Structural Vascular Changes in Hypertension
Role of Angiotensin II, Dietary Sodium Supplementation, and Sympathetic Stimulation, Alone and in Combination in Rats

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Abstract—Elevated circulating angiotensin (Ang) II levels, dietary sodium, and sympathetic stimulation are recurrent themes of hypertension research, but their in vivo interaction in physiologically meaningful doses has not been adequately investigated. In this study, the interaction of a subpressor dose of Ang II (50 ng \( \cdot \) kg \(^{-1} \) \cdot \) min \(^{-1} \) SC), 2% NaCl diet, and sympathetic stimulation in the form of overnight cold exposure was investigated in the development of hypertension and of structural vascular changes in male Sprague-Dawley rats. There were 8 experimental groups: sham operation and treatment (control), Ang II, 2% NaCl diet, cold exposure (5°C), Ang II plus 2% NaCl diet, Ang II plus cold exposure, cold exposure plus 2% NaCl diet, and Ang II plus 2% NaCl diet plus cold exposure (triple treatment). For each group, the duration of treatment was 12 weeks. Morphometric measurements of maximally dilated, in situ fixed, second-order (250 to 320 \( \mu \)m OD), intermediate-size (100 to 150 \( \mu \)m OD), and small (50 to 100 \( \mu \)m OD) mesenteric arteries were performed, and wall-to-lumen ratios (W/L) were calculated. During the 12-week study, the blood pressure (BP) load (the area under the systolic BP curve) of rats receiving the combined treatment of Ang II and 2% NaCl diet was increased \((P<0.05)\), and that of rats receiving the combined treatment of cold exposure and 2% NaCl diet was decreased \((P<0.05)\); there were no BP changes in the remaining groups of rats. The most pronounced changes among groups occurred in W/L of small resistance arteries. The W/L of small arteries increased in Ang II–treated \((P<0.01)\) and in cold-stressed rats \((P<0.01)\). The effect of Ang II was potentiated by the addition of a 2% NaCl diet. In contrast, the addition of 2% NaCl diet to cold stress reduced the W/L of small arteries \((P<0.01)\). No other positive or negative synergism occurred among groups, including the rats receiving triple treatment. The findings confirm the potentiation of the hypertensinogenic and vascular trophic effects of Ang II by a high-sodium diet but do not provide evidence for synergism between Ang II and sympathetic stimulation. The finding of hypotension and reduced W/L of small resistance arteries in rats receiving the combined treatment of cold stress and high-sodium diet is unique because there are few known nonpharmacological vascular “hypotrophic” stimuli. The ultimate test of the hypertensinogenic potential of pressor stimuli alone or in combination is their long-term administration in physiologically meaningful doses to experimental animals. (Hypertension. 2001;37:255-260.)

Key Words: arteries ■ circulation ■ mesenteric arteries

Essential hypertension is a disease of slow onset; significant elevations of blood pressure (BP) may not be detected until the third or fourth decade of life. In the early, so-called borderline stage of essential hypertension, a large number of hemodynamic, nervous system, and hormonal abnormalities have been detected, but no single alteration was identified as causative. It has been suggested some time ago that essential hypertension may be a “mosaic” of interactive stimuli that are initially subpressor but over time will lead to the gradual development of hypertension.\(^1,2\) There have been, however, few experimental models of these interactive stimuli that lead to the gradual development of hypertension.

Of all the potential causes of essential hypertension, 3 stimuli have stood the test of time and can be referred to as the recurrent themes of hypertension research: dietary sodium excess, increased sympathetic activity, and elevated circulating angiotensin (Ang) II levels. There are indications that these stimuli interact synergistically to raise BP.\(^3-6\) The evidence is strong in the case of dietary sodium supplementation and pressor doses of Ang II but is less well established in the case of initially subpressor doses of Ang II and dietary sodium supplementation or in the case of sympathetic activation combined with dietary sodium supplementation or with increased circulating Ang II levels. To our knowledge, the long-term, cumulative hypertensinogenic action of these 3 stimuli combined has not been investigated.

