Influence of Hypertension and Dietary Copper on Indexes of Copper Status in Rats

Timothy A. Garrow, Michael S. Clegg, Gay Metzler, and Carl L. Keen

The Dahl salt-sensitive rat was used to investigate the effect of hypertension on indexes of copper status and to determine the extent to which dietary manipulation of copper attenuated, or exacerbated, the rate of sodium chloride-induced hypertension. Weanling salt-sensitive rats were fed, in a 2 × 3 factorial design, one of six diets that contained one of three levels of copper (2.0 μg/g marginal, 12 μg/g adequate, or 50 μg/g supplemental) and either control (0.4%) or high (4%) levels of sodium. Diets were fed to the rats for 11 weeks. Rats fed the high sodium diets were characterized by high plasma copper concentrations and ceruloplasmin activities compared with their respective control sodium rats. The magnitude of the sodium-induced rise in plasma copper and ceruloplasmin was affected by dietary copper intake; however, dietary copper intake had no effect on the development of hypertension in the high sodium groups. These results suggest that altered copper metabolism is secondary, rather than primary, to the development of sodium chloride-induced hypertension in the salt-sensitive rat. Red blood cell superoxide dismutase activity was reduced in rats fed the low copper diets compared with the adequate and supplemented copper groups. At the lower levels of copper intake, sodium chloride-induced hypertension increased red blood cell superoxide dismutase activity in a manner consistent with the plasma copper and ceruloplasmin changes observed. However, at adequate or supplemental levels of dietary copper, red blood cell superoxide dismutase activity plateaued, suggesting possible saturation of copper at sites of hematopoiesis. (Hypertension 1991;17:793–797)

We have previously reported that severely hypertensive Dahl salt-sensitive (DS) rats are characterized by elevated plasma copper concentrations.1 Similarly, Berthelot et al2 reported elevated serum copper concentrations in the spontaneously hypertensive rat (SHR) relative to normotensive controls and Schedl et al3 reported a trend of increased serum copper concentrations in the SHR relative to the Wistar-Kyoto (WKY) control rat. Elevated serum copper concentrations have also been reported in humans characterized by either essential or pulmonary hypertension.4,5 Furthermore, a number of disease states, including peripheral vascular damage, congestive heart failure, and ischemic heart disease, which are associated with hypertension in humans, have been reported to be characterized, in part, by elevated serum copper concentrations.6–8 Although the observation of high plasma copper concentrations may lead to the conclusion that the copper status of an individual is adequate, it has been suggested that plasma copper may not be a reliable indicator of copper nutritional status since over 90% of plasma copper is associated with the acute phase protein ceruloplasmin (Cp).9

Consistent with the idea that high plasma copper concentrations may be masking soft tissue copper depletion, Wester10 reported that atherosclerotic arteries were characterized by low concentrations of copper in the intima and media. Dubick et al11 and Keen et al12 have reported significantly lower activities of the copper-dependent enzyme copper-zinc superoxide dismutase (SOD) in vessel walls from hypertensive patients with aneurysm disease compared with vessel walls from traffic victims. Supportive of these findings, Tilson13 reported that patients who died from aortic aneurysms had significantly lower liver copper concentrations compared with controls.

Taken together, these data suggest that a hypertensive condition may result in mobilization of copper from liver to the plasma. This development could eventually result in reduced levels or activities of soft tissue cuproenzymes such as copper-zinc SOD, which could be deleterious to the animal.

The current study was designed to further delineate the effects of hypertension on copper metabolism. A number of functional markers of normal copper metabolism including tissue and erythrocyte...
copper-zinc SOD activity, organ/plasma copper concentrations, plasma Cp, total plasma cholesterol, and plasma uric acid concentrations were measured and compared in hypertensive and normotensive DS rats. Additionally, the degree to which dietary manipulation of copper amplifies or attenuates changes in these parameters was examined.

Methods

Weanling DS rats were obtained from a commercial source (Brookhaven National Laboratory, Upton, N.Y.). All rats were individually housed in stainless steel hanging cages in a light- (12-hour light/dark cycle) and temperature- (22–23°C) controlled room. The care of the rats and the experimental procedures used were in accord with the Animal Welfare Acts and the National Institutes of Health documents entitled Principles for the Use of Animals and Guide for the Care and Use of Laboratory Animals. Initially, all rats were fed ad libitum a semipurified control diet containing 12 μg copper/g, 50 μg zinc/g, and 0.4% sodium (no sodium chloride added) and distilled deionized water for 5 days to allow for adaptation to the environment. After the adaptation period, initial blood pressure and body weights were measured, and animals were randomly assigned to one of the experimental dietary groups described below. Initial mean blood pressures were similar among the groups and averaged 92 mm Hg. During the study period, six groups of DS rats were fed in a 2×3 factorial design, ad libitum, one of six semipurified diets, which differed in copper and sodium concentrations. Diets contained one of three levels of copper (2 [marginal], 12 [adequate], or 50 [supplemental] μg/g diet) and either 0.4% (control) or 4.0% (high) sodium. A seventh group was fed the adequate copper, low sodium diet in amounts consumed by its high sodium counterpart and thus served as restricted-fed control. The detailed composition of the basal diet has been reported. Sodium was added to the high sodium diets as the chloride salt displacing the appropriate amount of carbohydrate. All animals had free access to distilled deionized water throughout the study. Body weights were recorded weekly and systolic blood pressures were measured by the indirect tail-cuff method biweekly.1

