Altered Na\(^{+}\)-K\(^{+}\) pump activity and plasma lipids in salt-hypertensive Dahl rats: relationship to Atp1a1 gene

JOSEF ZICHA,1 CERVANTES D. NEGRIN,2 ZDENKA DOBEŠOVA,1 FIONA CARR,2 MARTINA VOKURKOVA,1 MARTIN W. McBRIDE,1 JAROSLAV KUNEŠ,1 AND ANNA F. DOMINICZAK2

1Institute of Physiology, Academy of Sciences of the Czech Republic, Center for Experimental Research of Cardiovascular Diseases, CZ-142 20 Prague, Czech Republic; and 2Department of Medicine and Therapeutics, Gardner Institute, Western Infirmary, Glasgow G11 6NT, United Kingdom

Received 21 February 2001; accepted in final form 5 June 2001

Zicha, Josef, Cervantes D. Negrin, Zdenka Dobešová, Fiona Carr, Martina Vokurková, Martin W. McBride, Jaroslav Kuněš, and Anna F. Dominiczak. Altered Na\(^{+}\)-K\(^{+}\) pump activity and plasma lipids in salt-hypertensive Dahl rats: relationship to Atp1a1 gene. Physiol Genomics 6: 99–104, 2001.—A genetic variant of the gene for the α\(_{1}\)-isoform of Na\(^{+}\)-K\(^{+}\)-ATPase (Atp1a1) was suggested to be involved in the pathogenesis of salt hypertension in Dahl rats through altered Na\(^{+}\)-K\(^{+}\) coupling ratio. We studied Na\(^{+}\)-K\(^{+}\) pump activity in erythrocytes of Dahl salt-sensitive (SS/Jr) rats in relation to plasma lipids and blood pressure (BP) and the linkage of polymorphic microsatellite marker D2Arb18 (located within intron 1 and exon 2 of Atp1a1 gene) with various phenotypes in 130 SS/Jr × SR/Jr F\(_{2}\) rats. Salt-hypertensive SS/Jr rats had higher erythrocyte Na\(^{+}\) content, enhanced ouabain-sensitive (OS) Na\(^{+}\) and Rb\(^{+}\) transport, and higher Na\(^{+}\)-Rb\(^{+}\) coupling ratio of the Na\(^{+}\)-K\(^{+}\) pump. BP of F\(_{2}\) hybrids correlated with erythrocyte Na\(^{+}\) content, OS Na\(^{+}\) extrusion, and OS Na\(^{+}\)-Rb\(^{+}\) coupling ratio, but not with OS Rb\(^{+}\) uptake. In F\(_{2}\) hybrids there was a significant association indicating suggestive linkage (P < 0.005, LOD score 2.5) of an intragenic marker D2Arb18 with pulse pressure but not with mean arterial pressure or any parameter of Na\(^{+}\)-K\(^{+}\) pump activity (including its Na\(^{+}\)-Rb\(^{+}\) coupling ratio). In contrast, plasma cholesterol, which was elevated in salt-hypertensive Dahl rats and which correlated with BP in F\(_{2}\) hybrids, was also positively associated with OS Na\(^{+}\) extrusion. The abnormal Na\(^{+}\)-K\(^{+}\) stoichiometry of the Na\(^{+}\)-K\(^{+}\) pump is a consequence of elevated erythrocyte Na\(^{+}\) content and suppressed OS Rb\(^{+}\)-K\(^{+}\) exchange. In conclusion, abnormal cholesterol metabolism but not the Atp1a1 gene locus might represent an important factor for both high BP and altered Na\(^{+}\)-K\(^{+}\) pump function in salt-sensitive Dahl rats. F\(_{2}\) hybrids; erythrocyte ion transport; erythrocyte sodium content; plasma cholesterol; rat chromosome 2; α\(_{1}\)-Na\(^{+}\)-K\(^{+}\)-ATPase gene

SALT-SENSITIVE DAHL RATS SUBJECTED to excess salt ingestion represent a salt-dependent form of genetic hypertension. Numerous phenotypic differences were identified in both outbred and inbred Dahl rats (24), and several candidate genes were tested for cosegregation with blood pressure (BP) (25). Genetic studies found multiple quantitative trait loci (QTLs) linked with high BP of salt-hypertensive Dahl rats (6, 26).

