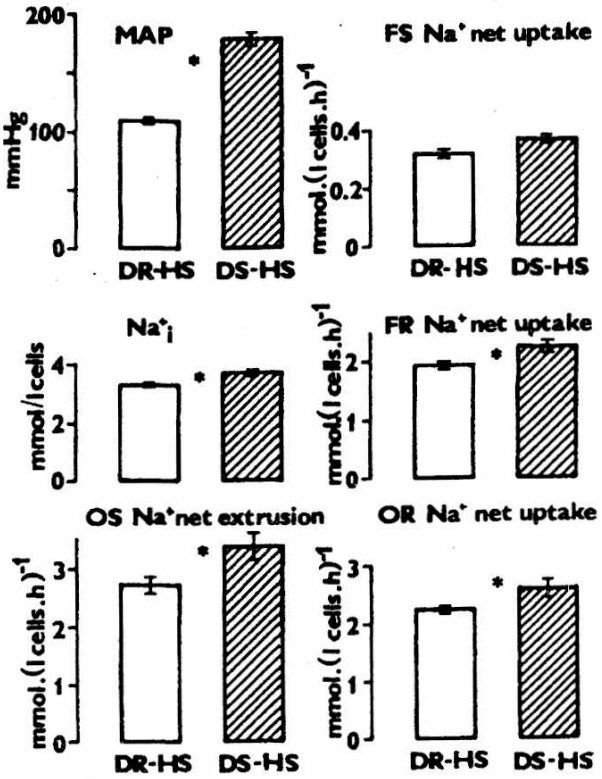


Fig. 7.
Mean arterial pressure (MAP), red cell Na^+ content (Na^+_i), ouabain-sensitive (OS) and -resistant (OR) as well as furosemide-sensitive (FS) and -resistant (FR) Na^+ transport in erythrocytes of young Dahl salt-sensitive rats (hatched bars) and salt-resistant rats (open bars) fed a high-salt diet. Red cells with native Na^+ content were incubated in saline media containing 5 mM ouabain, 1 mM furosemide and 3.5 mM RbCl. Asterisks indicate significant differences ($p<0.05$) between salt hypertensive and normotensive animals. For details see Zicha and Duhm (1990).

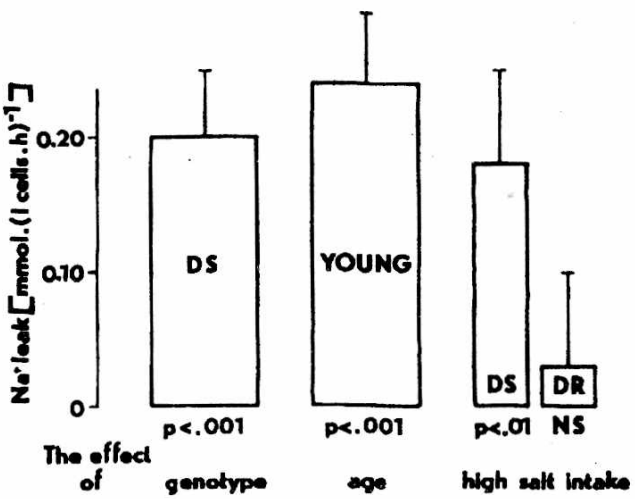


Fig. 8.
The factorial effects of genotype (DS vs DR), age (young vs old) and salt intake (high-salt vs low-salt diet) on furosemide-resistant Na^+ leak in erythrocytes of Dahl rats (DS – salt-sensitive, DR – salt-resistant). Red cells with native Na^+ content were incubated in saline media containing 5 mM ouabain, 1 mM furosemide and 5 mM RbCl. For details see Zicha and Duhm (1990).