On days 27, 48, and 69, tail blood samples were obtained for determination of plasma Cp oxidase activities. When mean blood pressures of approximately 190 mm Hg were attained in the rats consuming the 4.0% sodium diets, the study was terminated. Rats were anesthetized with carbon dioxide, opened along the ventral midline, and killed by cardiac puncture using heparinized syringes. An aliquot of blood was taken for hematocrit determination and the remaining blood was centrifuged at 1,500g for 20 minutes at 4°C. The plasma and red blood cells were stored at −70°C. Plasma was analyzed for copper concentration,15 uric acid,16 total cholesterol,17 and Cp oxidase activity.18 Copper-zinc SOD was assayed in red blood cell lysates after the removal of hemo-
globin by ethanol/chloroform extraction as described by Schacter et al.19 Values are expressed as activity per gram hemoglobin. Hearts and livers were removed and stored at −70°C until analyzed for SOD activities20 and copper concentrations.15

Data were analyzed by one-way and two-way analysis of variance. When analysis gave a significant F value (p<0.05), post hoc differences were evaluated using Fisher least significant difference (p<0.05).

Results

The restricted-fed group (0.4% sodium, 12 μg copper/g) did not differ from the ad libitum–fed group (0.4% sodium, 12 μg copper/g) in any parameter measured so their data were pooled. Independent of dietary sodium, dietary copper intake did not affect growth; however, rats consuming the high sodium diets had terminal body weights that averaged 18% lower than rats fed the low sodium diets (328±7 versus 398±4 g). Independent of dietary copper, rats consuming the high sodium diets had cardiomegaly and splenomegaly and also had lower hematocrits than rats fed the low sodium diets (data not shown).

Table 1 gives the terminal values obtained in this study. Rats fed high sodium diets had final blood pressures that were significantly higher than controls. Rats fed the low sodium and either the marginal or supplemental copper diets showed a moderate increase in blood pressure compared with the low sodium, adequate copper group. Independent of dietary sodium, liver copper concentrations were significantly lower in the rats fed the marginal copper diets than in the adequate or supplemental copper groups. Dietary copper and sodium had significant effects on plasma copper concentrations, plasma Cp, and red blood cell SOD activities. Specifically, the marginal copper groups had lower plasma copper concentrations, Cp activities, and red blood cell SOD activities than the other groups while the general effect of hypertension (high sodium) was to increase plasma copper and Cp compared with their control sodium counterparts. In addition, at the marginal level of copper intake, NaCl-induced hypertension increased red blood cell SOD activity in a manner consistent with the changes observed with plasma copper and Cp. Similar to liver, heart copper concentrations were lower in the marginal copper groups than in the adequate or supplemental copper groups; however, there was no influence of hypertension on this parameter (Table 1). Liver and heart copper-zinc SOD activities were lower in the marginal copper groups than in the adequate or supplemental copper groups. In contrast to our expectation, heart copper-zinc SOD activities tended to be higher in the high sodium groups compared with the control sodium groups; liver copper-zinc SOD activities were not consistently affected by sodium (Table 1). Heart and liver manganese SOD activities were unaffected by either dietary copper or sodium intake (data not shown). Figure 1 depicts changes in blood pressure in
TABLE 1. Final Systolic Blood Pressures and Indexes of Copper Status of Dahl Salt-Sensitive Rats Fed 0.4% or 4.0% Sodium and a 2, 12, or 50 μg Copper/g Diet

