Contribution of the Sympathetic Nervous System to the Hypertensive Effect of a High Sodium Diet in Stroke-Prone Spontaneously Hypertensive Rats

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SUMMARY In stroke-prone spontaneously hypertensive rats (SHRSP) plasma norepinephrine levels and vascular reactivity to norepinephrine are increased and intravascular volume is reduced during the developmental phase of hypertension. Since the accelerated rise in blood pressure following sodium-loading in SHRSP cannot be attributed to the volume-retaining properties of sodium, the effects of an increased dietary intake of sodium on biochemical parameters of sympathetic vascular tone were investigated. The following results were obtained. First, the increased reactivity of vascular smooth muscle was further augmented in sodium-treated SHRSP; the degree of supersensitivity was positively correlated to the plasma sodium concentration. After blockage of the neuronal uptake by 30 μM cocaine, no difference in vascular reactivity to norepinephrine was detected between SHRSP on a normal and SHRSP on a high-sodium diet. Second, the inactivation of norepinephrine by the neuronal uptake was impaired in rats on a high-sodium diet, the impairment being more pronounced in SHRSP than in Wistar-Kyoto (WKY) rats. This decreased inactivation could be expected to cause higher concentrations of the neurotransmitter at the receptor site if the transmitter release from the nerve ending remains constant. Third, the release of norepinephrine and epinephrine into the plasma was increased in sodium-loaded SHRSP but not in sodium-loaded WKY. Cold exposure exaggerates these differences between normotensive and hypertensive rats. These findings suggest that a high-sodium intake modifies the transmission of sympathetic impulses at the level of the nerve terminal in both WKY and SHRSP. In the normotensive rats, moderate impairment of norepinephrine inactivation, however, was balanced by an appropriate reduction in central sympathetic discharge following sodium-loading. In the hypertensive rats, the peripheral disturbance in norepinephrine inactivation due to sodium-loading was obviously not balanced by an adequate withdrawal of central sympathetic discharge. The resultant hemodynamic change was a further increase in the sympathetically mediated vasoconstriction, which is regarded as at least one of the main mechanisms of the sodium-dependent acceleration of hypertension in SHRSP. (Hypertension 4: 773-781, 1982)

KEY WORDS • sodium • stroke-prone spontaneously hypertensive rats • sympathetic activity • vascular reactivity • norepinephrine metabolism

The reciprocal relationship between intravascular volume and the degree of vasoconstriction is mediated by changes in the activity of vasopressor systems in response to changes in volume. Thus, salt-loading, which is accompanied by fluid volume expansion, leads to a suppression of the activity of vasopressor systems. Total peripheral resistance has been shown to decrease initially in salt-loaded normotensive animals and to rise only when the ability of the kidneys to excrete salt is impaired, e.g., after subtotal nephrectomy.

There are, however, experimental and clinical observations that may represent a disturbance in the normal regulatory mechanisms by which vasopressor activity is reduced to compensate for volume expansion: In the Brookhaven strain of rats (Dahl strain), dietary sodium-loading elicits a rise in blood pressure only in the "salt-sensitive" (DS) substrain but not in the salt-resistant (DR) rats. In DR rats, salt-loading is accompanied by a compensatory fall in total peripheral
resistance, whereas in DS rats salt-loading is accompanied by an increase in resistance. The exact mechanism of this different hemodynamic response to a high-sodium diet is as yet unknown. A similar inappropriate response of peripheral resistance to sodium-loading has been reported in humans with borderline hypertension.

The response of sympathetic activity to sodium-loading in experimental types of hypertension has been characterized by an increased turnover of norepinephrine combined with a decreased accumulation of tritiated norepinephrine due to a reduced storage capacity. More recently, neuronal uptake of norepinephrine has been demonstrated to be decreased in DOCA-salt hypertensive rats. On the basis of plasma catecholamines, this altered norepinephrine metabolism due to sodium-loading may not be detected under basal conditions but probably after a marked stimulation of norepinephrine release.

