Heritability of Erythrocyte Sodium Permeability: A Possible Genetic Marker for Hypertension

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Abstract. Individuals genetically susceptible to hypertension may have preexisting membrane defects influencing cell sodium permeability. Fourteen Minnesotan families of Northern European descent were selected as having one or both progenitors with either high (HP) or low (LP) erythrocyte sodium permeability. We earlier found that over one-half of the $^{22}\text{Na}^+$ influx into HP erythrocytes can be inhibited by micromolar amounts of furosemide, which has no apparent effect on LP erythrocytes. In these families, we find a significant midpoint parent/offspring correlation in the furosemide-sensitive component of erythrocyte $^{22}\text{Na}^+$ flux rates ($p < 0.001$). The relationship between parents and children in this metric trait is most consistent with a single locus, 2-allele system with variable expression, which suggests that enhanced furosemide-sensitive $^{22}\text{Na}^+$ influx may be inherited as an autosomal recessive trait. Individuals with HP erythrocytes tend to have increased blood pressure and/or a family history of hypertension. The results were confirmed in these family samples with HP and LP $^{22}\text{Na}^+$ influx (mmol/L RBC/hr): 0.404 ± 0.03 vs 0.232 ± 0.01 ($p < 0.001$); systolic blood pressure (mm Hg): 136 ± 4 vs 108 ± 4 ($p < 0.001$); and diastolic blood pressure (mm Hg): 89 ± 2 vs 69 ± 2 ($p <0.001$). These results may help to identify inherited factors involved in salt sensitive hypertension.

Keywords: erythrocyte sodium permeability, diuretics, ion transport, blood pressure, genetic linkage

Introduction

Studies indicate that genetic defects in sodium transport across cell membranes may be important in the development of primary hypertension in humans [1-3]. Increased monovalent cation permeability of erythrocytes has also been reported in spontaneously hypertensive rats [4-8]. In other studies, abnormalities of kidney and erythrocyte cation transport functions have been reported in Milan hypertensive rats [9-11]. Furthermore, renal transplant studies in several rat models indicate that hypertension may be causally associated with defective renal functions [12].

Some authors have suggested that variations in human erythrocyte sodium transport may be polygenically determined, showing significant correlation between siblings and between parents and offspring but not between spouses [13]. In an earlier investigation, the rate of influx of $^{22}\text{Na}^+$ into red blood cells (RBC) from several hundred adult Minnesotans was determined in our laboratory. In this population, erythrocyte sodium permeability varied from <0.15 to about 0.68 mmol/L RBC/hr. We assigned donors to the high permeability (HP) group with values >0.40 mmol/L RBC/hr and those with rates <0.28 mmol/L RBC/hr to the low permeability (LP) group [14]. In our study, two classes of agents, polyanions and sulfonamide loop diuretics, blocked the increased sodium permeability of HP erythrocytes but not of LP erythrocytes.
The inheritance of this presumably genetic trait has not been resolved. In the present study, we measured $^{22}$Na$^{+}$ flux rates in nuclear families, using 14 family groups with HP or LP progenitors, in order to test intra- and inter-family variations in erythrocyte sodium permeability and to seek a possible link to elevated blood pressure.

Materials and Methods

Families were selected by screening for erythrocyte Na$^{+}$ permeability in a large number of volunteers including laboratory and hospital personnel at the University of Minnesota and participants in a study on prevention of high blood pressure in children in Minneapolis and St. Paul, Minnesota. The 14 selected families, 6 having one or two parents with high permeability (HP) ($^{22}$Na$^{+}$ flux > 0.4 mmol/L RBC/hr) and 8 having both parents with low permeability (LP) ($^{22}$Na$^{+}$ flux < 0.28 mmol/L RBC/hr), were largely of Northern European ancestry. The families included a minimum of 5 members with at least 2 biological children.

Tris(hydroxyethyl)aminomethane (Tris), 3-N-morpholino propane-sulfonic acid (MOPS), ouabain, and furosemide were from Sigma Chemical Co., St. Louis, MO. All other chemicals were of reagent grade.