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dietary Na (% Na)</th>
<th>Dietary Cu (μg/g)</th>
<th>n</th>
<th>Final SBP (mm Hg)</th>
<th>Liver Cu (μg/g)</th>
<th>Plasma Cu (μg/g)</th>
<th>Heart Cu (μg/g)</th>
<th>Cpx units/l</th>
<th>Red blood cell SOD units/mg Hb</th>
<th>Liver ZnSOD (units/mg)</th>
<th>Heart Cu/ZnSOD (units/mg)</th>
<th>Plasma cholesterol (mg/dl)</th>
<th>Plasma uric acid (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.4</td>
<td>2</td>
<td>7</td>
<td>148±4*</td>
<td>1.6±0.2*</td>
<td>0.3±0.1*</td>
<td>2.2±0.2*</td>
<td>137±7*</td>
<td>681±63*</td>
<td>47±12*</td>
<td>15±3*</td>
<td>83±7*</td>
<td>2.3±0.3</td>
<td></td>
</tr>
<tr>
<td>4.0</td>
<td>2</td>
<td>8</td>
<td>191±5†</td>
<td>1.9±0.3*</td>
<td>0.5±0.1*</td>
<td>2.7±0.4*</td>
<td>37±14*</td>
<td>882±86*</td>
<td>57±14*</td>
<td>25±4*†</td>
<td>100±5†</td>
<td>1.8±0.1</td>
<td></td>
</tr>
<tr>
<td>0.4</td>
<td>12</td>
<td>24</td>
<td>130±1†</td>
<td>3.1±0.2†</td>
<td>1.1±0.1†</td>
<td>4.0±0.2†</td>
<td>138±5†</td>
<td>1,439±49†</td>
<td>101±3†</td>
<td>34±3††</td>
<td>79±3*</td>
<td>2.3±0.2</td>
<td></td>
</tr>
<tr>
<td>4.0</td>
<td>12</td>
<td>7</td>
<td>195±5†</td>
<td>3.5±0.2†</td>
<td>1.9±0.2†</td>
<td>4.2±0.1†</td>
<td>359±46†</td>
<td>1,584±70†</td>
<td>82±5‡</td>
<td>36±4††</td>
<td>99±7†</td>
<td>2.6±0.2</td>
<td></td>
</tr>
<tr>
<td>0.4</td>
<td>50</td>
<td>7</td>
<td>148±4*</td>
<td>3.2±0.2†</td>
<td>1.0±0.1†</td>
<td>3.8±0.6</td>
<td>124±5†</td>
<td>1,472±108†</td>
<td>110±4†</td>
<td>27±2†</td>
<td>87±2*†</td>
<td>2.8±0.6</td>
<td></td>
</tr>
<tr>
<td>4.0</td>
<td>50</td>
<td>7</td>
<td>189±2†</td>
<td>3.7±0.2†</td>
<td>2.6±0.2§</td>
<td>4.5±0.3</td>
<td>377±37††</td>
<td>1,514±73††</td>
<td>137±17§</td>
<td>43±5†</td>
<td>132±11†</td>
<td>2.1±0.2</td>
<td></td>
</tr>
</tbody>
</table>

Treatment effects (p values)
- Na <0.01 NS <0.01 0.06 <0.01 <0.06 NS 0.01 0.01 NS
- Cu <0.08 <0.01 <0.01 0.01 <0.01 <0.01 0.02 0.01 0.01 NS
- Na×Cu <0.01 NS <0.01 NS <0.01 NS 0.02 NS NS NS

Group values are mean ± SEM. Tissue copper levels expressed per gram wet weight. Cu, copper; Na, sodium; SBP, systolic blood pressure; CP, ceruloplasmin; SOD, superoxide dismutase; Zn, zinc.

*†‡§Different superscripts indicate significant differences at <p<0.05 (vertical comparison).

Discussion

The plasma data are consistent with our earlier report that sodium chloride-induced hypertension in the DS rat is accompanied by alterations in copper metabolism. Specifically, in each group of rats consuming high sodium diets, terminal plasma copper concentrations and cp activities were significantly elevated with respect to their control sodium controls. It appears from the serial plasma and blood pressure data (Figure 1) that elevation of plasma copper and cp activity is preceded by the elevation in blood pressure, perhaps signaling pathological developments. The observation that dietary copper manipulation had no impact on the magnitude or duration of the blood pressure rise indicates that changes in the adequate copper, low and high sodium groups over time and the mean cp activities on days 27, 48, 69, and 76 of rats fed these diets. On days 69 and 76, the hypertensive animals had significantly elevated levels of plasma cp oxidase activity compared with the normotensive controls (p<0.05). The elevation of plasma cp oxidase activity occurred after systolic blood pressure was already significantly elevated. Plasma cp oxidase activities were significantly correlated to plasma copper levels (p²=0.90; p<0.05).

Although plasma uric acid has been reported to be elevated in severely copper-deficient rats, neither marginal copper intake nor hypertension affected uric acid levels in the current study. Consistent with previous reports, there was a trend of increased total cholesterol in rats consuming the high sodium chloride diets compared with those fed the control sodium diets (Table 1). Marginal copper intake did not elevate total plasma cholesterol acid as has been reported for severely copper-deficient rats.

