Genetic control of renal thiazide receptor response to dietary NaCl and hypertension

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Fanestil, Darrell D., Duke A. Vaughn, Ronald H. Hyde, and Patricia Blakely. Genetic control of renal thiazide receptor response to dietary NaCl and hypertension. Am. J. Physiol. 276 (Regulatory Integrative Comp. Physiol. 45): R901–R904, 1999.—Excess NaCl increases blood pressure in some strains of animals but not others. An 8% NaCl diet did not change renal thiazide receptor (TZR) density in two salt-resistant normotensive rat strains (Wistar-Kyoto and Sprague-Dawley) [Fanestil, D. D., D. A. Vaughn, and P. Blakely. Am. J. Physiol. 273 (Regulatory Integrative Comp. Physiol. 42): R1241–R1245, 1997]. However, the renal response to salt differs in normal and hypertensive kidneys [Rettig, R., N. Bandelow, O. Patschan, B. Kuttler, B. Frey, and A. Uber. J. Hum. Hypertens. 10: 641–644, 1996]. Therefore, we examined two strains with salt-aggravated hypertension. Renal TZR did not change when Dahl-S (salt sensitive) animals became hypertensive with 8% dietary NaCl. In contrast, renal TZR decreased 34%, whereas blood pressure increased further, in SHR with 8% dietary NaCl. Blood pressure increased after N⁵-nitro-L-arginine in SHR, but renal TZR did not change, indicating the salt-induced decrease in TZR in SHR cannot be attributed nonspecifically to elevated arterial pressure. We conclude that the renal response to NaCl-induced increases in blood pressure can be genetically modulated independently of the genes that mediate either the primary hypertension or the salt sensitivity of the hypertension. This finding may be of use in future studies directed at identifying genotypes associated with salt-dependent hypertension.

SALT-SENSITIVE HYPERTENSION is influenced by genetic background. The two rat strains developed by Dahl (8), with blood pressure that is either salt sensitive (Dahl-S) or salt resistant (Dahl-R), are a classic example in rodents. The demonstration of salt-sensitive hypertension within substrains of spontaneously hypertensive rats (SHR) (14) may indicate that the phenomenon can be controlled by a few genes. Considerable evidence indicates that the kidneys have a prominent role in expressing this genetic predisposition in both Dahl and SHR strains (7, 16). We previously observed that the renal density of the thiazide-sensitive NaCl cotransporter [or thiazide diuretic receptor (TZR)] was greater after 4 wk of age in SHR than in Wistar-Kyoto (WKY) strains of animals (3). Moreover, in that study, we found that TZR was not altered in either kidney of animals with systemic arterial hypertension due to placement of a constriction clip on one renal artery (3), suggesting that renal TZR density does not respond to renal perfusion pressure. More recently, we reported (9) that renal handling of high salt intake (8% dietary NaCl) in normotensive strains of rats (Sprague-Dawley and WKY) did not involve changes in renal TZR (1, 15). Moreno et al. (13) simultaneously arrived at a similar conclusion, based on their finding that salt loading (2.92% dietary NaCl) did not alter renal expression of Na-Cl cotransporter mRNA. These observations do not exclude the possibility that salt-sensitive hypertension involves changes in renal TZR, because the renal response to salt differs in normal and hypertensive kidneys (16). Therefore, the current studies were undertaken to determine if the renal expression of salt-sensitive hypertension in rodent genetic hypertension might involve altered TZR. We report that the response of TZR to high dietary salt is aberrant in SHR but not in Dahl-S animals. Thus the renal response to an NaCl-induced increase in blood pressure is, at least in part, genetically mediated independently of the genes that mediate either the primary hypertension or the salt sensitivity of the hypertension.

METHODS

Male animals of the Dahl-S strain, purchased from Harlan Sprague Dawley, and SHR from the La Jolla colony, SHR-LJ, were maintained in the animal care facility at the University of California, San Diego (UCSD). All protocols and procedures were approved by the UCSD Animal Subjects Committee. Within each study, animals were of the same strain and age and, in addition, were matched by weight into control and experimental groups.

Sodium intake was varied by offering ad libitum access to one of two diets for 4 wk, beginning at 6 wk of age. The 1% NaCl diet (Teklad no. TD90220) contained 0.39% sodium, 0.72% potassium, 0.66% chloride, 0.86% calcium, and 0.15% magnesium, and the 8% NaCl diet (Teklad no. TD9023) contained 3.15% sodium, 0.71% potassium, 4.90% chloride, 0.86% calcium, and 0.14% magnesium.

The inhibitor of nitric oxide synthase, N⁵-nitro-L-arginine (L-NNA), 50 mg/l, was dissolved in distilled water. At 15 wk of age, SHR-L-NNA animals had free access to the drinking solution for the next 7 days, whereas age-matched control SHR animals ingested distilled water ad libitum.

