Role of Hypothalamic–Renal Noradrenergic Systems in Hypotensive Action of Potassium

Toshiro Fujita and Yuji Sato

To clarify the role of the renal and hypothalamic noradrenergic systems in the antihypertensive actions of dietary potassium supplementation in salt-loaded spontaneously hypertensive rats (SHR), we measured systolic blood pressure and norepinephrine turnover, which was determined from the rate of decline of tissue norepinephrine concentration after the administration of α-methyl-p-tyrosine, in 5-week-old SHR or age-matched Wistar-Kyoto (WKY) rats eating normal-NaCl (0.66%) or high-NaCl (8%) diet with supplementation of 8% KCl. In WKY rats, neither high-sodium nor high-potassium diets had an effect on blood pressure with no change in renal or hypothalamic norepinephrine turnover. In SHR, however, salt loading accelerated the development of hypertension. Potassium supplementation did not affect blood pressure in normal-sodium SHR but attenuated the rise in blood pressure with salt loads. Correspondingly, renal norepinephrine turnover in SHR was increased compared with that of WKY rats, and salt loading further potentiated the increased turnover in the kidney; however, no changes in hypothalamic turnover occurred. Potassium supplementation attenuated the rise in blood pressure with salt loads and the increased renal turnover. Stimulation of sympathetic discharge by cold exposure after the administration of α-methyl-p-tyrosine produced a marked depletion of norepinephrine in most tissues. The loss of norepinephrine was significantly greater in both kidney and hypothalamus of salt-loaded SHR than in those of normal-sodium SHR, but potassium could normalize this. Thus, potassium not only diminished the increased renal norepinephrine turnover in the kidney under normal conditions but also attenuated the augmented renal and hypothalamic norepinephrine turnover by cold stress in salt-loaded SHR. The data suggest that hypothalamic–renal noradrenergic systems might be involved in the hypotensive action of potassium in salt-loaded SHR. (Hypertension 1992;20:466–472)

KEY WORDS • sympathetic nervous system • hypothalamus • norepinephrine • cold • potassium

Accumulating experimental evidence suggests that dietary potassium intake plays an important role in the regulation of blood pressure as well as in the pathogenesis of hypertension in animals and humans.1–7 Moreover, the antihypertensive action of potassium might be linked to sodium intake; it could be exerted only under the condition of salt load since the supplementation of potassium attenuated salt-induced elevation of blood pressure in patients and animals with salt-sensitive hypertension,2,4–8 but it did not affect blood pressure in the sodium-depleted state.8,9 According to the linkage to sodium, the main mechanism for potassium-induced antihypertensive action might be related to natriuresis, although the hypotensive action of potassium is still multifactorial.1,4–9

Evidence suggests that the increase in renal sympathetic nervous system (SNS) activity decreases urinary sodium excretion via both renal arteriolar vasoconstriction and increased tubular sodium reabsorption.10,11 Increased renal SNS activity was also involved in the development of hypertension through sodium retention in spontaneously hypertensive rats (SHR) since renal denervation promoted sodium excretion and delayed the development of hypertension.12 Moreover, a recent study of Koeck et al.13 showed that high NaCl intake produced a greater increase in renal SNS activity and a greater antinatriuresis induced by stressful environmental stimulation (air jet to head) in SHR but not in normotensive Wistar-Kyoto (WKY) rats. The data suggest that enhanced renal SNS activity and antinatriuretic response to air stress in SHR on high salt intake are dependent on a centrally mediated facilitation of sympathetic neural outflow to the kidney. Correspondingly, we demonstrated that potassium supplementation in deoxycorticosterone acetate–salt hypertensive rats attenuates the antinatriuretic and antidiuretic responses to air stress.7 Moreover, it is consistent with the result of our previous experiment that potassium supplementation normalizes the abnormal renal and hypothalamic norepinephrine turnover in deoxycorticosterone acetate–salt rats.3 Thus, potassium-induced attenuation of the increased renal SNS activity via the functional changes in the central noradrenergic mechanism may contribute to the antihypertensive action of potassium, possibly through natriuresis.

