Salt-Induced Hypertension in Normotensive Spontaneously Hypertensive Rats

J. Michael Wyss, Sanya Roysommuti, Kathryn King, Inga Kadisha, Christopher P. Regan, Kathleen H. Berecek

Abstract Lifetime treatment with oral captopril prevents the development of hypertension in spontaneously hypertensive rats (SHR). We tested the hypothesis that this treatment also prevents the hypertensive response that occurs when untreated NaCl-sensitive SHR are placed on a high NaCl diet. Female SHR were continuously treated with oral captopril before conception and throughout lactation, and the offspring were similarly treated with oral captopril throughout life. At 6 weeks of age, treated male SHR were placed on an 8% (or remained on a 1%) NaCl diet, and systolic arterial pressure, heart rate, and body weight were monitored for 2 weeks. The 8% NaCl diet caused a rapid increase in arterial pressure in the lifetime captopril-treated rats, and 18 days after the initiation of the diet, the mean arterial pressure of this group was 136±7 mm Hg compared with 100±2 mm Hg in the 1% NaCl diet rats. The results of a second experiment confirmed the hypertensive effect of the high NaCl diet in lifetime captopril-treated SHR and demonstrated that after 18 days on the diet the dietary NaCl-induced hypertensive response was greater in magnitude in lifetime captopril-treated compared with untreated SHR. The results also demonstrated that lifetime captopril-treated Wistar-Kyoto rats, which are normotensive irrespective of captopril treatment, display no significant increase in arterial pressure when given a high NaCl diet. A third experiment demonstrated that rapidly progressing NaCl sensitivity is also present in female lifetime captopril-treated SHR. These data indicate that an imbalance in the renin-angiotensin system is not the major contributor to NaCl-sensitive hypertension in SHR and suggest that lifetime captopril treatment may unmask the early hypertensive response to dietary NaCl excess in the SHR. (Hypertension. 1994;23[part 1]:791-796.)

Key Words • renin-angiotensin system • angiotensin converting enzyme • hypertension, sodium-dependent • brain • kidney

Many clinical and experimental studies suggest that the renin-angiotensin system plays an important role in the pathogenesis of hypertension. Some of the strongest experimental evidence supporting this hypothesis is derived from studies that have administered angiotensin-converting enzyme (ACE) inhibitors (eg, captopril) peripherally or into the cerebral ventricles of spontaneously hypertensive rats (SHR), although some authors have not found a decrease in arterial pressure in SHR given intracerebroventricular captopril. Systemic (and in several experiments intracerebroventricular) administration of the inhibitors results in a reduction in arterial pressure and a significant alteration in brain angiotensin II (Ang II) and ACE activity. Recent studies have demonstrated that early exposure of SHR to ACE inhibitors causes a permanent reduction in arterial pressure even after the ACE inhibition is stopped later in life and that administration of ACE inhibitors to SHR from conception onward (lifetime treatment) prevents the development of hypertension in SHR. This antihypertensive effect appears to be due in part to alteration in the endogenous renin-angiotensin system in the brain, re-

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rate (indirectly measured in conscious, prewarmed, restrained rats by the tail-cuff method; Narco Biosystems) were recorded between 9 and 10 AM on the days indicated in each experiment. The arterial pressure of each rat was measured directly. To test the possible involvement of the sympathetic nervous system in the NaCl-sensitive hypertension present in lifetime captopril-treated male SHR, we withdrew 1 mL of arterial blood from resting animals and measured plasma catecholamine concentrations by radioenzymatic assay as previously reported.14 To confirm the findings of the initial experiment and compare the changes in arterial pressure with those of untreated SHR and to test the possibility that the captopril treatment induces NaCl sensitivity in normotensive rats, we placed 6-week-old untreated male SHR and WKY rats and 6-week-old lifetime captopril-treated SHR and WKY rats on either an 8% (or kept them on a 1%) NaCl diet. The arterial pressure of each group was measured (direct) 2 weeks after the initiation of the 8% NaCl diet. A final experiment tested the hypothesis that the dietary NaCl-sensitive hypertension present in lifetime captopril-treated male SHR is also exhibited by lifetime captopril-treated female SHR. At 6 weeks of age, female untreated SHR and lifetime captopril-treated SHR were placed on an 8% (or remained on a 1%) NaCl diet. The arterial pressure of each group was measured before (indirect) and 2 weeks after (direct) the initiation of the 8% NaCl diet.