Among multiple ion transport alterations reported in this rat strain (16, 18, 22, 28, 34), enhanced active transport by the Na\(^{+}\)-K\(^{+}\) pump and augmented passive membrane permeability for Na\(^{+}\) and Rb\(^{+}\) (K\(^{+}\) ions (cation leaks) were described in erythrocytes of these hypertensive animals (36, 39). Our recent study (38) revealed that ouabain-sensitive (OS) Na\(^{+}\) transport mediated by the Na\(^{+}\)-K\(^{+}\) pump, but not Na\(^{+}\) or Rb\(^{+}\) leaks, correlated with BP of young salt-loaded F\(_{2}\) hybrids obtained from the cross of salt-sensitive (SS/Jr) and salt-resistant (SR/Jr) Dahl rats.

The acceleration of OS Na\(^{+}\) and Rb\(^{+}\) (K\(^{+}\)) transport in erythrocytes of salt-hypertensive SS/Jr rats is accompanied by the abnormal Na\(^{+}\)-Rb\(^{+}\) (K\(^{+}\)) coupling ratio of the Na\(^{+}\)-K\(^{+}\) pump (38), which was suggested to play an important role in the pathogenesis of salt hypertension (21). This characteristic change in the function of the Na\(^{+}\)-K\(^{+}\) pump in the SS/Jr strain has already been reported by Canessa et al. (1) who ascribed it to the possible functional consequences of a single amino acid substitution in α\(_{1}\)-isoform of Na\(^{+}\)-K\(^{+}\)-ATPase (13) resulting from point mutation Q276L in the respective gene located on rat chromosome 2 (30). Recently, Herrera et al. (14) described that the transfer of R allele of this gene from salt-resistant Dahl rats into salt-sensitive animals attenuated the development of salt hypertension.

However, salt-hypertensive Dahl rats are characterized by hypercholesterolemia (4, 19) and elevated plasma triglyceride levels (20, 29). Our previous studies in genetically hypertensive rats with dyslipidemia (37) confirmed the importance of altered lipid metabolism for the occurrence of ion transport abnormalities in hypertension (for review, see Ref. 41). Epidemiological studies in humans indicated the dependence of OS Na\(^{+}\) transport on circulating triglycerides and cholesterol (8, 15), which might be due to lipid-dependent changes in Na\(^{+}\)-K\(^{+}\) pump kinetics (9). In fact, the in vitro modifications of cholesterol content in the mem-
brane of human erythrocytes (2, 17) influenced Na\(^+\)-K\(^+\) pump activity through the characteristic modulation of its kinetic parameters.

The aims of our study were 1) to describe the alterations of OS Na\(^+\) and Rb\(^+\) (K\(^+\)) transport in erythrocytes of salt-hypertensive SS/Jr Dahl rats and 2) to determine their possible association with BP and plasma lipids (triglycerides, cholesterol) in young salt-loaded SS/Jr × SR/Jr F\(_2\) hybrids. Using this set of F\(_2\) hybrids, we have tested a hypothesis that OS ion transport alterations and/or high BP are related to the polymorphism of the gene for \(\alpha_1\)-isoform of Na\(^+\)-K\(^+\)-ATPase (\textit{Atp1a1}) in salt-sensitive Dahl rats. We have also studied several markers located close to the proposed BP QTLs on chromosome 2 (26). Our studies were carried out in young rats, which are generally more susceptible to develop salt hypertension (40). This is also true for immature salt-sensitive Dahl rats in which high salt intake induced greater BP rise (5, 36) and more pronounced ion transport abnormalities (36, 39) than in adult animals.

**MATERIALS AND METHODS**

**Animals.** The experiments were carried out in age-matched male animals of two inbred strains, Dahl salt-sensitive (SS/Jr) and salt-resistant (SR/Jr) rats, from our own colony (initial breeding pairs were kindly provided by Dr. J. P. Rapp). The animals were housed under standard laboratory conditions (temperature 23 ± 1°C, 12-h light-dark cycle), drank tap water ad libitum and were fed either a low-salt (0.3% NaCl) or high-salt diet (8% NaCl) for 5 wk after weaning (at the age of 4 wk). The genetic analysis was carried out in SS/Jr × SR/Jr F\(_2\) hybrids (\(n = 130\)) fed a high-salt diet (8% NaCl) for 5 wk after weaning.

All procedures and experimental protocols were approved by the Ethical Committee of the Institute of Physiology, Academy of Sciences of the Czech Republic, and conform to the American Physiological Society’s Guiding Principles for Research Involving Animals and Human Beings.

**Experimental protocol.** On the day of experiment, BP was measured under light ether anesthesia by a direct puncture of the carotid artery (using Statham pressure transducer and Hewlett-Packard recorder). Hematocrit, hemoglobin, and cell Na\(^+\) and K\(^+\) content were determined in heparinized blood withdrawn from the abdominal aorta. Plasma triglycerides and total cholesterol levels were determined under light ether anesthesia by a direct puncture of the abdominal aorta.

