Hypertension-Induced Alterations in Copper and Zinc Metabolism in Dahl Rats

MICHAEL S. CLEGG, FAY FERRELL, AND CARL L. KEEN

SUMMARY It has been suggested that one risk factor in the development of hypertension and vascular disease may be abnormal copper and zinc metabolism. In the current study we tested the hypothesis that hypertension itself may result in alterations in the metabolism of these essential elements. Dahl salt-sensitive rats were fed diets containing 0.4 or 8.0% NaCl for 32 days. At the conclusion of the study, blood pressure was significantly higher in the rats fed a high NaCl diet than in controls. Liver, kidney, and heart copper concentrations were significantly lower in the rats fed a high NaCl diet compared with controls, while plasma copper levels were higher. In contrast, tissue zinc levels were higher in the rats fed a high NaCl diet than in controls, while plasma zinc levels were lower. It is hypothesized that alterations in copper and zinc metabolism may be one factor underlying tissue damage in these animals. (Hypertension 9: 624–628, 1987)

KEY WORDS • hypertension • copper • zinc • Dahl rats

ISCHEMIC heart disease is the primary cause of death in the industrialized world. While the etiology of ischemic heart disease is without question multifactorial, several potential risk factors have been suggested. During the last decade there has been considerable interest in the hypothesis that one of these factors may be copper deficiency. This hypothesis has been based on the dual observations that severe Cu deficiency in experimental animals can result in connective tissue defects and that the dietary intake of Cu is often below that recommended by health agencies.1,2 The risk of Cu deficiency is also thought to be increased by the consumption of excess zinc in the diet, as these two elements can be biologically antagonistic.1-3 In support of the hypothesis that abnormal Cu metabolism may be a factor underlying some types of vascular disease, we have recently shown that abdominal aortic tissue removed from patients suffering from abdominal aeurysms or occlusive disease is characterized by low activity of the copper-containing enzyme superoxide dismutase.4

A second factor that has been reported to result in an increased risk of cardiovascular disease is hypertension.1 Interestingly, abnormal trace element metabolism has been reported in some hypertensive patients,1,4-6 and one effect of dietary Cu deficiency has been reported to be an induction of hypertension.10 Thus, it is reasonable to suggest that these two risk factors may be interrelated in some way. In the current report we present evidence that the development of hypertension in an experimental animal model can result in altered Cu and Zn metabolism. Based on results presented we hypothesize that the induced alterations in Cu and Zn metabolism may contribute to some of the vascular pathology associated with hypertension.

Materials and Methods

The tissues analyzed in this study were collected from animals that were being used to investigate potential hypertension-induced changes in taste preference for calcium solutions. The taste preference study was performed to test the hypothesis that behavioral changes in appetite for calcium might be an adaptive response to hypertension. Data regarding the taste preference changes are presented elsewhere.11

Sixty-four weanling male Dahl salt-sensitive rats (DS) were obtained from a commercial source (Brookhaven National Laboratory, Upton, NY, USA). This rat strain is considered to be a model for salt-sensitive, low-renin hypertensive humans.12,13 Animals were housed individually in stainless steel hanging cages in a quiet, light-controlled (12 hour light/12 hour dark cycle) room at 23°C. All conditions concerning the care of the rats and the experimental procedures used...
followed the standards established by the Animal Welfare Acts and by the National Institutes of Health documents entitled Principles for the Use of Animals and Guide for the Care and Use of Laboratory Animals. The rats were assigned to dietary treatments and fed Purina 5012 rat chow (Ralston-Purina Co., St. Louis, MO, USA), a constant formula rat diet containing 1.01% calcium and either 0.4% (n = 31) or 8.0% NaCl (n = 33). The diets contained 55 μg of Zn and 12 μg of Cu per gram of diet. These diets were specifically formulated by Purina for use by researchers investigating the Dahl rat strains at Brookhaven National Laboratory.