Results

In the initial experiment the high NaCl diet did not affect the rate at which the lifetime captopril-treated SHR gained weight, and at 9 weeks of age the 1% and 8% NaCl diet groups were nearly identical in body weight (Fig 1b). The lifetime captopril treatment prevented hypertension in SHR on the 1% NaCl diet throughout the study (Fig 1a). In contrast, the high NaCl diet caused a rapid elevation of arterial pressure in lifetime captopril-treated SHR (Fig 1a). Five days after the 8% NaCl diet was initiated, the systolic arterial pressure of the 8% NaCl diet group was significantly increased from the pretreatment level and was significantly higher than that of the 1% NaCl group. Two weeks after the 8% NaCl diet was initiated, the average systolic arterial pressure of the 8% NaCl diet group was 47 mm Hg higher than that of the 1% NaCl diet group and 56 mm Hg higher than the initial arterial pressure of the 8% NaCl diet group. Eighteen days after the diet was initiated, mean arterial pressure was 136 ± 7 mm Hg in the 8% NaCl diet group compared with 100 ± 2 mm Hg in the 1% NaCl diet group (Fig 1a). Heart rate was initially decreased slightly by the 8% NaCl diet but then returned toward pretreatment levels. Eighteen days after the diet was initiated, heart rate was slightly faster in the 8% (compared with the 1%) NaCl diet group (Fig 1c). Plasma norepinephrine concentration was elevated significantly in lifetime captopril-treated SHR (765 ± 130 pg/mL plasma) compared with control rats (394 ± 53 pg/mL plasma). Neither epinephrine nor dopamine concentrations in the plasma were different between the groups. Ratios of heart weight to body weight (X10^3) were increased significantly in the lifetime captopril-treated rats receiving the 8% compared with the 1% NaCl diet (452 ± 10 compared with 375 ± 15, 792  Hypertension Vol 23, No 6, Part 1 June 1994
was closely correlated with mean arterial pressure but did not appear to be related directly to lifetime captopril treatment (Table). Neither diet nor captopril treatment affected body weights or kidney weight–body weight ratios in SHR (Table). The 8% NaCl diet had no significant effect on arterial pressure in the treated or untreated WKY rats (Fig 2). Similarly, neither captopril treatment nor diet affected heart weight in WKY rats. It should be noted that captopril treatment significantly reduced arterial pressure in WKY rats irrespective of diet (Fig 2).

In the final protocol we tested whether female lifetime captopril-treated SHR display NaCl sensitivity. In untreated female SHR, exposure to an 8% NaCl diet for 2 weeks did not elicit a significant increase in arterial pressure (187±4 mm Hg [8% NaCl diet] compared with 181±5 mm Hg [1% NaCl diet]; however, in the lifetime captopril-treated female SHR, the exposure to the 8% (compared with the 1%) NaCl diet caused a 25 mm Hg increase in mean arterial pressure (134±2 mm Hg [8% NaCl diet] compared with 109±4 mm Hg [1% NaCl diet]; P<.05). It should be noted that the groups on the 8% NaCl diet did not gain body weight as rapidly as their counterparts on the 1% NaCl diet (1% control, 183±5 g; 1% captopril, 203±6 g; 8% control, 155±6 g; 8% captopril, 169±7 g). None of the manipulations significantly altered heart rate.