In stroke-prone spontaneously hypertensive rats (SHRSP), an increased alpha-adrenergic tone has been shown to be of pathogenetic significance in the rise in blood pressure. Sodium-loading in these animals leads to an accelerated rise in blood pressure. The aim of the present study was to evaluate possible interrelationships among sodium-loading, changes in intravascular volume, sympathetically mediated vasoconstriction, and changes in blood pressure in SHRSP. Information about the activity of the sympathetic nervous system in this study is based on: plasma norepinephrine and epinephrine concentrations under basal conditions and after stimulation; norepinephrine uptake and metabolism by peripheral sympathetic nerve endings; and the responsiveness of resistance vessels to the neurotransmitter norepinephrine. By this approach it should be possible to identify the mechanism of a sodium-dependent modulation in sympathetic vascular tone. Furthermore, differences in the response of these biochemical parameters between WKY and SHRSP following sodium-loading may help to characterize the genetic susceptibility to the deleterious effects of a high-sodium intake in rats with spontaneous hypertension.

Material and Methods

Female SHRSP, bred in Heidelberg for more than 6 years (F-23 generation) and age-matched WKY rats were fed either a standard pellet chow (sniff R, 100 mmole sodium/kg) or a high-sodium diet (1500 mmole sodium/kg). Rats were housed individually in Macrolon cages at a constant room temperature of 24±1°C and a humidity of 60±3%. The light was on from 6 a.m. to 6 p.m.

Blood Pressure and Plasma Volume

Systolic blood pressure was measured weekly under light ether anesthesia by means of tail plethysmography for 5 weeks. At the end of the experiment, mean arterial blood pressure was also recorded by means of chronically implanted arterial catheters connected to a Statham P23Db pressure transducer. Blood pressure tracings from conscious rats were recorded on a Gould Brush Recorder 2400, and values for mean arterial pressure were obtained from the computerized integration of the pressure signal (Brush pressure computer, Model 13-4214-04; Gould, Inc., Instruments System Division, Cleveland, Ohio).

To determine plasma volume, rats were anesthetized with ether, and injected with 0.10 to 0.12 ml/100 g body weight (the exact amount was measured gravimetrically with an analytical balance) of a 1% solution of Evans blue (E. Merck, Darmstadt, West Germany) into the saphenous vein. At 10 and 60 minutes later, 0.2 ml blood was collected from an incision in the tail, and plasma concentration of the dye was measured photometrically (Perkin Elmer, Hitachi Company).

Plasma Catecholamines

To determine the plasma norepinephrine and epinephrine, blood (0.5 ml) was collected from an arterial catheter that had been implanted into the femoral artery 1 day earlier. Blood was collected outside the cage by free flow from the distal end of the arterial catheter into cooled microtubes containing 5 USP heparin and immediately centrifuged; 200 μl 0.6 N perchloric acid was added to each 200 μl of plasma. The mixture was centrifuged, and the 100 μl aliquots of supernatant were frozen at −80°C. The concentrations of norepinephrine and epinephrine were assayed radioenzymatically in each aliquot. After collecting 0.5 ml blood for measuring basal catecholamine levels, rats (Group 2) were exposed to cold (4°C) in order to stimulate sympathetic activity. After 30 minutes of cold exposure, blood was again collected for the determination of plasma catecholamines.

Uptake of 3H-Norepinephrine

The activities of the neuronal and extraneuronal uptake of norepinephrine were determined in an isolated perfused heart preparation. Rats were anesthetized with thiobutabarbital (Inactin 50 mg/kg i.p.). The hearts were rapidly excised, weighed, and then perfused with an oxygenated Tyrode’s solution containing tritiated norepinephrine (1.0 nM, 0.01 μCi/ml) with a flow rate of 3 ml/min. After an equilibration period of 40 minutes, uptake of norepinephrine was determined with labelled norepinephrine only (10−6M) and subsequently during cumulative doses (10−8 and 10−7M, 10 minutes each) of unlabelled norepinephrine. Finally, neuronal uptake was blocked by the addition of cocaine (30 μM) and 40 minutes later a combined blockade of neuronal and extraneuronal uptake was achieved by adding corticosterone (100 μM). The concentration of labelled norepinephrine, of its deaminated metabolite 3,4-dihydroxyphenylglycol (DOPEG), and of its O-methylated metabolites normetanephrine (NMN), 3-methoxy-4-hydroxymandelic acid (VMA), and 3-methoxy-4-hydroxyphenylglycol (MOPEG), were measured in both perfusate and effluent following a separation procedure described by Graefe et al.
In an initial step, the catechol compounds were adsorbed on an Al₂O₃ column at pH 8.2. The O-methylated metabolites, which were found in the effluent, could be separated by a Dowex 50 W × 4 column into two fractions, one containing VMA and MOPEG (effluent), the other containing NMN (6 n HCl ethanol eluate). The compounds absorbed on Al₂O₃ were eluted by 0.2 M acetic acid (norepinephrine and DOPEG). Norepinephrine and DOPEG were separated by absorption of norepinephrine on a Dowex 50 W × 4 column. DOPEG was found in the effluent whereas norepinephrine was eluted by 2 n HCl. The value for the fractions obtained by liquid scintillation counting were corrected for quench, but not for recovery, since it was more than 90% in all fractions.