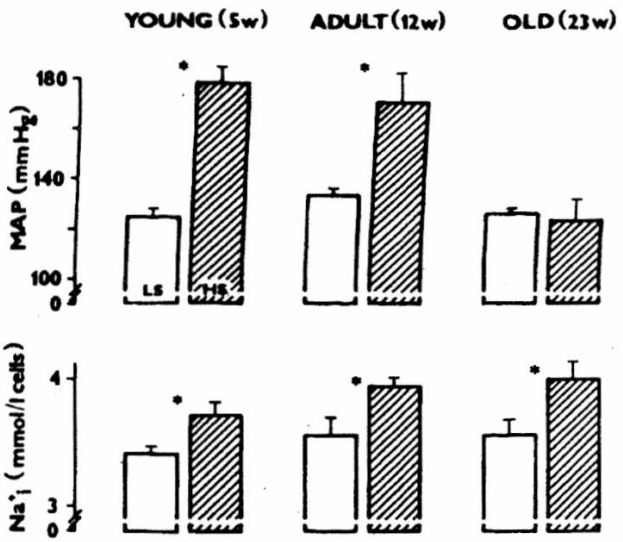


Fig. 9.
Mean arterial pressure (MAP) and red cell Na^+ content (Na^+_i) in Dahl salt-sensitive rats fed either low-salt diet (LS) or high-salt diet (HS) from the age of 5 (young), 12 (adult) or 23 weeks (old) for 7–10 weeks. Asterisks indicate significant differences ($p<0.05$) between LS and HS animals. For details see Zicha and Duhm (1990).

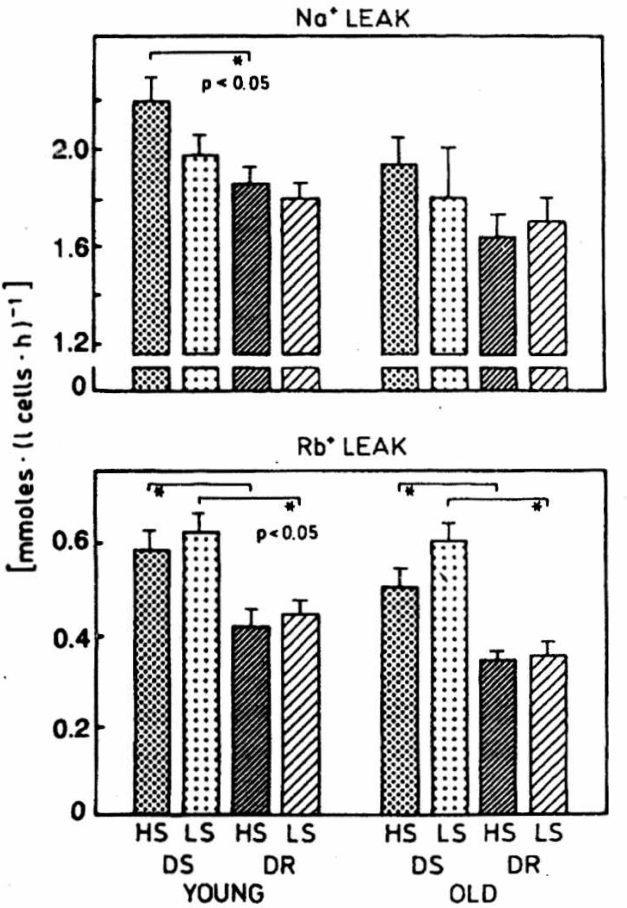


Fig. 10.
Furosemide-resistant Na^+ and Rb^+ leaks in Dahl salt-sensitive (DS) and salt-resistant (DR) rats fed low-salt diet (LS) or high-salt (HS) diet from the age of either 5 weeks (young) or 23 weeks (old). Red cells with native Na^+ content were incubated in saline media containing 5 mM ouabain, 1 mM furosemide and 5 mM RbCl. Asterisks indicate significant differences ($p<0.05$) between DS and DR rats. For details see Zicha and Duhm (1990).