Heparinized fresh blood samples were used for measurements of $^{22}$Na$^{+}$ flux rates. Plasma and buffy coat were removed by centrifugation for 3 min at 3000 rpm (1000 x g). The red blood cells were washed 3 times with a buffer (flux buffer) containing 145 mM KCl, 5 mM NaCl, 10 mM Tris base, 10 mM MOPS, 10 mM D-glucose, and 0.1 mM ouabain, pH 7.40 at 37°C, 300 ± 10 mOsm/L. Red blood cell membrane sodium permeability was measured according to the method developed in our laboratory [16]. The influence of furosemide or dithiorethiol (DTT) on red cell $^{22}$Na$^{+}$ flux rates was determined in washed cells preincubated at 37°C for 30 min with flux buffer containing 1 mM furosemide or 1 mM DTT [14,15]. Fresh buffer solutions were made in each DTT experiment since the reducing agent is oxidized easily under aerobic conditions.

Information on a family history of hypertension was obtained and blood pressure of all participants was measured. A standard clinical sphygmomanometer was used for measurement of blood pressure by healthcare professionals and specially trained technicians, with an average of two measurements taken on the right arm with the subject in a sitting position after 5 min at rest. Standard cuffs were used for adults. Children’s blood pressure was measured using pediatric cuffs with at least 120% of the upper arm diameter and long enough to encircle 90% of it. To minimize circadian blood pressure variations, blood pressure measurements were performed during the late afternoon (16:00 to 19:00 hr). Weight and height values were used to estimate body mass index (BMI) and subjects with BMI values >30 were excluded because for adults this is the cutoff point for obesity and since being overweight is one of the major risk factors for increased blood pressure.

The personnel who measured $^{22}$Na$^{+}$ flux rates were blinded to blood pressure levels and family history of hypertension, as well as the $^{22}$Na$^{+}$ flux rate of other family members. Written consent was obtained from all participants. Erythrocyte $^{22}$Na$^{+}$ flux rates with and without furosemide were compared by a paired t test. Complex segregation analysis was performed to test whether a major gene could explain the variation in red cell $^{22}$Na$^{+}$ flux rates. Complex segregation analysis depends critically on the distribution of the data, so that transformations of the data were made to (a) remove effects of age and sex, (b) remove skewness by use of a power transform [17], and (c) normalize so that the new variable has a zero mean with unit standard deviation.

Detection of a major gene in the presence of polygenic heritability was evaluated under the mixed model using the computer program POINTER [18,19]. In addition to the mean and variance, the mixed model includes the following additional parameters: (a) Q, the frequency of the major gene A; (b) T, the displacement, defined by the distance between the two assumed homozygous genotypic classes at the major locus, measured in standard deviation units on the scale of genetic liability; (c) D, the degree of dominance at the major locus on the liability scale (when D = 1 the allele A is dominant; when D = 0 the allele A is recessive); (d) H, the polygenic heritability; and (e) the transmission probabilities, defined by $T_1 = Pr( AA \rightarrow A )$, $T_2 = Pr( Aa \rightarrow A )$, and $T_3 = Pr( aa \rightarrow A )$. Under Mendelian segregation, the values of $T_1$, $T_2$, and $T_3$ should approach 1, 1/2, and 0, respectively.

Parameters were estimated by maximizing the likelihood of the phenotypes of the offspring conditional on the phenotypes of parents. The maximum estimate probabilities of all unknown parameters were computed under both a generalized model and under various null hypotheses (restricted models). Each null hypothesis was tested by the heritability ratio criterion, in which minus twice the predicted ratio is distributed asymptotically as a Chi-square, with the number of degrees of freedom being equal to the number of restrictions imposed upon the model that defines the null hypothesis.

This study was approved by the Institutional Human Subjects and Use Committee; all of the study protocols followed the NIH and University of Minnesota human subject study guidelines. Data are expressed as mean ± SE and p < 0.05 is considered statistically significant.