The rates of norepinephrine uptake and metabolism were calculated from the arteriovenous difference under steady-state conditions. The rates of neuronal uptake and metabolism were calculated from the differences before and after cocaine blockade. Extraneuronal uptake and metabolism were quantitated from the difference before and after additional blockade with corticosterone. Isolated Hindlimb Perfusion

Reactivity of the resistance vessels was studied in the isolated perfusion hindlimb preparation. After anesthesia with thiobutabarbital (Inactin, 50 mg/kg i.p.), the rats were prepared for hindlimb perfusion according to the method of Folkow et al. The hindlimbs were perfused at a constant temperature of 37°C and at pH 7.4 with an oxygenated Tyrode's solution (95% O₂, 5% CO₂) containing 2% (w/v) of an artificial colloid (Ficoll, Pharmacia Fine Chemicals, Freiburg, West Germany). The flow was kept constant (10 ml/min/100 g tissue) by a Harvard peristaltic pump (Harvard apparatus, Millis, Massachusetts). Changes in perfusion pressure as measured by a T-tube reflected changes in vascular resistance.

Cumulative dose-response curves to norepinephrine were established in a dose range from 10⁻²⁴ to 10⁻¹⁴M as final concentrations in the perfusate, starting from maximal dilatation. After establishing dose-response curves for norepinephrine and restoring maximum dilatation by rinsing with the perfusion medium, dose-response curves to norepinephrine were repeated in the presence of cocaine (30 μM).

**Statistics**

Results were expressed as means, ± SEM. Significance of differences between respective groups was assessed by analysis of variance in combination with Scheffe's test. Friedman's nonparametric test of paired values (χ²) was used to compare plasma catecholamines under different temperature conditions.

**Experimental Groups**

In Experiment 1, 15-week-old female SHRSP (n = 87) and weight-matched WKY rats (i.e., 14 weeks old, n = 96) were fed either a normal or a high-sodium diet for 30 days. Separate groups of rats were used for the determination of plasma volume, plasma catecholamine concentrations before and after cold stimulation, as well as for the hindlimb perfusion experiments with and without cocaine.

In Experiment 2, the metabolism of norepinephrine was assessed in 20-week-old female SHRSP (n = 8) and WKY (n = 18) after a period of 14 days of salt-loading.

In Experiment 3, plasma sodium concentration and vascular reactivity to norepinephrine were determined in 10-week-old female SHRSP after 2 weeks of a normal (100 mmole Na⁺/kg, n = 7), a high- (1500 mmole/kg, n = 7), or a low-sodium diet (<1 mmole/kg, n = 7).

**Results**

**Blood Pressure**

Table 1 illustrates the development of high blood pressure in SHRSP on a normal and a high-sodium diet during a salt-loading period of 30 days. As early as 5