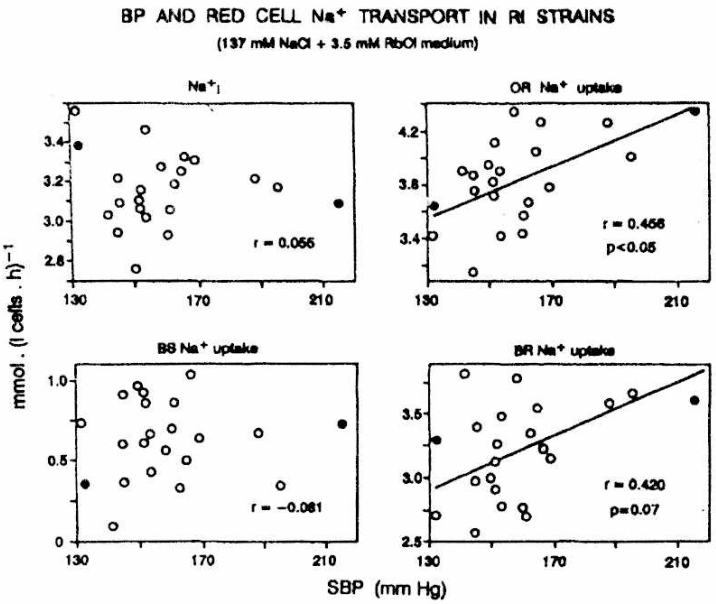


Fig. 11.
The relationship of systolic blood pressure (SBP) to red cell Na^+ content (Na^+_i), ouabain-resistant (OR), bumetanide-sensitive (BS) and bumetanide-resistant (BR) Na^+ net uptakes in erythrocytes of both progenitor strains (SHR and BN.lx, full dots) and 20 recombinant inbred strains (open circles). Erythrocytes were incubated in saline media containing 5 mM ouabain, 10 μM bumetanide and 3.5 mM RbCl. Progenitor strains differed significantly ($p<0.05$) in all parameters except of BR Na^+ net uptake.

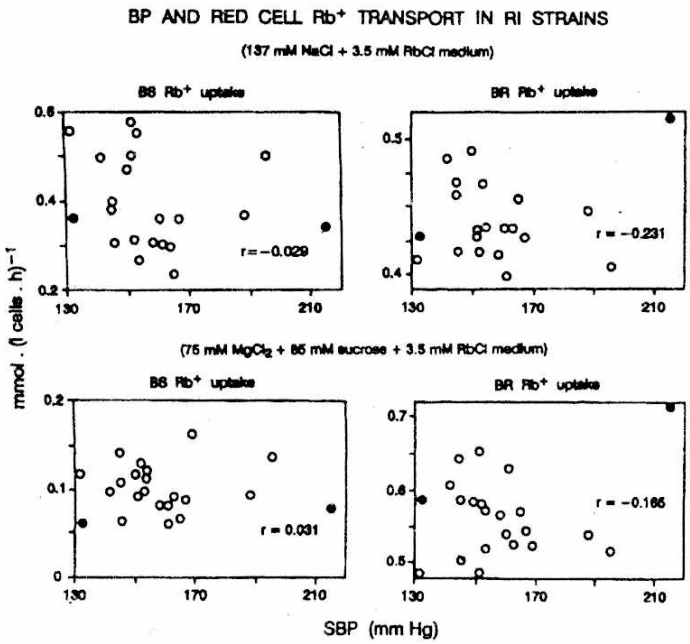


Fig. 12.
The relationship of systolic blood pressure (SBP) to bumetanide-sensitive (BS) and -resistant (BR) Rb^+ uptakes in erythrocytes of both progenitor strains (full dots) and recombinant inbred strains (open circles). Erythrocytes were incubated in either saline media (upper panels) or Mg^{2+}-sucrose media (lower panels) containing 5 mM ouabain, 10 μM bumetanide and 3.5 mM RbCl. Progenitor strains differed significantly ($p<0.001$) in BR but not in BS Rb^+ uptake.

indeed related to the difference in blood pressure or not. The only possible approach is to determine whether blood pressure of F_2 hybrids or recombinant inbred (RI) strains (a fixed F_2 generation) cosegregates with a certain trait (e.g. transport parameter) or whether hypertension occurrence is associated with particular gene(s) (Rapp 1987). As far as genetic hypertension in the rat is concerned, the only available set of RI strains has been produced by Dr. M. Pravenec (Department of Biological Experimental Models, Institute of Physiology, Czechoslovak Academy of Sciences, Prague) and Prof. V. Křen (Department of Biology, Faculty of General Medicine, Charles University, Prague) who used SHR and normotensive Brown-Norway (BN.lx) rats as progenitor strains (Pravenec *et al.* 1989). The major advantage of RI strains over F_2 hybrids is the reliable and reproducible determination of the quantitative phenotype in particular RI strains. Consequently, we can correlate the genes and traits determined in different experiments in order to evaluate their possible relationship. Such approach was used to prove the association of blood pressure with the polymorphism of renin and kallikrein genes (Pravenec *et al.* 1991a,b). On the other hand, altered platelet aggregation was found to be independent of blood pressure level in RI strains (Pravenec *et al.*, unpublished data).