**Ouabain experiments.** Ouabain (5 mmol/l) was used to inhibit Na\(^+\)-K\(^+\) pump activity through the characteristic modulation of its kinetic parameters.

**Materials and methods.** Cation transport mediated by the Na\(^+\)-K\(^+\) pump was studied as described in details elsewhere (37). Brieﬂy, erythrocytes were washed three times with incubation medium (in mmol/l: 140 NaCl, 5 glucose, 2.5 phosphoric acid, 10 MOPS, pH 7.4 at 37°C, 310 mosmol/l) and incubated in this medium supplemented with 3.5 mmol/l RbCl for 30 min at 37°C. Net Na\(^+\) movements and unidirectional Rb\(^+\) (K\(^+\)) fluxes were measured from the changes of cell Na\(^+\) and Rb\(^+\) content occurring during the incubation. OS ion transport was assessed at intracellular Na\(^+\) and extracellular Rb\(^+\) (K\(^+\)) concentrations that were close to those found in vivo. Ouabain (5 mmol/l) was used to inhibit OS Na\(^+\) net extrusion and Rb\(^+\) uptake, from which Na\(^+\)-Rb\(^+\) coupling ratio of the Na\(^+\)-K\(^+\) pump was calculated using a formula, i.e., Na:K ratio = OS Na\(^+\) extrusion/OS Rb\(^+\) uptake. The rate of OS exchange of extracellular Rb\(^+\) for intracellular K\(^+\) (1 Rb\(^+\) c/o 1 K\(^+\)) was calculated as follows: OS Rb\(^+\)-K\(^+\) exchange = OS Rb\(^+\) uptake − 2/3 OS Na\(^+\) extrusion. Red cell cation contents and transport rates were expressed per mean cell hemoglobin content found in particular animals.

**Genetic markers and genotyping.** Genomic DNA was extracted from livers of parental Dahl SS/Jr, Dahl SR/Jr, and SS/Jr × SR/Jr F\(_2\) animals by serial phenol-chloroform extraction. The molecular markers used in the current study consisted of polymorphic microsatellite markers typed by PCR. PCR primers were ﬂuorescently labeled (MWG-Biotech). Hotstar PCR was carried out using a thermal cycler (model PTC100, MJ Research) at 94°C for 15 min, 35 cycles of 1 min 94°C, 1 min at 55°C, then 72°C for 2 min with HOTSTAR enzyme (Qiagen). This was followed by a 30-min incubation at 60°C. PCR ampliﬁcation products were resolved on the ABI 377 DNA sequencer (Applied Biosystems, Foster City, CA), using Genescan 3.1 and Genotyper 2.1 software packages (Applied Biosystems).

We have studied nine microsatellite markers located on chromosome 2 (marked in Fig. 1) that were polymorphic in progenitor Dahl strains. These markers included intragenic marker D2Arb18 located within intron 1 and exon 2 of \(\alpha_1\)-Na\(^+\)-K\(^+\)-ATPase gene, markers located near to this gene (D2Rat49 and D2Mit14), markers close to BP1 (D2Rat52, D2Rat237, and D2Rat157) or BP2 loci (D2Mit6), and some more distant markers (D2Wox5 and D2Wox13).

**Fig. 1.** Composite genetic map of rat chromosome 2. Genes are indicated in italics, and markers investigated in the current study are indicated in bold.
Table 1. Body weight, basal blood pressure, plasma lipids, erythrocyte sodium content, and red cell ouabain-sensitive ion transport in SR/Jr and SS/Jr male rats fed either LS or HS diet for 5 wk from the age of 4 wk