Our recent study4 demonstrated that potassium supplementation for 4 weeks in 5-week-old SHR, a model of human essential hypertension, attenuates the development of hypertension with salt loads by improvement of impaired renal function for sodium excretion. Because it is well known that the abnormalities of renal...
SNS and central noradrenergic activity are implicated in the development and maintenance of hypertension in SHR, it can be speculated that a decreased renal ability for excreting sodium in NaCl-loaded SHR might be attributable to the increased renal SNS activity and that the improvement induced by potassium supplementation might be due to the attenuation of the increased renal SNS activity, which is intimately related to the changes in the central noradrenergic activity. Therefore, the present study was designed to evaluate the effects of potassium supplementation on noradrenergic activity in the hypothalamus (which is important to blood pressure control as well as temperature regulation) and in the kidney estimated from the rate of norepinephrine turnover in salt-loaded young SHR. Norepinephrine turnover was computed from the rate of fall of endogenous norepinephrine after blockade of norepinephrine biosynthesis. The results of these studies indicate that blood pressure could be reduced through potassium supplementation, which is associated with the restoration of the increased norepinephrine turnover in the kidney.

The stimulatory effect of cold exposure on the central and peripheral nervous systems and the crucial importance of sympathetic activation in the defense of body temperature in mammals are well recognized. Acute cold exposure increases norepinephrine turnover in the brain and various peripheral organs. In the present study, potassium-induced suppression of the augmented hypothalamic and renal norepinephrine turnover during cold stress in salt-loaded SHR emphasizes the potentiation of central noradrenergic mechanisms in hypotensive action of potassium.

Methods

Animal Preparations

Male SHR (n=98) and WKY rats (n=96) were purchased (Charles River Japan, Atsugi, Japan) at 5 weeks of age. All rats were maintained at constant temperature (23±1°C), humidity (60±5%), and light cycle (6 AM–6 PM). Five days after arrival, SHR and WKY rats were randomly divided into four groups as follows: the sodium-loaded groups were placed on an 8% NaCl diet (Oriental Yeast Co., Tokyo) with tap water to drink (high-sodium SHR and WKY); the potassium-loaded groups were placed on an 8% KCl diet (high potassium SHR and WKY); the sodium- and potassium-supplemented groups were placed on an 8% NaCl plus 8% KCl diet with tap water to drink (high sodium; high potassium SHR and WKY); the control groups were placed on a normal rat chow diet (0.66% NaCl and 1.34% KCl, MF: Oriental Yeast Co.) with tap water to drink (control SHR and WKY). These eight groups of rats each received diet and tap water ad libitum for 4 weeks. Systolic blood pressure was measured weekly in conscious, prewarmed, unrestrained rats (10 rats in each group) by the tail-cuff method using an electrosphygmomanometer and physiograph recorder (model PE-300, Narco BioSystems, Austin, Tex.). Body weight was also determined on the same day as the blood pressure determination.