This unique set of RI strains also enables to test the cosegregation of particular ion transport alterations with blood pressure. Several components of ouabain-resistant (OR) Na^+ and $K^+(Rb^+)$ transport were studied in both progenitor strains (SHR, BN.lx) and in 20 RI strains using erythrocytes incubated in saline as well as in Mg^{2+}-sucrose media (Bin Talib and Zicha, unpublished data). Progenitor strains differed in OR and FS Na^+ net uptake as well as in FR and BR Rb^+ uptake, the values being higher in SHR (Bin Talib *et al.* 1991, Orlov *et al.* 1991b,c). In contrast, red cell Na^+ contents were significantly greater in normotensive BN.lx rats (Bin Talib *et al.* 1991) what is similar to the difference reported by Bianchi et al. (1985) in Milan normotensive (MNS) and hypertensive rats (MHS) rats. It is evident (Fig. 11) that OR Na^+ uptake and bumetanide-resistant (BR) Na^+ leak cosegregated significantly with blood pressure. On the other hand, there was no evident relationship of blood pressure of RI strains to either bumetanide-sensitive (BS) Na^+ uptake (Na^+-K^+ cotransport) or red cell Na^+ content. Though the increased Na^+ leak did not result in the elevation of red cell Na^+ contents, this possibility cannot be excluded in other tissues involved in cardiovascular regulations. The absence of any significant cosegregation of Rb^+ leak (determined in erythrocytes incubated in either saline or Mg^{2+}-sucrose media) with blood pressure of RI strains was rather surprising because the difference between both progenitor strains was considerable (Fig. 12). Our data do not confirm earlier reports on the cosegregation of outward Na^+-K^+ cotransport with blood pressure that was found in F_2 MHSxMNS and SHRxWKY hybrids (Bianchi *et al.* 1985, Kotelevtsev *et al.* 1989). One of possible explanations might be the absence of a significant difference in BS or FS Na^+ efflux between SHR and BN.lx rats the erythrocytes of which were incubated in Mg^{2+}-sucrose medium.

The contrast between relatively higher rates of OR Na^+ uptake in strains with elevated blood pressure and their normal cell Na^+ contents suggests that OS Na^+ extrusion might be accelerated in these hypertensive strains. This is also true for SHR in which OR Na^+ uptake is high but red cell Na^+ content is lower than in normotensive BN.lx rats. Indeed Orlov *et al.* (1991b,c) demonstrated

elevated Na^+,K^+-ATPase activity in saponin-treated erythrocytes of SHR as compared to BN.lx rats. Of course, it remains to be established whether this difference is due to a greater number of pump sites and/or higher pump Na^+ turnover and whether these parameters also cosegregate with blood pressure in RI strains.

Conclusions

1. Salt-dependent as well as genetic hypertension are not always associated with an increased red cell Na^+ content.
2. Elevated red cell Na^+ content is usually caused by an increased Na^+ leak but not by a suppressed $Na^+ - K^+$ pump activity.
3. Na^+ leak (but not Rb^+ leak, $Na^+ - K^+$ cotransport or red cell Na^+ content) cosegregates with blood pressure in genetic hypertension.
4. There is no uniform elevation of red cell membrane passive permeability for Na^+ and K^+ ions because Na^+ leak can be enhanced without changes of $Rb^+(K^+)$ leak.
5. Transport rates mediated by the $Na^+ - K^+$ pump or $Na^+ - K^+$ cotransport should be estimated for *in vivo* cell and plasma Na^+ and K^+ concentrations in addition to the determination of kinetic parameters of the transport systems. Values obtained at a single set of unphysiological cation concentrations may lead to erroneous conclusions. A fall in the maximal velocity of the $Na^+ - K^+$ pump may be fully compensated at *in vivo* Na^+ and K^+ concentrations by an increased affinity for $Na^+{}_i$.
6. 1 $Rb^+{}_o(K^+{}_o)$: 1 $K^+{}_i$ and 1 $Na^+{}_o$: 1 $Na^+{}_i$ exchange mediated by these two above mentioned transport systems should be considered in the evaluation of unidirectional Na^+ and $K^+(Rb^+)$ fluxes.
7. Microsomal Na^+,K^+-ATPase activity (determined at saturating Na^+ concentrations) reflects rather the changes in maximal velocity than the actual alterations of *in vivo* transport rates.
8. Last three conclusions are also valid for other cell types.

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