Blood pressure was measured by indwelling arterial catheters, which were placed after 3 wk on the assigned diet (or 2 days before starting ingestion of L-NNA) using methodology described in detail previously (10). The reported mean arterial pressures were obtained the day or two before death at the end of the experimental period. Arterial blood, sampled via the indwelling catheter on the day of death, was analyzed by ion-selective electrodes for sodium, potassium, ionized calcium, and ionized magnesium. Plasma was analyzed for...
chloride using a commercially available kit (Sigma, St. Louis, MO).

Renal TZR density was determined by saturation analysis using the binding of [3H]metolazone to renal membranes, as previously described in detail (4): whole kidneys were homogenized in 10 ml ice-cold 50 mM Tris-PO4 buffer, pH 7.4, and membranes were prepared by centrifuging them for 5 min at 600 g and centrifuging the resulting supernatant two times at 45,000 g for 20 min. The final pellet was suspended in 10 ml buffer and diluted to achieve a final concentration of 0.8–1.0 mg protein/ml in the binding assay. Binding of [3H]metolazone to each membrane preparation was determined in duplicate at six concentrations of [3H]metolazone ranging from 0.313 to 10 nM. Specific binding of [3H]metolazone, defined by displacement with 10⁻² M hydroflumethiazide, was analyzed by the Scatchard equation to calculate the density of the binding using the EBDA program of McPherson (12). Protein was assayed by the Bradford Coomassie blue method (6), with bovine gamma globulin as the standard. Binding maximum is reported as picomoles of binding sites per milligram of membrane protein.

Statistical analyses were conducted with the StatView program (Abacus Concepts, Berkeley, CA) using Student’s unpaired t-test. Values are expressed as means ± SE.

RESULTS

Dietary NaCl in Dahl-S hypertensive rats. Mean arterial blood pressure in the Dahl-S animals ingesting 1% NaCl (145 ± 1.33 mmHg) was significantly (P < 0.0001) less than that in rats ingesting 8% NaCl (209 ± 8.93 mmHg) (Fig. 1). The plasma concentration of chloride was significantly higher (P = 0.001) in the 8% NaCl group (100.2 ± 0.458 mmol/l) than in the 1% NaCl group (97.1 ± 0.474 mmol/l). The animals ingesting 8% NaCl did not differ from the 1% NaCl animals with respect to final body weight or the plasma concentrations of sodium, potassium, ionized calcium, and ionized magnesium. Single kidney weight in the 8% NaCl group (0.554 ± 0.016 g/100 g body wt) was significantly (P < 0.0001) greater than in the 1% NaCl group (0.393 ± 0.010 g/100 g body wt). However, renal TZR density was not altered by dietary NaCl intake (1% = 1.05 ± 0.057 pmol/mg renal membrane protein, 8% = 1.09 ± 0.031 pmol/mg renal membrane protein; P not significant) (Fig. 2).

Dietary NaCl in SHR. Mean arterial blood pressure in the SHR animals ingesting 1% NaCl (137 ± 5.08 mmHg) was significantly (P < 0.0001) less than in those ingesting 8% NaCl (202 ± 9.07 mmHg) (Fig. 1). The plasma concentration of ionized calcium was significantly lower (P = 0.02) in the 8% NaCl group (1.08 ± 0.025 mmol/l) than in the 1% NaCl group (1.16 ± 0.013 mmol/l). The animals ingesting 8% NaCl did not differ from the 1% NaCl animals with respect to final body weight or the plasma concentrations of sodium, potassium, ionized calcium, and ionized magnesium.

Table 1. Values in SHR after L-NNA

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>L-NNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>9</td>
<td>8</td>
</tr>
<tr>
<td>Renal thiazide receptor</td>
<td>0.923 ± 0.058</td>
<td>1.08 ± 0.046*</td>
</tr>
<tr>
<td>Sodium</td>
<td>145.9 ± 0.261</td>
<td>143.0 ± 0.824†</td>
</tr>
<tr>
<td>Potassium</td>
<td>3.95 ± 0.063</td>
<td>4.43 ± 0.152‡</td>
</tr>
<tr>
<td>Chloride</td>
<td>99.0 ± 0.425</td>
<td>95.9 ± 1.21†</td>
</tr>
<tr>
<td>Ionized calcium</td>
<td>1.19 ± 0.012</td>
<td>1.17 ± 0.023</td>
</tr>
</tbody>
</table>

Values are means ± SE in mmol/l except N⁶-nitro-L-arginine (L-NNA) group renal thiazide receptor, in pmol/mg protein. SHR, spontaneously hypertensive rat. *P = 0.0513, †P = 0.05 vs. control.
sium, chloride, and ionized magnesium. Single kidney weight in the 8% NaCl group (0.517 ± 0.018 g/100 g body wt) was significantly (P = 0.0001) greater than in the 1% NaCl group (0.400 ± 0.007 g/100 g body wt). In contrast, renal TZR density was decreased by increasing dietary NaCl intake (1% = 0.803 ± 0.22 pmol/mg protein, 8% = 0.531 ± 0.056 pmol/mg protein; P = 0.0012) (Fig. 2).