In this study, we have investigated the interaction of initially subpressor doses of Ang II, dietary sodium supplementation, and sympathetic activation in the development of hypertension and of structural vascular changes in otherwise
normal adult rats. Structural vascular changes were measured because they are the hallmark of chronic hypertension.\(^7,8\) The suppressor doses of Ang II and of dietary sodium supplementation in rats have been well established,\(^6,9\) but the choice of experimental sympathetic stimulation has been difficult. Sympathetic stimulation had to be quantifiable and sustainable for 3 months. Previously, we found no cumulative effect of phenylephrine administration in initially suppressor doses on either the BP or small-artery structure of rats.\(^10\) We chose, therefore, exposure of rats to cold to chronically stimulate the sympathetic nervous system.\(^11,12\) Continuous exposure of rats to 5°C for several months has been shown to lead to mild-to-moderate hypertension that was sustained after removal from the cold. The stimulus, however, may be too stressful because young rats chronically exposed to cold fail to gain weight normally.\(^10\) In this study, cold stress was limited to overnight exposure. We hypothesized that Ang II administration, dietary sodium supplementation, and chronic sympathetic activation, which, by themselves have minimal or no effect on the BP of rats, will interact when combined to produce hypertension and structural vascular changes.

Methods

Design of Experiments
Pathogen-free male Sprague-Dawley rats (Sasco, Omaha, Neb) were used throughout these studies. Male rats were investigated to eliminate the changes in the renin-angiotensin system that accompany the estrous cycle in female rats. At the beginning of the experiments, the rats weighed 400 to 450 g. Young adult rats were chosen because Ang II treatment may influence the growth of rats independent of its pressor effect.\(^13\)

Eight experimental groups were investigated; each group received treatment for 12 weeks. The following treatments were administered (Tables 1, 2, and 3): sham operation and treatment (control), 50 ng · kg\(^{-1} \cdot \text{min}^{-1} \cdot \text{SC} \) Ang II, 2% NaCl diet (salt-fed), cold exposure (cold-stressed), 50 ng · kg\(^{-1} \cdot \text{min}^{-1} \cdot \text{SC} \) Ang II plus 2% NaCl diet, 50 ng · kg\(^{-1} \cdot \text{min}^{-1} \cdot \text{SC} \) Ang II plus cold exposure, cold exposure plus 2% NaCl diet, and 50 ng · kg\(^{-1} \cdot \text{min}^{-1} \cdot \text{SC} \) Ang II plus cold exposure plus 2% NaCl diet (triple treatment). On the basis of previous measurements of plasma Ang II concentrations of rats in our laboratory, the dose of Ang II used in this study is expected to raise plasma Ang II levels by 60% to 70%.\(^14\) The control rats were interspersed, 2 to 4 at a time, with the treated groups of rats.

Preparation of Rats
Alza model 2 ML4 (28-day) minipumps implanted subcutaneously were used to deliver Ang II for 4 weeks. To deliver Ang II for 12 weeks, the empty minipumps were surgically removed at weeks 4 and 8, and new ones were implanted. Procedures for filling and implantation of the minipumps have been reported.\(^15\) All the rats not receiving Ang II infusion were fitted with empty resterilized minipumps to reduce costs. Like the Ang II–containing minipumps, the empty minipumps were removed at weeks 4 and 8, and new ones were implanted. For cold exposure, the rats were placed in a 5°C room overnight (from approximately 6 PM to 6 AM) on weekdays and from Friday 4 PM to Saturday 10 PM on weekends. All rats were housed 2 per box. All rats not on 2% NaCl diet received “normal” sodium diet (0.7% NaCl). The diets were matched for other ingredients, including potassium (200 mmol/kg). The diets were prepared on order by Harlan Teklad.

Tail Systolic BP Measurements
Systolic BP (SBP) was measured in restrained, awake rats by the tail-cuff method (Narco Biosystems) between 8 and 11 AM as previously described.\(^14\) After insertion of the first minipump, the SBP of rats was measured weekly for 4 weeks and then every 2 weeks for the rest of the experiment. The BP load that rats were exposed to during the experiments was calculated as the area under the SPB curve.\(^14\) The rats were weighed to the nearest 1 g on the day of the final experiments.

Intra-Arterial BP Measurements
For direct measurement of BP, rats were fitted with a femoral artery catheter 7 to 10 days before the end of the 12-week treatment period.\(^14\) After a 2-day recovery period, the arterial catheter was connected to a pressure transducer (Kent Scientific Corp.), and the mean arterial BP (MAP) was monitored continuously for 2 hours between 8 AM and 12 PM on 3 separate days.\(^14\) During monitoring, rats moved freely in their boxes. The average MAP was calculated from collected data for each day, and the average daily MAP during 3 days of measurements was calculated.

For comparison of BPs in the cold and at room temperature, in 6 rats receiving the combined treatment of overnight cold exposure and high-sodium diet for 4 to 8 weeks, MAP on day 2 of monitoring was measured in the cold room (5°C) instead of in the laboratory.