<table>
<thead>
<tr>
<th></th>
<th>SR/Jr</th>
<th>HS</th>
<th>SS/Jr</th>
<th>HS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body wt, g</td>
<td>218 ± 9</td>
<td>225 ± 8</td>
<td>271 ± 10†</td>
<td>201 ± 20+</td>
</tr>
<tr>
<td>Systolic BP, mmHg</td>
<td>137 ± 3</td>
<td>138 ± 3</td>
<td>159 ± 3†</td>
<td>189 ± 6†</td>
</tr>
<tr>
<td>Mean BP, mmHg</td>
<td>113 ± 2</td>
<td>107 ± 4</td>
<td>131 ± 3†</td>
<td>153 ± 7†</td>
</tr>
<tr>
<td>Diastolic BP, mmHg</td>
<td>91 ± 3</td>
<td>85 ± 4</td>
<td>108 ± 3†</td>
<td>124 ± 6†</td>
</tr>
<tr>
<td>Pulse pressure, mmHg</td>
<td>46 ± 3</td>
<td>53 ± 3</td>
<td>52 ± 2</td>
<td>64 ± 5+</td>
</tr>
<tr>
<td>Plasma triglycerides, mmol/l</td>
<td>0.43 ± 0.11</td>
<td>0.61 ± 0.16</td>
<td>1.20 ± 0.16†</td>
<td>1.49 ± 0.29†</td>
</tr>
<tr>
<td>Plasma cholesterol, mmol/l</td>
<td>1.81 ± 0.18</td>
<td>1.88 ± 0.07</td>
<td>2.05 ± 0.12</td>
<td>2.90 ± 0.35††</td>
</tr>
<tr>
<td>Erythrocyte Na+, mmol/l RBC</td>
<td>3.38 ± 0.05</td>
<td>3.40 ± 0.06</td>
<td>3.50 ± 0.05</td>
<td>3.77 ± 0.04††</td>
</tr>
<tr>
<td>OS Na+ net extrusion, mmol·l⁻¹·h⁻¹</td>
<td>3.50 ± 0.09</td>
<td>3.48 ± 0.10</td>
<td>3.91 ± 0.10†</td>
<td>5.48 ± 0.44††</td>
</tr>
<tr>
<td>OS Rb⁺ uptake, mmol·l⁻¹·h⁻¹</td>
<td>4.28 ± 0.13</td>
<td>4.26 ± 0.13</td>
<td>4.47 ± 0.13</td>
<td>6.08 ± 0.56††</td>
</tr>
<tr>
<td>OS Na⁺:Rb⁺ ratio</td>
<td>0.83 ± 0.03</td>
<td>0.82 ± 0.03</td>
<td>0.89 ± 0.04</td>
<td>0.93 ± 0.03†</td>
</tr>
<tr>
<td>OS Rb⁺:K⁺ exchange, mmol·l⁻¹·h⁻¹</td>
<td>1.94 ± 0.13</td>
<td>1.93 ± 0.16</td>
<td>1.87 ± 0.16</td>
<td>2.43 ± 0.27</td>
</tr>
<tr>
<td>OS Rb⁺:K⁺ exchange, % OS Rb⁺ uptake</td>
<td>44.8 ± 1.9</td>
<td>44.6 ± 2.4</td>
<td>41.0 ± 2.5</td>
<td>38.1 ± 1.3†</td>
</tr>
</tbody>
</table>

Values are means ± SE. BP, blood pressure; SR/Jr, Dahl salt-resistant rats; SS/Jr, Dahl salt-sensitive rats; HS, high salt (8% NaCl); LS, low salt (0.34% NaCl); RBC, red blood cells; and OS, ouabain-sensitive. †Significantly different (P < 0.05) from LS rats. ††Significantly different (P < 0.05) from SR/Jr rats.

Genetic linkage analysis. We evaluated the linkage between particular phenotypes and the genetic loci examined, by using a comparison of Dahl SS homozygotes, SR heterozygotes, and RR homozygotes by means of ANOVA test. Microsatellite markers were mapped relative to each other by using the MAPMAKER/EXP 3.0 computer package with an erasure detection procedure. Genetic distances were calculated with the Haldane mapping function. QTLs affecting a given phenotype were mapped relative to the genetic markers by using the MAPMAKER/QTIL 1.1 computer package obtained from Dr. Eric Lander (Whitehead Institute, Cambridge, MA).

Statistical analysis. Results are expressed as means ± SE. The statistical differences between particular groups or genotypes were evaluated by one-way ANOVA followed by the least significant difference test. Linear correlation analysis was used to evaluate relationship of ion transport parameters with BP or plasma lipids. Holm’s sequentially rejective multiple test procedure was used to adjust the level of significance of individual test for the number of comparisons being made.

RESULTS

Progenitors. Salt-sensitive Dahl rats fed a high-salt diet had substantially elevated BP (both systolic and diastolic), augmented pulse pressure, increased erythrocyte Na⁺ content and elevated plasma cholesterol and triglycerides compared with salt-loaded SR/Jr rats which remained normotensive (Table 1). OS Na⁺ net extrusion and Rb⁺ uptake mediated by the Na⁺-K⁺ pump were enhanced in erythrocytes of salt-hypertensive SS/Jr rats in which a significantly higher Na⁺:Rb⁺ coupling ratio of the Na⁺-K⁺ pump was demonstrated. Although the absolute rate of OS 1 Rb⁺, K⁺ exchange tended to be increased, its relative contribution to total OS Rb⁺ uptake was significantly decreased in salt-hypertensive SS/Jr rats (Table 1).