Renal and Hypothalamic Norepinephrine Turnover

Four weeks after the start of each diet, tissue norepinephrine turnover was measured by a nonisotope method, as previously reported. Briefly, the inhibitor of catecholamine biosynthesis, α-methyl-DL-p-tyrosine (α-MPT) methyl ester hydrochloride (Sigma Chemical Company, St. Louis, Mo.), was administered intraperitoneally, and the rate of tissue norepinephrine depletion was evaluated. After the first intraperitoneal injection of α-MPT (240 mg α-MPT dissolved in saline per kilogram body weight), eight to nine rats from each group were killed at 3 hours, and the remaining rats were reinjected with a booster dose of α-MPT (120 mg/kg) at 3 hours and killed at 6 hours; eight animals from each group were not reinjected and served as the t₀ reference. Rats were killed by decapitation without anesthesia. Brains and kidneys were removed immediately, and the hypothalamus was dissected from the brain according to the method of Glowinski and Iversen. Tissue samples were quickly excised, frozen on dry ice, and stored at −40°C until assay. When ready for assay, tissue samples were weighed and homogenized in ice-cold 0.4N perchloric acid (Wako Pure Chemical, Osaka, Japan). The homogenates were centrifuged at 40,000g for maximum for 10 minutes, and supernatants were frozen at −40°C until norepinephrine assay, as previously reported. Norepinephrine concentration was measured by trihydroxindole methods after separation by high-performance liquid chromatography. Norepinephrine turnover rate was evaluated by linear regression analysis of the decline of the log tissue norepinephrine concentration over time after tyrosine hydroxylase inhibition. The rate constant of amine loss (k) was taken as the slope of log norepinephrine concentration versus time multiplied by 1/0.434 according to the method of Brodie et al. In the present study, to assess norepinephrine turnover, α-MPT was used to inhibit tyrosine hydroxylase, the rate limiting enzyme in norepinephrine biosynthesis. With the norepinephrine biosynthesis inhibited, tissue norepinephrine levels must be decreased according to the removal rate from various tissues, and thus endogenous norepinephrine levels decline exponentially. The slope of the decline is almost identical with that of the decline in specific activity after labeling with [3H]norepinephrine, another method of assessing norepinephrine turnover. Therefore, the decline of endogenous norepinephrine after the blockade of norepinephrine biosynthesis is an indication of norepinephrine turnover.

Exposure to Cold

After the first injection of α-MPT, the animals were placed in individual cages; some were kept in a cold room at 4°C for 6 hours; others were kept at 23°C for the same period of time. The animals were killed at 6 hours. The condition chosen to elicit sympathetic stimulation such as exposure to cold is known to result in increased SNS activity and release of catecholamines. Exposure to 4°C is known to be sufficient to cause marked and prolonged elevations of urinary catecholamines. In the present study, the increment of norepinephrine turnover by cold exposure was estimated from the remaining norepinephrine concentration at 6 hours after the first injection of α-MPT during cold exposure, expressed as percentage of the value at 6 hours after α-MPT during the ambient temperature.
Statistical Analysis

Data are presented as mean±SEM. One-way analysis of variance (ANOVA), with Bonferroni adjustment of the p value, was used when comparing the changes in systolic blood pressure with sodium loads and potassium supplementation and analyzing the difference between SHR and WKY rats. Two-way ANOVA was used to analyze the difference between the vehicle and sodium lines (Fv), the effects of potassium supplementation (Fk), and the differential effects of potassium supplementation on each of the two lines (interaction: Fvk), and the Bonferroni method was used for comparisons between individual means. A value of p<0.05 was considered significant.

Results

Blood Pressure

Figure 1 shows systolic blood pressure in SHR and WKY rats eating high-sodium, high-potassium, and high-sodium-high-potassium diets. In high-sodium SHR, the diet significantly accelerated from week 2 the rise in systolic blood pressure, which thereafter continued to rise, reaching 215±3 mm Hg at week 4. However, the high-sodium-high-potassium diet significantly attenuated the salt-induced acceleration of hypertension from week 3, although the high-potassium diet did not affect systolic blood pressure in the normal-sodium SHR group throughout the study. As a result, systolic blood pressure at week 4 was significantly lower in high-sodium, high-potassium SHR as compared with that in the sodium SHR (155±5 versus 215±3 mm Hg, p<0.01). The present results of potassium-induced changes in systolic blood pressure measured by the tail-cuff method in SHR and WKY rats are consistent with those of direct measurements of arterial pressure in our previous study with the same design as the present study. It is suggested that salt loading promotes the development of hypertension in SHR and that potassium supplementation slowly attenuates the salt-induced acceleration of hypertension in SHR, but potassium loading does not affect blood pressure in salt-unloaded SHR. In WKY rats, systolic blood pressure was not significantly altered by any of the treatments throughout the study.