In Situ Tissue Fixation
The in situ fixation of the mesenteric vascular bed of rats was performed at maximal vasodilatation and 55 to 60 mm Hg perfusion pressure as previously reported.\(^14,15\) To achieve maximal vasodilatation, chloralose-anesthetized rats were given intravenous papaverine, 500 μg/rat, and 1 to 2 minutes later the rats were given an overdose of papaverine (2 to 3 mg IV per rat). Perfusion pressure was kept at 55 to 60 mm Hg because we have found in previous experiments that after maximal systemic vasodilatation, MAP falls to this level in normotensive rats.\(^16\) The mesenteric vascular bed was perfused sequentially with Krebs-Ringer solution containing 75 μg/ml papaverine for 8 to 10 minutes, with fixative containing 2.5% glutaraldehyde, 1.86% sucrose, and 0.063 mol/L phosphate buffer.

### Table 1. Tail Systolic Blood Pressures of Treated and Control Rats

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Baseline</th>
<th>wk 1</th>
<th>wk 2</th>
<th>wk 3</th>
<th>wk 4</th>
<th>wk 6</th>
<th>wk 8</th>
<th>wk 10</th>
<th>wk 12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>21</td>
<td>116±3</td>
<td>118±3</td>
<td>124±4</td>
<td>122±4</td>
<td>121±4</td>
<td>119±3</td>
<td>121±3</td>
<td>119±3</td>
<td>113±2</td>
</tr>
<tr>
<td>AngII</td>
<td>15</td>
<td>112±3</td>
<td>119±4</td>
<td>122±4</td>
<td>124±4</td>
<td>127±4</td>
<td>123±3</td>
<td>125±4</td>
<td>123±3</td>
<td>121±3</td>
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<tr>
<td>2% NaCl</td>
<td>10</td>
<td>110±4</td>
<td>113±3</td>
<td>120±2</td>
<td>119±3</td>
<td>123±3</td>
<td>120±3</td>
<td>123±3</td>
<td>119±2</td>
<td>117±2</td>
</tr>
<tr>
<td>Cold-stress</td>
<td>10</td>
<td>116±3</td>
<td>120±4</td>
<td>115±4</td>
<td>116±4</td>
<td>120±3</td>
<td>114±3</td>
<td>116±3</td>
<td>113±2</td>
<td>115±3</td>
</tr>
<tr>
<td>AngII+2% NaCl</td>
<td>15</td>
<td>114±3</td>
<td>116±3</td>
<td>126±5</td>
<td>134±4</td>
<td>136±4</td>
<td>129±5</td>
<td>141±6</td>
<td>134±5</td>
<td>138±7</td>
</tr>
<tr>
<td>AngII+cold</td>
<td>11</td>
<td>116±3</td>
<td>116±4</td>
<td>118±4</td>
<td>131±7</td>
<td>132±5</td>
<td>132±4</td>
<td>130±3</td>
<td>122±4</td>
<td>129±3</td>
</tr>
<tr>
<td>Cold+2% NaCl</td>
<td>12</td>
<td>110±3</td>
<td>109±3</td>
<td>105±3</td>
<td>108±2</td>
<td>104±2</td>
<td>105±2</td>
<td>110±3</td>
<td>106±2</td>
<td>111±2</td>
</tr>
<tr>
<td>AngII+cold+2% NaCl</td>
<td>13</td>
<td>120±3</td>
<td>114±6</td>
<td>110±4</td>
<td>120±4</td>
<td>127±6</td>
<td>121±4</td>
<td>120±3</td>
<td>122±5</td>
<td>118±4</td>
</tr>
</tbody>
</table>

Values are mean±SEM.
Wall-to-lumen ratio (mean±SEM) of second-order and intermediate-size and small resistance mesenteric arteries of treated rats compared with that of control rats (C); number of rats is shown at base of each bar. All indicates Ang II treatment; Na, 2% NaCl diet; and Cld, cold. *P<0.01; †P<0.05; ‡P<0.001.

(pH 7.4, 400 mOsm) for 15 minutes, and with 0.200 mol/L phosphate buffer for 8 to 10 minutes. At the end of perfusion, the entire small intestine and mesenteric vascular arcade were removed for further processing.