<table>
<thead>
<tr>
<th>Days after dietary regimen</th>
<th>WKY (n = 15) Normal diet</th>
<th>WKY (n = 12) High-salt diet</th>
<th>SHRSP (n = 15) Normal diet</th>
<th>SHRSP (n = 13) High-salt diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>111.3 ± 3.4 ns</td>
<td>112.0 ± 4.5</td>
<td>115.4 ± 4.6 ns</td>
<td>154.7 ± 3.8</td>
</tr>
<tr>
<td>5</td>
<td>112.5 ± 3.5 ns</td>
<td>118.3 ± 2.2</td>
<td>159.3 ± 2.5 p &lt; 0.05</td>
<td>172.0 ± 4.1</td>
</tr>
<tr>
<td>30</td>
<td>120.5 ± 2.7 ns</td>
<td>121.4 ± 3.6</td>
<td>167.7 ± 4.0 p &lt; 0.001</td>
<td>216.4 ± 3.5</td>
</tr>
<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>108.5 ± 1.2 p &lt; 0.05</td>
<td>120.2 ± 2.4</td>
<td>161.1 ± 5.0 p &lt; 0.001</td>
<td>201.1 ± 7.5</td>
</tr>
<tr>
<td>Plasma volume (ml/100 g body weight)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>3.95 ± 0.05 ns</td>
<td>4.01 ± 0.05</td>
<td>3.80 ± 0.06 ns</td>
<td>3.89 ± 0.03</td>
</tr>
</tbody>
</table>
days after sodium-loading, the accelerated rise in blood pressure in SHRSP rats can be observed, as indicated by a mean increase in systolic blood pressure of 13 mm Hg above that of SHRSP rats on a normal-sodium diet. This differential pressure development was progressively greater with continuation of sodium-loading and reached 50 mm Hg after a further period of 25 days. In WKY rats, salt-loading did not affect systolic blood pressure significantly. Values for mean arterial blood pressure recorded in separate but identically treated groups revealed a similar increase in blood pressure in the sodium-loaded SHRSP, above the levels in SHRSP on a normal-sodium diet. A small but significant rise in mean arterial pressure was observed in sodium-loaded WKY controls, above the values obtained in WKY rats on a normal-sodium diet (table 1).

**Plasma Volume**

Table 1 shows the relative plasma volumes/100 g body weight at the end of the 30 days of sodium-loading in both WKY and SHRSP as compared with their respective controls on a normal sodium diet. Sodium-loading caused an expansion of plasma volume of about 3% in both WKY and SHRSP. This increase was already demonstrable 4 days after initiation of salt-loading (data not shown). Plasma volumes were, however, lower in SHRSP as compared to WKY on both the normal- and high-sodium diets.

**Plasma Catecholamines**

The concentrations of the plasma catecholamines norepinephrine and epinephrine are given in table 2. There were no significant changes under basal conditions in plasma epinephrine concentration after salt-loading in either WKY or SHRSP. Plasma norepinephrine concentrations, however, were higher in SHRSP on a normal-sodium diet as compared to SHRSP on a normal-sodium diet, whereas plasma levels tended to be lower in sodium-loaded WKY as compared to their controls.

The difference in plasma norepinephrine levels in response to salt-loading became more pronounced between SHRSP and WKY after exposure to cold (table 2). After such stimulation of sympathetic outflow, plasma norepinephrine was about two times higher in SHRSP on a high-sodium diet as compared with SHRSP on a normal diet. Moreover, plasma epinephrine concentration (table 2) did not rise in WKY, but increased by 150% in SHRSP on a normal intake and by 500% in SHRSP on a high-sodium diet.

**Reactivity of Vascular Smooth Muscle to Norepinephrine**

The dose response curve to norepinephrine without blockade of the neuronal uptake (fig. 1 left) showed a marked displacement to the left after sodium loading. This type of supersensitivity in response to sodium loading was abolished after cocaine blockade of the neuronal uptake of norepinephrine (fig. 1, right). As

### Table 2. Plasma Concentration of Norepinephrine and Epinephrine 30 Days after Start of Sodium-Loading in WKY and SHRSP

<table>
<thead>
<tr>
<th>Plasma concentration (ng/liter)</th>
<th>WKY (n = 12) Normal diet</th>
<th>WKY (n = 12) High-salt diet</th>
<th>SHRSP (n = 12) Normal diet</th>
<th>SHRSP (n = 9) High-salt diet</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Room temp</td>
<td>Cold temp</td>
<td>Room temp</td>
<td>Cold temp</td>
</tr>
<tr>
<td>Norepinephrine</td>
<td>192.4</td>
<td>±19.1</td>
<td>372.9</td>
<td>±40.6</td>
</tr>
<tr>
<td>Epinephrine</td>
<td>101.3</td>
<td>±23.1</td>
<td>142.5</td>
<td>±34.8</td>
</tr>
</tbody>
</table>

Values are given under basal conditions (i.e., room temperature) and after exposure to cold (+4°C) for each group.