Response to inhibition of nitric oxide synthase in SHR. Inhibition of nitric oxide synthase by administration of L-NNA for 7 days increased mean arterial blood pressure in SHR to 233 ± 3.50 mmHg (n = 6) versus 181 ± 2.71 mmHg (n = 7; P < 0.0001) in the control SHR animals. Plasma sodium and chloride concentrations were slightly lower, whereas the plasma potassium concentration was significantly higher in the L-NNA group (Table 1). The 17% increase in renal TZR density associated with the increase in blood pressure produced in SHR by L-NNA, which represented a marginal level of statistical significance (P = 0.0513; Table 1), contrasts with the 34% decrease in TZR associated with the increase in blood pressure produced in SHR by the 8% NaCl diet (Fig. 2).

**DISCUSSION**

The renal response to ingestion of a diet containing 8% NaCl in normotensive rats (Sprague-Dawley and WKY strains) in a prior study did not involve a change in the renal density of TZR (9). Concurrently, Moreno et al. (13) used different methodology to arrive at a similar conclusion. Our current study tested for derangements in TZR in salt-loaded, salt-sensitive hypertensive animals. These studies were based on the knowledge that hypertensive SHR have greater renal TZR density than do WKY (3) and the findings that the renal adjustments to excretion of salt are abnormal in salt-sensitive hypertension (16). Dahl-S animals developed severe hypertension (209 mmHg) with, as in normotensive strains, no change in renal TZR density (Figs. 1 and 2). In contrast, salt-induced hypertension of similar magnitude (202 mmHg) in SHR was accompanied by a 34% decrease in renal TZR (Figs. 1 and 2). Salt loading increased renal weight in both Dahl and SHR strains to a similar extent (single kidney weights per 100 g body wt were not significantly different between Dahl and SHR on either salt intake). Thus the decrease in TZR density in SHR versus Dahl cannot be attributed to differential changes in overall renal size. A decrease in TZR of 34% if expressed as reduced reabsorption of NaCl at its locus in the distal convoluted tubule (5, 15), would facilitate urinary excretion of the excess dietary NaCl in SHR. Following this line of reasoning leads to the presumption that normotensive rat strains and the Dahl-S strain, when presented with a large dietary NaCl load, decrease reabsorption of NaCl predominantly in nephron segments other than the distal convoluted tubule. In this regard, it is of interest to note that thiazide treatment 1) prevents salt-dependent hypertension in Dahl-S animals (11, 18, 19) but 2) does not prevent salt-dependent (4% dietary NaCl) strokes in stroke-prone SHR (17). Although studies on the efficacy of thiazides on salt-accelerated hypertension in non-stroke-prone SHR are not available, thiazide diuretic treatment of SHR on normal diets has not yielded consistent lowering of blood pressure (reviewed in Ref. 2).

Blood pressure also increased markedly in SHR animals ingesting L-NNA, an inhibitor of nitric oxide synthase. The absolute level of mean arterial blood pressure (233 ± 3.50 mmHg) slightly exceeded that measured in SHR ingesting high salt (202 ± 9.07, P < 0.01) in the prior study, perhaps due to the older age of the animals used in the L-NNA study. However, the increase in blood pressure in SHR vs. L-NNA in SHR was not accompanied by a decrease in renal TZR. This finding of a lack of effect of arterial pressure on renal TZR is similar to our prior finding that renal perfusion pressure did not alter TZR in animals with two-kidney, one-clip hypertension (3). We propose that the decrease in renal TZR found in NaCl-loaded SHR is not secondary to a nonspecific effect produced by any increase in arterial pressure.

**Perspectives**

The renal response of salt-resistant normotensive [Sprague-Dawley and WKY in our prior study (9), Wistar in another study (13)] and salt-sensitive Dahl-S rat strains to high NaCl (8% NaCl) intake does not include a decrease in renal TZR, despite the differing effects of the salt on blood pressure. In contrast, renal TZR in the SHR strain is decreased 34% by NaCl-aggravated hypertension. A similar elevation of blood pressure in SHR produced by inhibition of nitric oxide synthase does not decrease renal TZR. These data provide evidence that renal responses to salt loading are not uniform and are therefore under genetic control that can be independent of renal responses to increased renal perfusion pressure. This observation 1) indicates that caution is needed when interpreting effects of dietary NaCl across strains of animals and 2) may be of use in future studies directed at identifying genotypes associated with salt-dependent hypertension.

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