Body Weight

At the start of the study, there were no body weight differences among the four groups. From week 1, the net weight gain was slightly but significantly lower in both high-sodium and high-potassium groups than in the control diet-fed groups, and the influence was additive in the case of concomitant loading with the high-sodium, high-potassium diet. The reduced rate of weight gain for 4 weeks, however, was consistent in WKY rats and SHR. Initial/final body weight was as follows: control SHR, 94±2/234±2 g; high sodium SHR, 95±2/221±3 g; high potassium SHR, 94±2/220±3 g; high sodium, high potassium SHR, 93±3/204±3 g; control WKY, 91±2/223±2 g; high sodium WKY, 91±2/214±2 g; high potassium WKY, 92±2/213±2 g; high sodium, high potassium WKY, 92±1/194±3 g.
Our first relevant observation, in keeping with previous studies, is that salt loading accelerated the development of hypertension in SHR but not in WKY rats.5,23-26 Whereas potassium supplementation effectively prevented salt-induced elevation of blood pressure in SHR.5,26 It is well known that stroke-prone SHR have an increased salt sensitivity, resulting in high blood pressure; however, a recent study demonstrated that SHR could be divided into salt-sensitive and salt-resistant SHR. Since the rate at which salt loading accelerates the development of hypertension in SHR depends on multiple factors such as the amount of salt ingested, the age at institution of high-sodium intake, and the sex of the animals,23,25,26 it is suggested that young SHR used in the current study have an increased sensitivity of blood pressure to changes in salt intake. Moreover, the antihypertensive action of potassium supplementation could be exerted under the condition of salt loads in salt-sensitive hypertensive SHR, but not in WKY rats. It suggests that renal SNS activity is increased in young SHR, which is consistent with the finding of Judy et al.,31 and that salt loading further enhances the increased renal SNS activity in SHR. In our previous study,6 salt loading increased renal vascular resistance in SHR, leading to the increased salt sensitivity of blood pressure, possibly through the lowered slope of the pressure-urinary sodium excretion relation. Thus, it is speculated that the increased renal SNS activity with salt loads might play an important role in sodium retention and the resultant blood pressure rise in SHR since renal SNS activity is one of the most important factors regulating sodium excretion in the urine by not only the direct effect on

### Table 1. Renal and Hypothalamic Norepinephrine Content and Rate Constant of Norepinephrine Efflux in Sodium- and Potassium-Supplemented Wistar-Kyoto Rats and Spontaneously Hypertensive Rats

<table>
<thead>
<tr>
<th>Variable</th>
<th>Vehicle</th>
<th>Na</th>
<th>K</th>
<th>Na,K</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Kidney</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial NE content (ng)</td>
<td>220±7.4</td>
<td>231±11.9</td>
<td>230±9.8</td>
<td>259±10.1</td>
</tr>
<tr>
<td>( \kappa ) (hr(^{-1}))</td>
<td>0.070±0.011</td>
<td>0.064±0.014</td>
<td>0.078±0.013</td>
<td>0.095±0.015</td>
</tr>
<tr>
<td><strong>Hypothalamus</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial NE content (ng/mg)</td>
<td>1,705±78</td>
<td>1,503±60</td>
<td>1,602±68</td>
<td>1,573±77</td>
</tr>
<tr>
<td>( \kappa ) (hr(^{-1}))</td>
<td>0.147±0.012</td>
<td>0.125±0.011</td>
<td>0.124±0.009</td>
<td>0.118±0.008</td>
</tr>
</tbody>
</table>

WKY, Wistar-Kyoto rats; SHR, spontaneously hypertensive rats; NE, norepinephrine; \( \kappa \), rate constant of NE efflux. Values are mean±SEM for groups of eight to nine rats. Effects of line (\( F_A \), vehicle versus sodium), potassium (\( F_B \)), and interaction (\( F_{AB} \)) effects of potassium in vehicle-treated and sodium-loaded rats: kidney in SHR: \( F_A=12.086 \) (\( p<0.01 \)), \( F_B=13.043 \) (\( p<0.01 \)), \( F_{AB}=18.968 \) (\( p<0.01 \)). No significant effects of line, potassium, and interaction were found for NE content or rate constant in hypothalamus of SHR nor in both kidney and hypothalamus of WKY rats.