Discussion

The results of this study demonstrate that although lifetime treatment with an ACE inhibitor normalizes arterial pressure in SHR on a basal NaCl diet, the treatment does not prevent or even blunt the increase in arterial pressure caused by a high NaCl diet in this model. The time course of the NaCl-sensitive hypertension in SHR appeared to be rapid in the initial experimental group. In the lifetime captopril-treated SHR, the high NaCl diet significantly increased systolic arterial pressure 5 days after treatment was initiated, and after 2 weeks of the diet, mean arterial pressure was elevated by 36 mm Hg. A direct comparison of the

### Heart Rate, Body Weight, Heart Weight–Body Weight Ratio, and Average Single Kidney Weight–Body Weight Ratio of Study Rats*

<table>
<thead>
<tr>
<th>Strain and Captopril</th>
<th>NaCl Diet</th>
<th>HR, bpm</th>
<th>BW, g</th>
<th>HW/BW (×10²)</th>
<th>KW/BW (×10²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHR</td>
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<td>180±5</td>
<td>478±21</td>
<td>501±130</td>
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<tr>
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<td>404±14</td>
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<td>550±23†</td>
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<tr>
<td></td>
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<td>167±5</td>
<td>392±20†</td>
<td>455±29</td>
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<td>398±5</td>
<td>168±6</td>
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<tr>
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<tr>
<td></td>
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<td>315±6</td>
<td>266±10</td>
<td>485±19</td>
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<td>390±27</td>
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<td></td>
<td>Yes 8%</td>
<td>296±8</td>
<td>238±8</td>
<td>481±28</td>
<td>...</td>
</tr>
</tbody>
</table>

HR indicates heart rate; bpm, beats per minute; BW, body weight; HW, heart weight, KW, kidney weight; SHR, spontaneously hypertensive rats; and WKY, Wistar-Kyoto rats.

*Study rats were untreated SHR and WKY rats and lifetime captopril-treated SHR and WKY rats after 18 days on an 8% or 1% NaCl diet.

†P<.05 compared with all other SHR groups. HR and BW of all WKY groups were significantly different from all SHR groups.
dietary NaCl-induced hypertensive response in treated and untreated SHR in the second study demonstrated that after 2 weeks on the diets, treated SHR displayed a significantly greater response to the dietary NaCl excess. In 10 previous studies of the effects of an 8% NaCl diet on arterial pressure in untreated SHR, the arterial pressure of SHR on a high compared with a basal NaCl diet was not significantly different after 5 days of the diet, and after 2 weeks of the diet the mean arterial pressure difference was never greater than 29 mm Hg (eg, see References 11 and 19). Although comparisons with previous data are always tenuous, the present results together with the previous findings suggest that lifetime captopril-treated SHR respond more rapidly to an 8% NaCl diet than do untreated SHR.

The cause of this apparent acceleration in the hypertensive response to the high NaCl diet may simply reflect the lack of a compensatory angiotensin response in treated SHR. Untreated SHR respond to a high NaCl diet by a rapid reduction in circulating angiotensin levels, an effect that tends to reduce arterial pressure, likely through initial NaCl-induced increase in renin secretion to the high NaCl diet. In contrast, in the lifetime captopril-treated SHR, Ang II production is blocked and thus the initial NaCl-induced rise in arterial pressure is not opposed by a simultaneous reduction in Ang II. Eventually, both groups would be expected to suppress Ang II to a similar extent and attain similar total increases in arterial pressure. This suggests that lifetime captopril treatment primarily unmasks the early component of dietary NaCl sensitivity in SHR. In human patients treated long term with ACE inhibitors, this effect would be expected to increase arterial pressure lability by augmenting the responsiveness to acute variations in dietary NaCl intake. Whether the NaCl-sensitive hypertension eventually reaches a higher level in the lifetime captopril-treated compared with control SHR also must be confirmed by future studies; our previous studies suggest that in untreated SHR the maximum NaCl-induced hypertensive response is approximately 40 mm Hg, and this is reached after 3 to 4 weeks of an 8% NaCl diet (eg, see Reference 11).