Results

Fig. 1 shows histograms of $^{22}$Na$^{+}$ flux rates in 4 representative families, 3 families having a progenitor with HP red cells and 1 having both LP red cell progenitors. This figure demonstrates a pattern consistent with familial aggregation of furosemide-sensitive red cell $^{22}$Na$^{+}$ flux rates. All other family data show a similar pattern. $^{22}$Na$^{+}$ influx was inhibited significantly in all samples of HP red cells by furosemide pretreatment (p < 0.001),
Fig. 1. Histograms of red blood cell $^{22}\text{Na}^+$ flux rates in 4 kindreds showing the pattern of familial aggregation. The data support a strong parent-offspring and/or sibling resemblance with some intermediate mixtures of both parents. Erythrocyte $^{22}\text{Na}^+$ flux rates with and without furosemide were compared by a paired t test. $^{22}\text{Na}^+$ influx (mmol/L RBC/hr) was inhibited in all samples of high permeability erythrocytes (HP RBC) with 1 mM furosemide pretreatment ($p < 0.001$). $^{22}\text{Na}$ = furosemide-sensitive $^{22}\text{Na}^+$ flux rate; FS = furosemide-insensitive $^{22}\text{Na}^+$ flux rate. JPM, HH, RC: progenitors’ initials with HP RBC; GC: progenitor’s initial with low permeability erythrocytes (LP RBC); F = father; M = mother; 1-5 = offspring; GMM = maternal grandmother; BM = mother’s brother; SM = mother’s sister; SF = father’s sister.

Fig. 2. $^{22}\text{Na}$ flux rate of midpoint parental values vs offspring’s values, compared using the Statview Regression Statistical package. The values show a significant correlation (regression coefficient, $r = 0.76$, $p < 0.001$). Midpoint of parents’ $^{22}\text{Na}^+$ flux rate (mmol/L RBC/hr) = average value of both parents.

Fig. 3. $^{22}\text{Na}^+$ flux rates and blood pressure comparisons. HP: erythrocytes (RBC) with high permeability; LP: low permeability RBC; BP: blood pressure. Data expressed as mean ± SE; each HP and LP group had 9 subjects; groups were compared by a paired t test.
Fig. 4. $^{22}\text{Na}^+$ flux rates and family history of hypertension. As an example of the 4 kindred, 3 generation pedigrees shown in this diagram, there is a strong correlation between $^{22}\text{Na}^+$ flux rates (mmol/L RBC/hr) and blood pressure. All other family samples showed similar patterns. H = hypertensive; P = progenitor; $^{22}\text{Na}^+$ flux rates (mmol/L RBC/hr) shown as numbers; ? = unknown $^{22}\text{Na}^+$ flux rate; no FH = no family history of hypertension.

Fig. 5. Effect of dithiothreitol on $^{22}\text{Na}^+$ flux rates. Pretreatment with dithiothreitol (DTT) at ≥1 mM concentrations reduced sodium permeability of HP erythrocytes but was not effective in LP erythrocytes. Subjects PH, JS, and MH were hypertensive with HP erythrocytes; subjects MC and JL were normotensive with LP erythrocytes.
while families with LP red cells showed little or limited reduction in $^{22}\text{Na}^+$ influx (Fig. 1). In accord with this, there was no significant relationship between parents and offspring in furosemide-insensitive $^{22}\text{Na}^+$ influx. In the parent-offspring flux rate comparison, offspring values for erythrocyte sodium permeability are significantly correlated with their parents’ midpoint values ($r = 0.76; p <0.001$) (Fig. 2). All offspring showed HP flux rates with fathers having HP RBC and mother with high intermediate flux rates, while offspring with both parents with LP RBC showed LP flux rates.

These results support a strong parent-offspring and sibling resemblance in erythrocyte sodium permeability. Results of complex segregation analyses are consistent with this finding (Table 1).

The hypothesis of “no genes” is rejected ($X^2 = 10.95$), implying a significant familial resemblance between parents and offspring. The hypothesis of “no major gene” is also rejected ($X^2 = 10.95$). The hypothesis of “no polygene” is not rejected, as the generalized single major locus model converged to the mixed model (with Mendelian transmission probabilities). It should be noted, however, that the test of Mendelian transmission is rejected ($X^2 = 22.70$), implying that the familial transmission of Na influx may not solely be due to a single gene.