### Table 3. Reactivity of Resistance Vessels to Norepinephrine either with or without Blockade of the Neuronal Uptake by 30 μM Cocaine

<table>
<thead>
<tr>
<th>Blockade of the neuronal uptake by 30 μM cocaine</th>
<th>WKY (n = 6) Normal diet</th>
<th>WKY (n = 6) High-salt diet</th>
<th>SHRSP (n = 9) Normal diet</th>
<th>SHRSP (n = 9) High-salt diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration of norepinephrine required for half maximal contraction (ED50, μM)</td>
<td>3.185 ± 0.491</td>
<td>2.120 ± 0.573</td>
<td>2.400 ± 0.525</td>
<td>1.695 ± 0.548</td>
</tr>
<tr>
<td>p &lt; 0.05</td>
<td>0.278 ± 0.348</td>
<td>0.146 ± 0.075</td>
<td>0.189 ± 0.019</td>
<td>0.189 ± 0.019</td>
</tr>
</tbody>
</table>

As an index of vascular smooth sensitivity to norepinephrine the respective values of the ED50 are presented from both WKY and SHRSP fed either a normal or a high sodium diet for 30 days.
an index of the degree of sensitivity of vascular smooth muscle to norepinephrine, the dose required for half maximal contraction (ED50) was diminished in salt-loaded SHRSP during perfusion without cocaine. Blockade of the neuronal uptake by cocaine resulted in a more pronounced reduction of the ED50 of SHRSP on a normal-sodium diet as compared to that obtained from SHRSP on a high-sodium diet; after blockade of the neuronal uptake, the ED50 in the sodium-loaded group was no longer different from that found in the group on a normal-sodium diet (table 3). In sodium-loaded normotensive WKY, the ED50 was also reduced as compared to WKY on a normal-sodium diet. Addition of cocaine to the perfusate also abolished this difference in vascular reactivity to norepinephrine in WKY.

**Plasma Sodium Concentration and Vascular Reactivity to Norepinephrine**

When the individual concentrations of plasma sodium in SHRSP under different sodium intake regimens were plotted against the responsiveness of vascular smooth muscle to norepinephrine (as determined by the ED50 of norepinephrine in the isolated perfused hindlimb) a positive linear correlation was calculated (fig. 2).

**Figure 1.** Cumulative dose-response curves obtained in the isolated perfused hindlimb preparation to increasing concentrations of norepinephrine in stroke-prone spontaneously hypertensive rats (SHRSP) on a normal-sodium diet (○ ○) and on a high-sodium diet (● ● ● ●). The dose-response curves on the left were performed without addition of cocaine to the perfusion medium, the two curves on the right, after perfusion of hindlimbs with the addition of 30 µM cocaine. From the dose-response curves, the ED50's have been evaluated by graphical analysis, representing the dose of norepinephrine at which half maximal vasoconstriction has been achieved. The values for the normal and high-sodium SHRSP groups are listed below the respective symbols. *p < 0.05; **p < 0.01.

**Figure 2.** Relationship between plasma sodium concentration and vascular reactivity to norepinephrine in SHRSP. Sodium diets are: ▲ low (1 mmole/kg), ● normal (100 mmole/kg), ■ high (1500 mmole/kg). Vascular reactivity to norepinephrine is characterized by the ED50 calculated from dose-response curves obtained in the isolated perfused hindlimb preparation (ordinate). On the abscissa, plasma sodium concentration is given. A linear correlation is found.
Inactivation and Metabolism of Norepinephrine

Total norepinephrine uptake at increasing doses of norepinephrine in the perfusate is shown in Table 4. At lower concentrations of norepinephrine in the perfusate (10⁻⁹ and 10⁻⁸M), the uptake was reduced in SHRSP on a high-sodium diet; at higher concentrations of norepinephrine (10⁻⁷M) this difference was no longer of statistical significance (p > 0.05). In WKY on a high-sodium diet, the values for norepinephrine uptake, despite a tendency similar to that observed in sodium-loaded SHRSP, were not statistically different from those obtained in WKY on a normal-sodium diet. The release of deaminated metabolites was reduced from hearts of sodium-loaded rats, both in WKY and SHRSP (Table 4). The differences between the respective control animals on a normal-sodium diet and the sodium-loaded animals were more pronounced in SHRSP than in normotensive WKY.