All animals were fed their respective diets and distilled deionized water ad libitum throughout the study, which lasted a total of 32 days. Animals in the 8.0% (n = 33) NaCl group were subdivided into three groups that were tested for taste preferences for distilled-deionized water (n = 15), water containing calcium chloride (0.002–0.05 M; n = 9), or water containing calcium lactate (0.002–0.05 M; n = 9) during the first 3 weeks of the study. Body weights were recorded regularly throughout the study, and four blood pressure measurements were obtained from each animal using the indirect tail-cuff method.14

On Day 32 of the study animals were anesthetized with ether. Animals were opened along the ventral midline, exposing the diaphragm and heart. The diaphragm was cut, and blood was removed from the heart by cardiac puncture using heparinized syringes. An aliquot of blood was taken from each sample for determination of hematocrit values. The remaining blood was centrifuged at 1500 g for 20 minutes at 4°C. Plasma was collected using trace element-free plastic pipettes and stored frozen at —20°C in acid-washed plastic vials. Liver, kidney, heart, and spleen were collected from each animal, weighed, and stored frozen at —20°C in acid-washed plastic vials until analyzed.

Plasma was diluted 1:5 with 0.1 N HNO3, and analyzed for mineral concentrations. Tissue samples were wet-ashed with concentrated nitric acid, prepared, and analyzed for trace elements as described by Clegg et al.15 Cu and Zn concentrations were determined by flame atomic absorption spectrophotometry (Model IL551; Instrumentation Laboratories, Lexington, MA, USA). In addition to Cu and Zn concentrations, the levels of iron, calcium, magnesium, sodium, and potassium were determined in these tissues; however, these data are not shown due to the lack of significant changes in their concentrations.

Data were analyzed by one-way analysis of variance. When analysis gave a significant F value (p < 0.05), post hoc differences were evaluated using Scheffe’s test (p < 0.05).

**Results**

There were no significant differences between the 8% NaCl + calcium lactate and 8% NaCl + calcium chloride groups; therefore, data from these groups were combined. Thus, for statistical purposes there were three groups: 0.4% NaCl (control), 8% NaCl, and 8% NaCl + calcium.

Both groups of rats fed a high NaCl diet had significantly retarded growth and higher terminal systolic blood pressure compared with controls (Table 1 and Figure 1), and rats in the high NaCl + calcium group had slightly higher blood pressures than did rats in the high NaCl-distilled water group. Rats in both of the high NaCl groups were characterized by low hematocrits and splenomegaly, suggesting the occurrence of a hemolytic anemia in these animals (see Table 1). There was no evidence of infection in any of the animals.

<table>
<thead>
<tr>
<th>Table 1. Body, Heart, and Spleen Weights and Hematocrits of DS Fed 0.4% or 8% NaCl for 32 Days</th>
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<td><strong>Diet</strong></td>
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<td>Hematocrit (%)</td>
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Values are means ± SE.
*p < 0.05, compared with control values.

**Figure 1.** Systolic blood pressure of DS fed diets containing 0.4% NaCl (■; n = 31), 8.0% NaCl (■■; n = 15), or 8.0% NaCl + Ca (■■■; n = 18). Values are mean ± SE. Values significantly different (p < 0.05) from controls (0.4% NaCl) are indicated by an asterisk.
Plasma and soft tissue Cu and Zn concentrations are shown in Figures 2 through 4. Plasma Cu concentrations were significantly higher in rats fed the high NaCl diet than in controls, while plasma Zn concentrations were significantly lower in rats fed a high NaCl diet than in controls (see Figure 2). Liver, kidney, and heart Cu concentrations were significantly lower in rats fed a high NaCl diet than in control rats (see Figure 3). Liver and heart Zn concentrations were significantly higher in rats fed a high NaCl diet than in controls. Kidney Zn concentrations were not significantly different among the groups (see Figure 4).

Discussion

The results from this study show that NaCl-induced hypertension in DS is accompanied by marked alterations in plasma and soft tissue Cu and Zn metabolism. A possible explanation for the observed changes in tissue Cu and Zn metabolism is that the stress of the hypertensive condition resulted in an increase in glucocorticoid synthesis and release. Cousins has shown that glucocorticoids have profound effects on Zn and Cu metabolism that are similar to the effects we have observed in the hypertensive Dahl rat.

Glucocorticoids can stimulate synthesis of the Cu-containing protein ceruloplasmin in the liver. Once ceruloplasmin is synthesized, it is transported into the blood; thus, an increase in liver ceruloplasmin synthesis could result in a reduction in liver Cu concentrations with a concomitant increase in plasma Cu concentrations.