Most individuals with HP RBC had elevated blood pressure or a family history of hypertension. As shown in many other studies on increased sodium permeability among hypertensives, there was a trend to have increased blood pressure when an individual had HP erythrocytes. The results were confirmed in the family samples: HP and LP comparisons in $^{22}\text{Na}^+$ influx (mmol/L RBC/hr): $0.404 \pm 0.03$ vs $0.232 \pm 0.01 (p <0.001)$; SBP (mm Hg): $136 \pm 4$ vs $108 \pm 4 (p <0.001)$; DBP (mm Hg): $89 \pm 2$ vs $69 \pm 2 (p <0.001)$ (Fig. 3). All data in both groups were normally distributed as shown by the small SE in a paired t test.

In the 4 kindred, 3 generation pedigree diagram, there is strong correlation between $^{22}\text{Na}^+$ flux rates (mmol/L RBC/hr) and blood pressure and/or family history of hypertension (Fig. 4). All other family data show a similar pattern.

**Discussion**

Despite the intra-individual variability of blood pressure, there is strong evidence of familial aggregation. One in four Americans has a diastolic blood pressure >90 mm Hg and there are familial correlations for both systolic and diastolic blood pressure [20] but no correlation has been found between spouses, even after decades of marriage [13,21], or between parents and adopted children [22]. This indicates that predisposition to essential hypertension may be genetically determined although environmental factors, especially in childhood, are likely to play a role as contributing factors to influence blood pressure.

![Table 1. Segregation analysis of sodium permeability under the mixed model.](image)

<table>
<thead>
<tr>
<th>Model</th>
<th>-2ln(L)+c</th>
<th>Parameters</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
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<tr>
<td>Mendelian</td>
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<td>—&gt;0</td>
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<tr>
<td>T2 = 0.5</td>
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D = degree of dominance; T = displacement; Q = gene frequency; H = heritability; T2 = transmission probability (Aa —> A)
Many risk factors have been postulated for essential hypertension, but salt intake may be the most prominent causal factor in controlling blood pressure, especially among individuals genetically predisposed to essential hypertension. Several epidemiological studies suggest that excessive dietary sodium intake may be causative of hypertension [23,24]. Other studies indicate that inherited abnormalities of ion transport across the cell membranes may be responsible for the development of essential hypertension [1,20,25-35]. Animal studies also support the importance of inherited abnormalities [4-6,10,36].

Different authors have reported major genetic determinants. Williams et al [13] suggested that total heritability of erythrocyte sodium transport may exceed 90%. Various human leukocyte antigen (HLA) types may be related to the risk of essential hypertension, although this is still controversial. However, many authors report that certain HLA-A and/or -B as well as DR antigens have increased frequency in human and experimental hypertension [36-40]. Antigens B12, B15, and B18 on chromosome 6 were found more frequently than others in hypertension among different populations [37-40]. A study by Heise et al [41] suggests that the MN locus on chromosome 4 may fall in a region controlling blood pressure variations in hypertension, while others suggested that increased kallikrein may have protective effects [42]. In genetic linkage study reviews, a gamma subunit of the epithelial sodium channel was implicated in the physiological variation of systolic blood pressure [43-45]. Others reported that the beta and gamma subunits of the epithelial sodium channel on chromosome 16p12 are linked with sodium-dependent forms of low and high blood pressure [46], and might be involved in marked phenotypic variations in blood pressure, serum potassium levels, and age of onset of hypertension [47]. Erythrocyte Na-K co-transport abnormalities were also observed in the cells of hypertensive individuals [33,48-50]. In other family and twin studies, blood pressure phenotypes were strongly affected by genetic factors [51,52]. Genetic causes of salt sensitivity include single gene mutations and are often manifested by significant family history of hypertension [33-35,53,54].

Variations in cellular Na\(^+/K^+\)-ATPase activity may play an important role in regulation of ion transport, fluid balance, and control of blood pressure levels [33-35,54-59]. Membrane-bound Na\(^+/K^+\)-ATPase, the enzyme responsible for the maintenance of Na\(^+\) and K\(^+\) ion electrochemical gradients across the plasma membrane, is composed of two subunits: \(\alpha\), which is the catalytic subunit, and \(\beta\), which maintains the structural/conformational stability. The enzyme is regulated by many hormones, such as catecholamines, aldosterone, and thyroid hormones [35]. Various functional activities of the ATPase are also based on the genetic variations [33,53,55,59] and genetically linked on human chromosome 19 [60]. It has been suggested that a circulating inhibitor of Na\(^+/K^+\)-ATPase may act as an endogenous ouabain, which may be responsible for an increased cellular sodium level and result in increased vascular resistance [54,58]. Induced ouabain sensitivity can be achieved by cerebrovascular injection of potassium, resulting in decreased blood pressure and heart rate [56]. In hypertensive patients, Na\(^+/K^+\)-ATPase is decreased and intracellular Na\(^+\) content is increased [61]. Reduced Na\(^+/K^+\)-ATPase activity induces a Ca\(^{++}\) elevation in vascular smooth muscle cells and sympathetic neurons that trigger increased vascular tone, resulting in increased peripheral resistance [61].