F₂ hybrids. Both systolic and diastolic BP of young salt-loaded SS/Jr × SR/Jr F₂ hybrids correlated with plasma triglycerides (r = 0.32, P < 0.001 and r = 0.19, P < 0.05, respectively) and total cholesterol levels (r = 0.54, P < 0.001 and r = 0.24, P < 0.01, respectively). Table 2 shows the relationships of erythrocyte Na⁺ content and OS ion transport parameters to diastolic BP and plasma cholesterol (similar results were obtained for systolic BP, data not shown). It is evident that BP correlated with both erythrocyte Na⁺ content and OS Na⁺ net extrusion, but it had no significant relationship to OS Rb⁺ uptake. There was a significant correlation between BP and the Na⁺:Rb⁺ coupling ratio of the Na⁺-K⁺ pump in erythrocytes of young salt-loaded Dahl F₂ hybrids. The apparent discrepancy between OS Na⁺ and Rb⁺ (K⁺) movements might be explained by the existence of another mode of Na⁺-K⁺ pump action, i.e., OS 1 Rb⁺, K⁺ exchange. This mode of pump action is suppressed in hypertensive F₂ hybrids as evidenced by its borderline negative correlation with diastolic BP (r = −0.176, P < 0.05). OS Na⁺ net extrusion also correlated positively with plasma cholesterol, but this was not the case for OS Rb⁺ uptake and OS Na⁺:Rb⁺ coupling ratio (Table 2).

Genetic linkage analysis. Table 3 evaluates putative cosegregation of measured phenotypes with the genotype at selected loci on rat chromosome 2. The results obtained in our cohort of young salt-loaded SS/Jr ×
Table 3. Cosegregation of blood pressure, plasma cholesterol level, erythrocyte Na⁺ content, and particular parameters of ouabain-sensitive ion transport with alleles of selective loci on rat chromosome 2 in male F2 population raised on HS diet from the age of 4 wk

<table>
<thead>
<tr>
<th>Genotype</th>
<th>n</th>
<th>MAP, mmHg</th>
<th>PP, mmHg</th>
<th>CHOL, mmol/l</th>
<th>Na⁺, mmol/l</th>
<th>OS Na⁺, mmol-l⁻¹·h⁻¹</th>
<th>OS Rb⁺, mmol-l⁻¹·h⁻¹</th>
<th>OS Na⁺:Rb⁺ Ratio</th>
<th>OS Rb⁺:K⁺ Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>D2Arb18</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RR</td>
<td>33</td>
<td>135 ± 3</td>
<td>56 ± 2</td>
<td>1.65 ± 0.06</td>
<td>3.62 ± 0.04</td>
<td>3.92 ± 0.12</td>
<td>4.70 ± 0.09</td>
<td>0.84 ± 0.03</td>
<td>2.08 ± 0.11</td>
</tr>
<tr>
<td>SR</td>
<td>36</td>
<td>136 ± 2</td>
<td>65 ± 2</td>
<td>1.78 ± 0.05</td>
<td>3.74 ± 0.03</td>
<td>3.93 ± 0.08</td>
<td>4.90 ± 0.07</td>
<td>0.81 ± 0.02</td>
<td>2.28 ± 0.08</td>
</tr>
<tr>
<td>SS</td>
<td>31</td>
<td>133 ± 2</td>
<td>66 ± 2†</td>
<td>1.82 ± 0.06</td>
<td>3.69 ± 0.05</td>
<td>3.93 ± 0.10</td>
<td>4.69 ± 0.09</td>
<td>0.85 ± 0.02</td>
<td>2.07 ± 0.10</td>
</tr>
<tr>
<td>F test</td>
<td>NS</td>
<td>P &lt; 0.005</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D2Mit6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RR</td>
<td>31</td>
<td>138 ± 3</td>
<td>61 ± 3†</td>
<td>1.85 ± 0.07</td>
<td>3.75 ± 0.05</td>
<td>3.91 ± 0.10</td>
<td>4.74 ± 0.12</td>
<td>0.84 ± 0.03</td>
<td>2.14 ± 0.12</td>
</tr>
<tr>
<td>SR</td>
<td>70</td>
<td>136 ± 2</td>
<td>65 ± 2</td>
<td>1.77 ± 0.04</td>
<td>3.69 ± 0.03</td>
<td>3.89 ± 0.07</td>
<td>4.83 ± 0.07</td>
<td>0.81 ± 0.02</td>
<td>2.24 ± 0.08</td>
</tr>
<tr>
<td>SS</td>
<td>28</td>
<td>131 ± 3</td>
<td>58 ± 2</td>
<td>1.64 ± 0.07</td>
<td>3.66 ± 0.05</td>
<td>4.03 ± 0.13</td>
<td>4.77 ± 0.08</td>
<td>0.85 ± 0.03</td>
<td>2.08 ± 0.11</td>
</tr>
<tr>
<td>F test</td>
<td>NS</td>
<td>NS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D2Wox5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RR</td>
<td>36</td>
<td>140 ± 3</td>
<td>64 ± 2</td>
<td>1.89 ± 0.06</td>
<td>3.75 ± 0.05</td>
<td>3.88 ± 0.11</td>
<td>4.78 ± 0.10</td>
<td>0.82 ± 0.02</td>
<td>2.19 ± 0.10</td>
</tr>
<tr>
<td>SR</td>
<td>67</td>
<td>133 ± 2</td>
<td>63 ± 2</td>
<td>1.70 ± 0.04</td>
<td>3.70 ± 0.03</td>
<td>3.88 ± 0.08</td>
<td>4.88 ± 0.07</td>
<td>0.80 ± 0.02†</td>
<td>2.29 ± 0.08‡</td>
</tr>
<tr>
<td>SS</td>
<td>27</td>
<td>135 ± 4</td>
<td>59 ± 2</td>
<td>1.72 ± 0.07</td>
<td>3.63 ± 0.04</td>
<td>4.08 ± 0.11</td>
<td>4.60 ± 0.08</td>
<td>0.89 ± 0.03§</td>
<td>1.88 ± 0.11§</td>
</tr>
<tr>
<td>F test</td>
<td>NS</td>
<td>NS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE; n = number of animals of a given genotype. MAP, mean arterial pressure; PP, pulse pressure; CHOL, plasma cholesterol; Na⁺, erythrocyte Na⁺ content; OS Na⁺, ouabain-sensitive Na⁺ net extrusion; OS Rb⁺, total ouabain-sensitive Rb⁺ uptake; OS Na⁺:Rb⁺, coupling ratio of the Na⁺-K⁺ pump; OS Rb⁺:K⁺, ouabain-sensitive Rb⁺:K⁺ exchange. Significantly different from rats with RR genotype: †P < 0.05, ‡P < 0.01. Significantly different from rats with SS genotype: §P < 0.01. NS, nonsignificant (P > 0.05).