The biological reasons for increased liver ceruloplasmin synthesis during periods of tissue injury and stress are not clear. It has been proposed that ceruloplasmin acts as an anti-inflammatory agent because of its ferroxidase and superoxide dismutase activity. A second function that has been ascribed to this protein is the transfer of Cu to extrahepatic tissue through a receptor-mediated process. However, in the current study it was observed that, despite high Cu plasma levels, extrahepatic tissue Cu concentrations were lower in the hypertensive rats than in controls. One interpretation of this result is that in the hypertensive condition, there is a reduction in tissue uptake of Cu, possibly due to alterations in the ceruloplasmin receptor proteins. Regardless of the biochemical lesion underlying the lower tissue Cu concentrations in the hy-
pertensive rats, the observation of the lower values is important because extrahepatic tissue stores of Cu are thought to be minimal. Thus, a reduction in extrahepatic tissue Cu concentrations may reflect a reduction in the activities of Cu metalloenzymes, such as Cu,Zn-superoxide dismutase, cytochrome C oxidase, and lysyl oxidase. This idea is consistent with our recent observation of low Cu,Zn-superoxide dismutase activity in abdominal aortic tissue from hypertensive patients undergoing operation for treatment of occlusive disease or aneurysms. Significantly low hepatic Cu concentrations, and low skin Cu concentrations, have also been reported to occur in patients with abdominal aortic aneurysms. Based on these findings, it is reasonable to suggest that hypertension-induced reductions in the activities of Cu enzymes such as superoxide dismutase and lysyl oxidase may result in vessel pathology secondary to the hypertension.

One sign of Cu deficiency can be an iron-refractory anemia. However, while it is tempting to suggest that the anemia observed in the hypertensive rats was the result of a hypertension-induced Cu deficiency, we do not think this was the case. The primary mechanism by which Cu deficiency has been suggested to result in anemia is through a reduction in serum ceruloplasmin activity, which leads to a reduction in the incorporation of iron into transferrin, resulting in a reduction of iron delivery to sites of hematopoiesis. In the current study plasma Cu concentrations were increased in the hypertensive animals; thus, it is unlikely that the anemia was the result of decreased iron delivery to hematopoietic sites.

A final observation that can be drawn from the Cu data is that measurement of circulating levels of this element in the hypertensive patient may be misleading, as high levels do not provide evidence that soft tissue Cu concentrations are normal. Thus, better methods for assessing soft tissue Cu concentrations need to be established.

As discussed for Cu, the biological reasons for the increased Zn concentration in soft tissue and the concomitant reduction in plasma Zn levels during periods of injury and stress are not known. However, with regard to hypertension, it is interesting that the serum activity of angiotensin converting enzyme in rats and guinea pigs has been found to be dependent on serum Zn concentrations. The two roles for angiotensin converting enzyme in animals are to catalyze the formation of angiotensin II and to degrade bradykinin. Angiotensin II acts to increase blood pressure, while bradykinin acts to reduce it; thus, a reduction in angiotensin converting enzyme activity would be expected to have a hypertensive effect. Indeed, hypotension has been reported to be a sign of prolonged Zn deficiency in rodents. Thus, it may be hypothesized that the hypertension-induced change in plasma Zn concentration may be a positive response to the hypertensive condition. Glucocorticoids increase the synthesis of the liver Zn-binding protein metallothionein. An increase in the amount of this protein in liver could result in increased liver Zn concentrations and decreased plasma Zn concentrations.

In addition to glucocorticoids, epinephrine has been shown to increase serum ceruloplasmin levels in both intact and adrenalectomized rats. This observation is particularly important in light of the fact that elevated plasma catecholamine levels have been demonstrated in up to 40% of patients with essential hypertension. Increased serum Cu levels have been reported in some humans with essential hypertension. Epinephrine can also stimulate the synthesis of metallothionein. Thus, overall, hypertension-induced increases in glucocorticoids and epinephrine may be acting in concert to produce some of the changes observed in the hypertensive animal with respect to Zn and Cu metabolism.

In summary, the results of this study show a marked effect of hypertension on Cu and Zn metabolism in the DS. The hypertension-induced changes in plasma Zn concentrations may represent a positive response of the animal to the hypertensive condition, while the changes observed in Cu metabolism may result in additional vessel pathology. The finding of altered Cu and Zn metabolism in the hypertensive Dahl rat is consistent with reports of altered Cu and Zn metabolism in hypertensive humans.

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Hypertension-induced alterations in copper and zinc metabolism in Dahl rats.
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Hypertension. 1987;9:624-628
doi: 10.1161/01.HYP.9.6.624

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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the World Wide Web at:
http://hyper.ahajournals.org/content/9/6/624

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