The cocaine-sensitive portion of norepinephrine uptake, which may correspond to the neuronal uptake of norepinephrine, was reduced in SHRSP in response to sodium-loading (Table 5). The small reduction in neuronal uptake observed in WKY on a high-sodium diet was not statistically significant.

Discussion

The blood-pressure-elevating effects of sodium chloride have generally been attributed to the volume-retaining properties of sodium.² ³ In SHRSP, however, a dissociation between the state of sodium balance, the amount of intravascular volume, and the development of high blood pressure has already been demonstrated.¹⁰-¹² Whereas higher amounts of sodium are retained during the early phase of hypertension in SHRSP, the intravascular volume does not show a concomitant expansion but, in contrast, is even reduced as compared to age-matched WKY.¹² Neonatal sympathectomy by 6-OH dopamine results in a further slight increase in sodium retention in SHRSP and may be responsible for the expansion of the intravascular volume after chemical sympathectomy; the rise in blood pressure, however, is not accelerated, but abolished for the first 10

| TABLE 4. Total Norepinephrine Uptake and Release of Deaminated Metabolites from Isolated Perfused Rat Hearts (WKY and SHRSP on either a Normal or a High Sodium Diet) at increasing Concentrations of Norepinephrine in the Perfusate |
|---|---|---|---|---|
| **Concentration in perfusate (n)** | Normal diet | High-salt diet | Normal diet | High-salt diet |
| **WKY (n = 9)** | **SHRSP (n = 9)** |
| Total norepinephrine uptake (pmol/min/g tissue) | | | | |
| Norepinephrine | | | | |
| 10⁻⁹ | 1.366 ± 0.005 | 1.326 ± 0.013 | 1.159 ± 0.004 | 0.817 ± 0.007 |
| 10⁻⁸ | 13.87 ± 0.42 | 14.38 ± 0.96 | 11.69 ± 0.42 | 8.26 ± 0.82 |
| 10⁻⁷ | 118.8 ± 12.3 | 90.02 ± 20.2 | 103.4 ± 9.2 | 91.0 ± 4.4 |
| Release of deaminated metabolites (DOPEG, DOMA: pmol/min/g tissue) | | | | |
| Norepinephrine | | | | |
| 10⁻⁹ | 0.320 ± 0.025 | 0.267 ± 0.031 | 0.180 ± 0.008 | 0.100 ± 0.020 |
| 10⁻⁸ | 3.17 ± 0.28 | 2.25 ± 0.29 | 1.89 ± 0.13 | 1.02 ± 0.20 |
| 10⁻⁷ | 28.43 ± 2.49 | 21.67 ± 2.77 | 17.14 ± 1.06 | 10.00 ± 2.18 |

| TABLE 5. Absolute Heart Weights and Cocaine-Sensitive Uptake of Norepinephrine of Hearts from WKY and SHRSP on either a Normal or High-Sodium Diet |
|---|---|---|---|
| **WKY (n = 9)** | **SHRSP (n = 9)** |
| Absolute heart weight (g) | Normal diet | High-salt diet | Normal diet | High-salt diet |
| 0.988 ± 0.030 | 1.017 | 1.049 ± 0.025 | 1.061 ± 0.056 |
| Cocaine-sensitive (neuronal) uptake of norepinephrine (pmol/min/g tissue) | Normal diet | High-salt diet | Normal diet | High-salt diet |
| 122.7 ± 5.7 | 106.8 | 96.1 ± 7.7 | 70.0 ± 2.9 |
weeks after systemic denervation. Since these observations are difficult to reconcile with the hypothesis that sodium affects blood pressure only insofar as it changes body fluid volumes, the effects of dietary sodium-loading in SHRSP on blood pressure and plasma volume have been investigated.