SR/Jr F2 hybrids indicate that D2Arb18 was significantly associated with pulse pressure but not with mean arterial pressure. There was no significant relationship of this marker for Atp1a1 gene with erythrocyte Na⁺ content or any parameter of OS Na⁺ and Rb⁺ transport (including Na⁺:Rb⁺ coupling ratio of the Na⁺-K⁺ pump) (Table 3). Almost the same results were obtained for D2Rat49 and D2Mit14 (located in close vicinity of Gca and Atp1a1 genes), as well as for D2Rat52, D2Rat237, and D2Rat157 (located closer to Camk2d gene) (data not shown). A log of the odds ratio score (LOD) plot of rat chromosome 2 for pulse pressure reached peak values of about 2.5 (P < 0.005) between D2Rat49 and D2Arb18 (data not shown). However, these values are only suggestive of linkage but do not prove a significant linkage.

It should be mentioned that D2Mit6 was not significantly associated with any phenotype of interest (Table 3). On the other hand, D2Wox5 (and partially also D2Wox13) were related to the altered stoichiometry of the Na⁺-K⁺ pump. It is evident that F2 rats with SS genotype had higher OS Na⁺:Rb⁺ coupling ratio and decreased OS 1 Rb⁺:1 K⁻ exchange than the animals with the SR or RR genotype. This cannot be explained by the differences in plasma cholesterol, which tended to be lower in rats with the SR and SS genotype compared with RR rats (Table 3).

**DISCUSSION**

Our study demonstrated that plasma cholesterol, erythrocyte Na⁺ content, OS Na⁺ extrusion, and Na⁺:Rb⁺ coupling ratio of the Na⁺-K⁺ pump were elevated in young salt-hypertensive SS/Jr Dahl rats. All the above phenotypes correlated with systolic and diastolic BP in young salt-loaded SS/Jr × SR/Jr F2 hybrids. It should be pointed out that in our F2 hybrids not only BP and erythrocyte Na⁺ content but also OS Na⁺ extrusion and OS Na⁺:Rb⁺ coupling ratio were positively related to plasma cholesterol levels. In contrast, there was no significant linkage of the α₁-Na⁺-K⁺-ATPase locus with BP or any parameter of OS Na⁺ or Rb⁺ (K⁺) transport. Thus our data do not support the hypothesis that the polymorphism of Atp1a1 gene had a major impact on either Na⁺-K⁺ pump function or BP levels in Dahl rats.