The results of the third experiment demonstrate that compared with the response in untreated male SHR, untreated female SHR display no significant increase in arterial pressure after 2 weeks on the high NaCl diet. This is in agreement with our past studies which indicated that the development of NaCl-sensitive hypertension is much slower in female than male SHR. In contrast, in the lifetime captopril-treated female SHR, exposure to a high NaCl diet for 2 weeks causes a 25 mm Hg increase in mean arterial pressure. The mechanisms that apparently blunt the NaCl sensitivity in the female compared with male SHR require further study.

The present data indicate that the NaCl sensitivity of lifetime captopril-treated SHR is not due to a primary effect on heart rate or body weight or to a direct effect on cardiac or renal hypertrophy. Furthermore, preliminary studies from this laboratory suggest that renal function is not compromised by captopril treatment and that baroreceptor reflex responses to arterial pressure changes are undisturbed by captopril treatment. Although many studies have found that antihypertensive treatment with ACE inhibitors is beneficial to renal function in SHR, one abstract suggests that renal function is severely compromised when such treatment is given to newborn rats. We find no indication that renal damage is present in lifetime captopril-treated SHR or WKY rats. At 1 year of age these rats are healthy and able to complete learning tasks as rapidly as age-matched Sprague-Dawley rats and much more rapidly than age-matched SHR. In an extension of the learning study, more than 60% of lifetime captopril-treated SHR reached 24 months of age (more than 20% reached 30 months of age) and appeared to be as healthy as age-matched Sprague-Dawley rats. In contrast, no untreated SHR rat has lived beyond 24 months of age, and more than 50% die before 18 months of age. Second, in a recent study on renal function in 8-week-old lifetime captopril-treated and control SHR on a basal NaCl diet, we found no significant difference in resting or stimulated natriuresis or diuresis. Finally, in a preliminary study of NaCl handling in 8-week-old treated and untreated SHR on 1% or 8% NaCl diets, captopril treatment did not increase proteinuria (lifetime captopril-treated SHR, 8.9±1.9 mg/24 h; control untreated, 11.1±2.8 mg/24 h). Although comparisons with previous data are always tenuous, the present results with previous findings suggest that lifetime captopril-treated SHR respond more rapidly to an 8% NaCl diet than do untreated SHR.

Our data suggest that the structural changes in the cardiovascular system of the maturing SHR are related more directly to arterial pressure than to either the activity of the renin-angiotensin system (ACE inhibition experiments) or dietary NaCl excess. This is in agreement with the recent findings of Harrap et al, who suggest that although the interaction between Ang II activity and sodium excretion contributes importantly to the regulation of arterial pressure in SHR, the final mediator of cardiac and vascular hypertrophy is arterial pressure.

The role of the renin-angiotensin system in NaCl-sensitive hypertension is not the same in all models of hypertension. Several studies have demonstrated that in the SHR blockade of ACE reduces arterial pressure and that if the treatment is initiated in young SHR, the hypotensive effect extends long after the treatment is discontinued. More recently, blockade of the angiotensin subtype 1 (AT1) receptor (the primary receptor subtype responsible for the systemic actions of angiotensin) has been shown to result in the same effect as captopril, suggesting that the beneficial effect of captopril is due to inhibition of Ang II production. Whereas short-term, postnatal treatment of young SHR with ACE inhibitors causes a long-term hypotensive effect, neither short-term nor lifetime treatment with ACE inhibitors prevents dietary NaCl sensitivity in SHR (see Reference 15 and present results). In contrast to the ineffectiveness of ACE inhibition on NaCl sensitivity in SHR, treatment with either ACE inhibitors or an AT1 receptor antagonist prevents NaCl-sensitive hypertension in the reduced renal mass model. Thus, the renin-angiotensin system appears to contribute to some forms of NaCl-sensitive hypertension.