The results presented in this paper demonstrate a similar degree of intravascular volume expansion in WKY and SHRSP during both the early and later stages of an increased sodium intake. Hence, it is unlikely that the accelerating effect of sodium on the course of hypertension in SHRSP can be explained merely in terms of volume expansion.

The further aim of this investigation, therefore, was to elucidate alternative mechanisms by which an increased sodium intake may cause an acceleration in the development of high blood pressure in SHRSP. Among the vasopressor systems, the sympathoneuronal system appeared to be one plausible candidate for promoting hypertensive effects of sodium in SHRSP, since this system has been demonstrated to be intimately linked to the development of hypertension in these rats. The increased alpha-adrenergic tone in SHRSP appears to be responsible for the higher degree of vasoconstriction, since other vasopressor systems such as the renin-angiotensin-aldosterone system and the vasopressin system are even suppressed in SHRSP during the development of hypertension in SHRSP.

The data presented provided evidence for a further stimulation of sympathetically mediated vasoconstriction due to salt-loading in SHRSP. Norepinephrine concentration in plasma was markedly increased in SHRSP following sodium-loading and became even more marked after cold stress. Elevated levels of circulating norepinephrine did not, however, allow discrimination between an increased frequency of sympathetic discharge with consequently higher amounts of neurotransmitter available to react with the receptors or an impairment of norepinephrine inactivation, both of which would result in an increased overflow of the neurotransmitter into the plasma.

In SHRSP on a high-sodium diet, the inactivation of norepinephrine was, in fact, reduced. The activity of the neuronal uptake for norepinephrine was decreased in sodium-loaded animals; this implies that, even in the presence of an identical central firing rate reaching the sympathetic nerve endings, the concentration of norepinephrine at the receptor site and the portion of the neurotransmitter from the neuronal uptake for norepinephrine was decreased. Both the quantitative and qualitative differences in catecholamine release, and inactivation between WKY and SHRSP on a high-sodium diet, suggest a disordered regulation of sympathetically induced vasoconstriction in response to sodium-loading in SHRSP. These findings may be explained by assuming two different sites of action of sodium on the sympathetic nervous system in SHRSP. First, there is a measured impairment of neuronal uptake at the level of the sympathetic nerve ending; this effect might quantitatively account for the observed increase in peripheral plasma norepinephrine concentration in SHRSP. For any given central activity of sympathetic discharge the relative amount of norepinephrine at the receptor site in plasma will be increased.

The second site of action of sodium on the sympathetic nerve ending is proposed on the basis of indirect evidence. The metabolism of norepinephrine at the sympathetic nerve ending was altered in a similar way...
by sodium in WKY, albeit quantitatively less so than in SHRSP. If predictions can be made from the sodium-induced changes in norepinephrine metabolism, increased plasma norepinephrine values would also have been expected in sodium-loaded WKY. The observation that norepinephrine levels are not elevated following sodium-loading in normotensive rats suggests a functioning feedback loop between the norepinephrine-induced effect on the target organ and the central sympathetic discharge. A similar observation has been made in corticosterone-treated rats, in which a primary enhancement of reactivity of resistance vessels to norepinephrine has led to suppression of central sympathetic discharge. An alternative hypothesis would be an effect of sodium on central sympathetic discharge independent of its effect on the peripheral sympathetic nervous system. To summarize the effect of sodium in normotensive rats, there was a moderate increase in plasma norepinephrine values in WKY, albeit quantitatively less so than in SHRSP. If predictions can be made from the sodium-dependent impairment of norepinephrine inactivation which was balanced by an appropriate reduction in central sympathetic discharge, with the consequence that sympathetically induced vasoconstriction was held constant or may even be reduced by some extent in WKY.

In contrast, in SHRSP the peripheral disturbance in norepinephrine inactivation due to sodium-loading was obviously not balanced by an adequate withdrawal of central sympathetic discharge. The hemodynamic consequence was a further increase in sympathetically mediated vasoconstriction leading to a further increment in blood pressure. The assumption of an additional effect of sodium on central sympathetic outflow is supported by the observation that not only sympatho-neuronal but sympathoadrenergic activity was stimulated in SHRSP, but not in WKY, following sodium-loading. Previous reports without sodium-loading have already suggested that, in SHRSP, central sympathetic outflow cannot be inhibited adequately by physiological stimuli such as activation of the baroreceptor reflex.