Herrera and Ruiz-Opazo (13) reported 1079A→T point mutation in the Atp1a1 gene in the SS/Jr strain. This mutation would result in Q276L substitution in the ATPase protein with possible alterations in the function of the pump and increased reabsorption of Na⁺ in the kidney (13, 14, 21). Moreover, the genomic region containing Na⁺-K⁺-ATPase has been linked to hypertension is several F2 crosses (3, 7, 26, 31, 32). However, other groups failed to confirm the existence of this mutation using either direct sequence analysis of PCR-generated fragments from SS/Jr genomic DNA or Eae I digests of the fragments (33). More recently, Harris (12) used first nucleotide change analysis, a method that can detect point mutations in a mixed population of PCR products, even in the presence of PCR bias, and established that the SS/Jr strain and all other strains tested do not have 1079T transversion. Taken together with the current functional data, these results eliminate the putative A1079T transversion in Atp1a1 gene as a causative factor contributing to hypertension in genetically hypertensive rat strains and point to other candidate genes on rat chromosome 2. Lack of the association of Atp1a1 gene with basal BP is in accordance with the earlier findings of Rapp and Dene (27), who did not find a significant influence of α₁-Na⁺-K⁺-ATPase locus on systolic BP in three segregating populations of Dahl rats (derived from SS/Jr and SR/Jr rats) which were raised on 8% NaCl diet from weaning. It should, however, be noted that the genetic background of the normotensive control strain used in a given segregation study might substantially modify BP effects of particular genes as was demonstrated by Rapp and coworkers (7, 27).
There is an alternative possibility that high BP in Dahl rats could be caused by another gene(s) on rat chromosome 2, where two different BP QTLs were described (26). One of them, which comprises the gene for angiotensin II AT1 receptor (Agtr1b), was suggested to be associated with BP in several different F2 populations (3, 7, 11). However, we did not find any significant association of D2Mit6 (located within this BP QTL) with BP (or Na\(^{+}\)-K\(^{+}\) pump function) in our F2 cross. The other BP QTL on chromosome 2 is in the region spanning D2Mgh15 and D2Mit15, where the genes for guanylate cyclase A (Gca), \(\alpha_1\)-Na\(^{+}\)-K\(^{+}\)-ATPase isofrom (Atp1a1), and calmodulin-dependent protein kinase II-\(\beta\) (Camk2d) are located. Guanylate cyclase A locus was found to be associated with BP in SHR × BN recombinant inbred strains (23) and various F2 populations (31, 32) including salt-loaded SS/Jr and SS/Jr × MNS F2 hybrids (7). Camk2d locus, which is located at the opposite end of this BP QTL, is associated with the enhanced BP response to calcium channel antagonist in a backcross population of Lyon hypertensive rats (35).

Our data showed no significant linkage between systolic or diastolic BP and the putative BP locus close to Gca, Atpa1, and Camk2d genes, but there was a suggestive linkage of pulse pressure to D2Arb18 and D2Rat49 loci (LOD score = 2.5, \(P < 0.005\)). This seems in agreement with Clark et al. (3), who demonstrated the linkage of this locus with both systolic and diastolic BP in WKY × SHRSP F2 cross.

Our most surprising observation was the absence of a linkage between \(\alpha_1\)-Na\(^{+}\)-K\(^{+}\)-ATPase locus and the respective intermediate phenotype, i.e., OS ion transport in our young salt-loaded SS/Jr × SR/Jr F2 hybrids. Elevated Na\(^{+}\):Rb\(^{+}\) coupling ratio of the Na\(^{+}\)-K\(^{+}\) pump in SS/Jr rats was originally suggested (1) to be a consequence of altered function of \(\alpha_1\)-Na\(^{+}\)-K\(^{+}\)-ATPase isofrom due to a point mutation in its gene (13, 30). We have observed a tendency to higher OS Na\(^{+}\):Rb\(^{+}\) coupling ratio in SS/Jr rats fed a low-salt diet, but its values were significantly increased only in SS/Jr rats subjected to high salt intake (Table 1). Although we have demonstrated a positive correlation of OS Na\(^{+}\):Rb\(^{+}\) coupling ratio with diastolic BP in young salt-loaded Dahl F2 hybrids, there was no significant linkage of polymorphic markers (located within or close to Atp1a1 gene) with any parameter of OS ion transport (Table 3). Thus our data suggest that factors other than the Atp1a1 gene locus are responsible for the observed acceleration of OS ion transport and for the altered Na\(^{+}\):Rb\(^{+}\) stoichiometry of the Na\(^{+}\)-K\(^{+}\) pump in erythrocytes of salt-hypertensive SS/Jr rats.