\*\( p<0.01 \) compared with each treatment in WKY (by one-way analysis of variance).
hypothalamic norepinephrine turnover under normal conditions was not altered by salt loading, although salt loading could enhance the increased norepinephrine turnover under the environmental stimuli of cold exposure in both the kidney and hypothalamus of SHR. According to the absence of salt-induced changes in hypothalamic norepinephrine turnover under normal conditions, it should be noted that we measured norepinephrine turnover in total hypothalamus, which consists of both anterior hypothalamus containing sympathoinhibitory neurons and posterior hypothalamus containing sympathoexcitatory neurons. In fact, Chen et al recently reported that salt loading in salt-sensitive SHR decreased norepinephrine turnover in anterior hypothalamus but not in posterior hypothalamus. However, cold exposure might stimulate sympathoexcitatory neurons in posterior hypothalamus, which could be reflected by the occurrence of the increased total hypothalamic norepinephrine turnover, possibly leading to the increased renal SNS activity. Then stress-induced hyperresponse of hypothalamorenal noradrenergic systems may mediate the abnormal renal tubular reabsorption of sodium, resulting in the aggravation of hypertension by salt loading. These hypotheses are supported by a previous experiment wherein salt loading produced the augmented antinatriuretic response to air stress in SHR.

The most important observation of the present study is that potassium supplementation in salt-loaded SHR not only attenuated the increased renal NE turnover under normal conditions but also normalized the augmented hypothalamic and renal NE turnover during cold exposure. It led us to the speculation that the normalization of the increased renal SNS activity is mediated by changes in the central nervous system. Potassium-induced normalization of the abnormal hypothalamorenal noradrenergic systems may originate at two points: the hypothalamus and the kidney. Central action of potassium supplementation is supported by several studies where potassium supplementation normalized not only hyperresponses of blood pressure to intracerebroventricular infusion of hypertonic saline or angiotensin II in Dahl salt-sensitive rats but also the increased response of renal SNS activity to electrical stimulation of the hypothalamus in deoxycorticosterone acetate–salt rats. Then it seems reasonable to suggest that salt-loaded SHR might have an enhanced hypothalamic noradrenergic activity to environmental stimuli such as cold stress, which is translated into increased SNS activity to peripheral organs such as the kidney. Alternatively, the augmented antinatriuretic response to air stress would be likely as a result of the increased hypothalamic noradrenergic activity.
evidence suggests that hypothalamic noradrenergic activity is influenced by afferent nerves in the kidney, and the stimulated afferent inputs to the central nervous system may further increase efferent sympathetic nerve activity, including renal SNS activity (renorenal reflex). This excitatory renorenal reflex might contribute to the development of hypertension, since renal denervation in one-kidney, one clip Goldblatt hypertensive rats significantly decreases blood pressure associated with the decrease in both hypothalamic NE content and plasma NE concentration. In the present study, therefore, it is suggested that the inhibition of sodium retention, the change in potassium balance with potassium supplementation, or both in salt-loaded SHR may be responsible for the normalization of abnormal hypothalamic noradrenergic systems via renorenal reflex, resulting in the interception of the positive feedback loop to accelerate hypertension. However, the causal relation between changes in hypothalamic and renal NE turnover and blood pressure after potassium supplementation remains to be established; it is possible that potassium-induced changes in NE turnover might not reflect a direct but an indirect effect of changes in blood pressure. Therefore, further studies are warranted to clarify the role of hypothalamic renorenadnergic systems in the antihypertensive action of potassium in salt-loaded hypertensive animals and humans.

In summary, the present study indicates that salt loading in SHR increases renal NE turnover, which is intimately related to the hypothalamic noradrenergic mechanism. This relation may contribute to the salt-induced acceleration of hypertension. Moreover, the attenuation of the increased renal NE turnover, possibly through the changes in hypothalamic noradrenergic activity, may be involved in the antihypertensive effects of potassium supplementation in NaCl-loaded SHR.

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