Although the mechanism or mechanisms underlying the contribution of angiotensin to SHR hypertension are not fully resolved, increasing evidence suggests that the nervous system is involved in the pathogenesis. Many studies have indicated that some neurons in the
brain produce Ang II and use it as a neurotransmitter/neuromodulator to transfer information to other neurons or to glial cells in the brain.20-29 Furthermore, the intrinsic brain renin-angiotensin system contributes importantly to the homeostatic regulation of arterial pressure, and abnormalities in this system appear to play a role in the pathogenesis of hypertension in SHR, on a basal NaCl diet.34-36 Also, intracerebroventricular administration of ACE inhibitors to young SHR has been reported by some investigators to normalize arterial pressure.1-3 Furthermore, lifetime treatment of SHR with captopril prevents the development of hypertension, an effect that appears to be due at least in part to an alteration in the endogenous renin-angiotensin system in the brain.9

Previous data from our laboratory indicate that the NaCl sensitivity of SHR is closely related to an alteration in an area of the brain that is very responsive to Ang II (ie, the anterior hypothalamus). These studies also demonstrate that blockade of the AT1 receptor in the anterior hypothalamic area with DuP 753 (an AT1 receptor antagonist) causes a significant reduction in arterial pressure that is markedly greater in SHR that have been on an 8% compared with a 1% NaCl diet for 2 weeks.13,14 Before the present study, we interpreted these data to mean that an abnormality in the renin-angiotensin system in the hypothalamus of SHR contributed to a decrease in sympathoinhibition and a resulting rise in arterial pressure. However, the current results do not confirm this role of angiotensin in NaCl-sensitive hypertension in the SHR. Although it is possible that captopril does not cross the blood-brain barrier effectively in the anterior hypothalamus of SHR, studies by Keep and Jones46 strongly suggest that captopril gains access to the brain, and in the present study, the lifetime captopril-treated SHR likely are exposed to the drug before the blood-brain barrier is fully competent.

Our previous studies strongly suggest that an overactivity of the sympathetic nervous system is responsible for the NaCl-sensitive hypertension in untreated SHR (eg, References 11 and 19). Two findings suggest that the same mechanism underlies the NaCl-induced hypertension in the lifetime captopril-treated SHR. First, in the lifetime captopril-treated SHR in the present study, a high NaCl diet elevates both arterial pressure and plasma norepinephrine concentration, suggesting that the release of norepinephrine from sympathetic nerve terminals is increased by the high NaCl diet in captopril-treated SHR. Our previous studies have demonstrated that a high NaCl diet elevates plasma norepinephrine by approximately the same extent in untreated SHR.18 Second, we have recently conducted preliminary experiments in which we tested the contribution of the sympathetic nervous system to the dietary NaCl-exacerbated hypertension in 8-week-old lifetime captopril-treated SHR that had been on a high NaCl diet for 2 weeks. In awake, freely moving rats (n=6 per group), ganglionic blockade with hexamethonium (100 mg/mL in saline; 1 mg/kg per minute for 10 minutes) decreased arterial pressure to the same level in lifetime captopril-treated SHR that had been on the high (before, 125±2 mm Hg; after, 72±5 mm Hg) compared with the basal (before, 100±4 mm Hg; after, 71±5 mm Hg) NaCl diet. This indicates that at this point in the course of the hypertension the dietary NaCl-mediated exacerbation of hypertension in the lifetime captopril-treated SHR is largely dependent on the sympathetic nervous system. Although these data suggest an important contribution of the sympathetic nervous system to NaCl-sensitive hypertension in both the lifetime captopril-treated and untreated SHR, future studies comparing the central and peripheral nervous system mechanisms underlying this form of hypertension are needed.

In summary, despite the powerful, dramatic effect of lifetime captopril treatment on basal arterial pressure in SHR on a basal NaCl diet, the treatment has little or no effect on the hypertensive response of SHR to dietary NaCl excess. Thus, the lifetime captopril-treated SHR may be a useful model in which the mechanisms of NaCl-sensitive hypertension can be dissociated from the mechanisms that regulate the non-NaCl-sensitive components of genetic hypertension.

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References


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