The acceleration of OS Na\(^{+}\) net transport in salt-hypertensive Dahl rats, which also correlated with BP of our F2 hybrids, seems to result from lipid-dependent changes in the kinetic properties of the Na\(^{+}\)-K\(^{+}\) pump. The increased affinity \(K_m\) of the Na\(^{+}\)-K\(^{+}\) pump for internal Na\(^{+}\) together with the decrease of its maximal velocity \(V_{max}\) are responsible for the accelerated OS Na\(^{+}\) net extrusion seen in erythrocytes of young salt-hypertensive Dahl rats when studied at physiologically low values of erythrocyte Na\(^{+}\) content (39). The depletion of membrane cholesterol content in human erythrocytes augmented \(V_{max}\) and reduced \(K_m\) of the Na\(^{+}\)-K\(^{+}\) pump (2, 17). This implies that the kinetic changes of the Na\(^{+}\)-K\(^{+}\) pump disclosed in young salt-hypertensive Dahl rats (39) are probably due to membrane cholesterol enrichment. This is fully compatible with a positive correlation between OS Na\(^{+}\) net extrusion and plasma cholesterol in our F2 hybrids (Table 2).

The increased OS Na\(^{+}\):Rb\(^{+}\) coupling ratio in erythrocytes of salt-hypertensive Dahl rats reflects the relative attenuation of another mode of Na\(^{+}\)-K\(^{+}\) pump action, i.e., OS 1 Rb\(^{+}\)-1 K\(^{+}\) exchange, which has no net transport effects. The contribution of this mode to the total OS Rb\(^{+}\) uptake is decreased by high cell Na\(^{+}\) content, low extracellular K\(^{+}\) concentration, and elevated concentration of inorganic phosphate. The stepwise increase of cell Na\(^{+}\) content in erythrocytes of normotensive Sprague-Dawley rats was indeed accompanied by a major gradual rise of OS Na\(^{+}\):Rb\(^{+}\) coupling ratio (10). Both increased erythrocyte Na\(^{+}\) content and decreased plasma K\(^{+}\) levels, which are characteristic findings in salt-hypertensive SS/Jr rats (39), favor the reduction in the rate of OS 1 Rb\(^{+}\);1 K\(^{+}\) exchange. Its contribution to the total OS Rb\(^{+}\) uptake was significantly reduced not only in salt-hypertensive SS/Jr animals (Table 1) but also in hypertensive F2 rats, as indicated by the inverse relationship of OS 1 Rb\(^{+}\);1 K\(^{+}\) exchange to their diastolic BP. It is evident that the acceleration of OS Na\(^{+}\) net extrusion (the true transport mode of Na\(^{+}\)-K\(^{+}\) pump action driven by increased internal Na\(^{+}\) concentration) is accompanied by the attenuation of other modes of Na\(^{+}\)-K\(^{+}\) pump action that serve no net transport purpose.

In conclusion, abnormal cholesterol metabolism seems to be an important factor for both high BP and altered Na\(^{+}\)-K\(^{+}\) pump activity in salt-hypertensive Dahl rats. The causative role of a possible mutation within the gene for the \(\alpha_1\)-isoform of Na\(^{+}\)-K\(^{+}\)-ATPase was not confirmed, because the polymorphism at the intragenic marker was not associated with BP (both systolic and diastolic) or Na\(^{+}\)-K\(^{+}\) pump activity. Although our study favors the hypothesis of Orosz and Hopfer (21), that enhanced sodium reabsorption by accelerated Na\(^{+}\)-K\(^{+}\) pump activity might play an important role in salt hypertension development, our data suggest that the changes in Na\(^{+}\)-K\(^{+}\) pump function result from the alterations in lipid metabolism and/or Na\(^{+}\)/K\(^{+}\) distribution across the cell membrane.

It would be highly desirable to perform a total genome scan in this set of F2 hybrids to disclose particular QTLs for multiple phenotypes determined.

Statistical assistance of Niall H. Anderson and Jaroslav Vorlíček is acknowledged.

This work has been accomplished within the framework of EU-HYPGEN II Concerted Action of BIOMED2 Program (EC), with further support from the Grant Agency of the Czech Republic Research Grant 305/00/1638 (to Z. Dobesová) and British Heart Foundation Program Grant RG/97009 (to A. F. Dominiczak).

This work has been presented in a preliminary form during the symposium on Genetics of Experimental and Human Hypertension held in Toledo, OH, August 17–18, 2000.
REFERENCES