Besides the effects that sodium exerts on sympathetic activity, there is also a sodium-dependent type of supersensitivity of target organs to exogenously administered norepinephrine. Since the amount of norepinephrine that will reach the receptor site after intravenous infusion also depends on the activity of the uptake mechanisms, the reduced neuronal uptake in sodium-loaded SHRSP allowed a higher portion of the drug infused to reach the receptors, thereby increasing the response of target organs to a given dose. The degree of supersensitivity of vascular smooth muscle to norepinephrine was linearly related to the plasma sodium concentration. The relationship also included values obtained from sodium-deprived SHRSP, indicating a subsensitivity to norepinephrine in SHRSP on a low-sodium diet, the exact mechanism of which remains to be solved. A similar relationship between the concentration of sodium in plasma and corresponding changes in vascular reactivity have also been reported in humans.

Thus, the majority of our findings that indicate an increased sympathetic vascular tone in SHRSP following sodium-loading may be interpreted as the consequence of an impaired inactivation of norepinephrine (mainly due to a reduced neuronal uptake), not opposed by an adequate withdrawal of central sympathetic discharge.

The observation of a marked impairment of the inactivation of norepinephrine in consequence of a high-sodium intake may also explain why in sodium-dependent forms of experimental hypertension the turnover of norepinephrine was found to be increased, with a simultaneous reduction in its storage. Although the dependency of norepinephrine uptake activity on sodium concentration in the perfusate has been demonstrated in acute experiments, it remains unclear whether chronic changes in sodium availability may also affect the metabolism of norepinephrine. The increased sensitivity of vascular smooth muscle in spontaneously hypertensive rats has been related to an inherently lower membrane potential because of a reduced intracellular potassium concentration in SHR; a further increase in vascular reactivity occurs after sodium-loading and is the consequence of a sodium-dependent impairment of norepinephrine uptake. In combination with enhanced sympathetic activity, an increase in sympathetically mediated vasoconstriction results; its hemodynamic consequence, an increase in total peripheral resistance, has been reported earlier in SHR, in Dahl salt-sensitive rats, and in hypertensive patients, which was the opposite reaction to that found in normotensive individuals following salt-loading.

It has been suggested that deleterious effects of high-sodium intake on neurogenic control of vascular resistance and arterial pressure in humans may be determined by an underlying genetic susceptibility. For the experimental-models of hypertension, the salt-induced increase in resistance has been found to result from an increased neurogenic vasoconstrictor tone only in Dahl salt-sensitive rats but not in rats with spontaneous hypertension (SHR). These authors, however, investigated the effect of sodium-loading on adult SHR with established hypertension, whereas our study was performed during the developmental phase of hypertension in a substrain of SHR with an accelerated rise in blood pressure (SHRSP).

Thus, it may be concluded that a high-sodium intake in SHRSP accelerated the rise in blood pressure by increasing the amount of norepinephrine available to react with the receptor. One major determinant of the elevated sympathetic activity is an impairment of norepinephrine inactivation in consequence of a diminished neuronal uptake, leading to higher plasma concentrations of norepinephrine and to a supersensitivity of target organs to norepinephrine. Recent studies in human essential hypertension, as well as in experimental hypertension, suggested the occurrence of a natriuretic ouabain-like substance, which might be responsible for the further increase in total peripheral resistance on a high-sodium intake in the hypertensive subjects. Since ouabain impairs neuronal uptake of norepinephrine in a dose-dependent manner, the
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aforementioned studies may provide some arguments for the mechanism by which sodium may exert its effect on the peripheral norepinephrine metabolism. A second component, a central effect of sodium on sympathetic outflow, may be assumed on the basis of indirect evidence.

The different pattern of the regulation of sympathetic impulse transmission may help to clarify the underlying pathophysiological events occurring in salt-sensitive and salt-resistant individuals. In humans, evidence has accumulated for a similar difference existing in sympathetic activity following salt-loading between different hypertensive individuals. Further studies are necessary to unravel the volume-independent effects of electrolytes on the regulation of total peripheral resistance.

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