Using these observations as a possible lead, we re-examined earlier data derived from several hundred Minnesotans in Minneapolis and St. Paul. The resulting bell-shaped distribution of values for \(^{22}\)Na\(^+\) influx [14] bore a resemblance to a population blood pressure distribution curve from the Hypertension Detection and Follow-up Program Cooperative Group [62]. In light of these results, we attempted to define familial relationships for erythrocyte sodium permeability among first degree family members in 14 Minnesotan families. As hypothesized, we found a strong familial aggregation and significant correlation between parent-offspring in \(^{22}\)Na\(^+\) influx (\(p<0.001\)), which suggests that sodium permeability is genetically determined. In our current study, many young offspring with a family history of hypertension showed increased red cell sodium permeability even as teenagers and these individuals may be at
risk of hypertension at a later age. However, the relation between the normal and furosemide-dependent cation transport rates and blood pressure control still remains obscure because as we and others show, cation permeability changes have been reported in different directions among hypertensives [increased: 16,27-29,32,41,48-50,63-65; decreased: 36; and normal: 3,26,43,45,66].

When similar determinations of erythrocyte permeability were extended to black Americans with essential hypertension, no hypertension-associated increase in sodium permeability was found [30]. Additional studies suggest that there may be racial differences that affect hypertension, such as renal response to furosemide [67], and reduced endothelial release of bioactive nitric oxide (NO) [68]. In this regard, the large African American Heart Failure Trial compared the anti-hypertensive effects of BiDil, an orally administered NO enhancing medicine that combines isosorbide dinitrate and hydralazine [69]. It was found that this agent was preferentially effective in blacks, further supporting the concept of reduced endothelial NO generation in endothelial cells of blacks. NO can react with sulfhydryl groups yielding nitrosothiols and, upon decomposition, oxidized thiols.

Our current results indicate that pretreatment of erythrocytes with a SH-reducing agent, DTT, reduces sodium permeability selectively in Caucasian HP erythrocytes (PH, JS and MH, all hypertensives) but has no effect on normotensive LP erythrocytes (JL and MC) (Fig. 5). This indicates that oxidized sulfhydryl groups (or nitrosothiols) in erythrocyte membrane proteins may be responsible for at least some of the exaggerated sodium permeability seen in patients with essential hypertension.

In the current study, it may be a limitation that the population we used was largely of Scandinavian origin. However, limited genetic diversity may have been beneficial in the current study by minimizing subtype heterogeneity in defining the relationship of Na\(^{+}\) permeability and the hypertensive phenotype as shown in other studies [53,59]. Future studies employing more molecular techniques and subjects of different ethnic origin may be required for detection of the loci and gene product(s) involved in governing red cell sodium permeability and predisposition to essential hypertension. A search for associated restriction fragment length polymorphisms as markers, as well as attempts to determine membrane protein variations associated with HP and essential hypertension phenotypes, should be pursued, including studies among people of different ethnic origins.

In summary, individuals having HP and LP erythrocytes showed \(^{22}\)Na\(^{+}\) flux rates in a pattern of familial aggregation in the current study. The results support a strong parent-offspring and/or sibling resemblance with some intermediate mixtures of both parents. Furthermore, there is strong correlation between \(^{22}\)Na\(^{+}\) flux rates and blood pressure levels. A search for inherited factors involved in salt-sensitive hypertension may permit an improved understanding of the mechanisms involved in salt-genetic hypertension. Thus, \(^{22}\)Na\(^{+}\) flux rates as heritable phenotypes may be used to detect the genetic etiology of hypertension at the prehypertensive stage.

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