**Discussion**

The plasma data are consistent with our earlier report that sodium chloride–induced hypertension in the DS rat is accompanied by alterations in copper metabolism. Specifically, in each group of rats consuming high sodium diets, terminal plasma copper concentrations and Cp activities were significantly elevated with respect to their control sodium controls. It appears from the serial plasma and blood pressure data (Figure 1) that elevation of plasma copper and Cp activity is preceded by the elevation in blood pressure, perhaps signaling pathological developments. The observation that dietary copper manipulation had no impact on the magnitude or duration of the blood pressure rise indicates that changes in
copper metabolism occur secondary to the development of hypertension.

The changes in plasma copper levels were highly correlated with increases or decreases in plasma Cp activity \(^{2} \text{(r} = 0.98, \text{p} < 0.05)\), this relation held across all dietary groups. Cp is a copper-containing glycoprotein enzyme postulated to have many diverse metabolic roles including free radical quenching, mobilization of iron stores, and transport and donation of copper to extrahapatic sites.\(^{9}\)

Cp is an acute-phase protein and as such takes part in the acute-phase response that occurs naturally in response to tissue injury or infection.\(^{9}\) Cp, like many other acute-phase proteins, is both transcriptionally and translationally responsive to several monokines, including interleukin 1 \(\beta\) (IL-1\(\beta\)), interleukin 6 (IL-6) tumor necrosis factor \(\alpha\), and hepatocyte stimulating factor, which are released by activated macrophages at or near the inflammatory site.\(^{9,23-25}\) Acute effects of monokines on Cp synthesis may be supported chronically by elevated levels of glucocorticoids and epinephrine,\(^{9,26}\) which are characteristic of hypertensive animals including humans.\(^{27,28}\)

In the present study, hypertensive animals were not characterized by low liver copper concentrations, findings which are in contrast to our previous report.\(^{1}\) In our opinion, the dichotomy between the previous and current reports may reside with the degree of hypertension induced in the animals during the two studies. In the former study, the hypertensive DS rats had terminal mean systolic blood pressures greater than 210 mm Hg, whereas in the present study, rats were killed before they obtained mean blood pressures of 200 mm Hg. Thus, we suggest that as the elevation of blood pressure and severity of hypertension increases with time, copper is mobilized from hepatic stores in increasing amounts into its circulating form, Cp. Eventually, this state will be reflected by low liver copper concentrations.

Despite the lack of a statistical effect of dietary copper intake on the rate and severity of the observed hypertension in the DS rat, both the normotensive marginal and normotensomal supplemental copper groups showed increases (albeit small relative to salt-loading effects) in blood pressure relative to the normotensive control (12 \(\mu\)g copper/g diet) group. These data confirm the reports of others that both a dietary deficiency and a dietary excess of copper can result in the elevation of blood pressure.\(^{29-31}\)

The mechanisms underlying the effects of copper on blood pressure regulation are unknown; recent work suggests that one effect of severe copper deficiency on blood pressure regulation may be in part due to a reduction in circulating erythrocyte and soft tissue copper-zinc SOD activities, which are correlated with significant reductions in the synthesis of prostaglandin I \(_2\) favoring vasoconstriction.\(^{32,33}\)

Copper deficiency can also be postulated to affect blood pressure via an increased turnover of endothelium-derived relaxing factor secondary to copper deficiency-associated reductions in copper-zinc SOD activity since the turnover of endothelium-derived relaxing factor is in part dependent on the concentration of superoxide radicals.\(^{34}\) A third mechanism by which copper deficiency could be postulated to influence blood pressure regulation would be via an associated reduction in Cp activity, as this enzyme also has SOD activity.\(^{35,36}\)

It should be stressed that, although the rate of development of hypertension in the DS rat is not affected by relatively large variations in dietary copper intake and tissue copper concentrations, this does not rule out the possibility that in normotensive animals, marginal copper diets may result in an elevation in systolic blood pressure as a consequence of decreased synthesis or stability of vasodilator compounds. In addition, it can be predicted that the rate and severity of hypertension-induced vascular damage could be amplified in copper-deficient animals due to their compromised antioxidant status.

Finally, it is interesting to note that it has been suggested that red blood cell SOD activity may be a valuable indicator of copper status.\(^{37}\) However, the current results suggest that the relation between plasma copper or Cp and red blood cell SOD is hyperbolic and therefore the measurement of erythrocyte SOD activity may not be a reliable indicator of copper status. Specifically, under marginal copper nutritive and states of prolonged physiological stress, red blood cell SOD activity levels may be high due to the chronically elevated plasma copper associated with Cp that may not correlate with soft tissue copper concentrations.

References

37. Institute of Medicine, Food and Nutrition Board: Recommended Dietary Allowances, ed 10. Washington, DC, National Academy of Sciences, 1989

KEY WORDS: sodium-dependent hypertension • ceruloplasmin • superoxide dismutase • copper • atherosclerosis • coronary disease • Dahl rats
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