Morphometric Measurements

Proceeding from the duodenum, mesenteric vascular arcades with Y-configuration of the second-order branches of the superior mesenteric artery were identified. A minimum of 2 second-order arteries were dissected free and cleared of adhering tissue. A 3-mm-long midsegment of each artery was dehydrated through graded series of ethanol and embedded in epoxy resin. Semithin sections, 1 μm thick, with 2 to 4 arteries per rat were averaged. The thickness of individual smooth muscle cells was measured at ×1000 magnification with oil immersion at 4 points along the vessel wall.13 Cell thickness was measured at the midpoint of the nucleus along the short axis of vascular smooth muscle cells. Cell thickness measurements were averaged for each rat.

For morphometric measurements of small (<150 μm OD) resistance arteries, four to six 2- to 3-cm segments of mesenteric-intestinal junction were embedded in paraffin as previously described.14,15 Cuts were made at right angles and parallel to the mesenteric intestinal junction; this resulted in cross-sectional cuts of mesenteric arteries just proximal to their entry into the intestinal wall. Sections of 4-μm thickness were stained with hematoxylin and eosin and examined in a light microscope. Two criteria were used to select arteries for morphometric measurements. First, by inspection, the thickness of the vessel wall had to be uniform throughout its circumference. Second, only vessels with a long- to short-axis ratio of <1.50 were measured. In this way, the error caused by calculating the diameter by averaging the maximal and minimal diameters would be 3%.17 Morphometric measurements were performed on two categories of resistance arteries, one with external diameter of 100 to 150 μm (intermediate size) and the other with external diameter of 50 to 100 μm (small). External and lumen diameters along the long axis and short axis were measured under ×450 magnification with a filar micrometer. Measurement of external diameter extended from the margin of the outer media to the margin of the outer media of the opposing wall. The 2 measurements of external and lumen diameters were averaged. The observer was blinded as to the treatment that the rats received. Measurements were made on a minimum of 3 cross-sectionally cut arteries in each of the 2 vessel categories investigated (see above). Wall thickness and W/L and the mean of each parameter were calculated for the 2 categories of vessels for each rat.

Statistical Analysis

Results are presented as mean±SEM. One-factor ANOVA for multiple comparisons (Fisher’s least significant difference) was used to compare body weights, BP loads (the area under the weekly systolic BP curve), MAPs, and W/Ls of second-order, intermediate, and small resistance arteries of treated rats with those of control rats (Table 2). Statistical analysis of small and intermediate-size artery dimensions was restricted to W/Ls because sampling bias could not be excluded, considering that measurements were made on only cross-sectionally cut arteries.18 Random sampling may result in measurement of different-size arteries from rat to rat.18 Also, these arteries were categorized on the basis of their diameter, which precludes meaningful comparison of their overall dimensions. W/Ls,

### Table 2. Body Weights and Indirect and Direct Blood Pressures of Treated and Control Rats

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Body Wt, g</th>
<th>BP Load, mm Hg×No. of wks</th>
<th>MAP, mm Hg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>21</td>
<td>540±10</td>
<td>1435±29</td>
<td>122±2 (14)</td>
</tr>
<tr>
<td>AngII</td>
<td>15</td>
<td>558±21</td>
<td>1477±41</td>
<td>123±2 (8)</td>
</tr>
<tr>
<td>NaCl</td>
<td>10</td>
<td>488±16</td>
<td>1414±19</td>
<td>118±2 (8)</td>
</tr>
<tr>
<td>Cold-stress</td>
<td>10</td>
<td>498±9</td>
<td>1397±33</td>
<td>116±4 (6)</td>
</tr>
<tr>
<td>AngII+NaCl</td>
<td>15</td>
<td>513±17</td>
<td>1580±40*</td>
<td>130±3* (8)</td>
</tr>
<tr>
<td>AngII+cold</td>
<td>11</td>
<td>542±21</td>
<td>1518±34</td>
<td>128±3 (8)</td>
</tr>
<tr>
<td>Cold+NaCl</td>
<td>12</td>
<td>491±9</td>
<td>1281±19*</td>
<td>118±2 (7)</td>
</tr>
<tr>
<td>AngII+cold+NaCl</td>
<td>13</td>
<td>536±10</td>
<td>1439±36</td>
<td>123±2 (11)</td>
</tr>
</tbody>
</table>

Values are mean±SEM. Numbers in parentheses represent number of rats with successful direct monitoring of MAP.

*P<0.05 by 1-factor ANOVA for multiple comparisons with control values.
on the other hand, are relatively unchanged despite differences in lumen diameter as long as a small range of diameters is being measured (as in the present study) and are therefore less affected by sampling bias than measurements of external and lumen diameter and wall thickness.\textsuperscript{18} Null hypotheses were rejected at a level of P<0.05.

The W/Ls of small resistance arteries of subgroups of Ang II–treated (n=8), salt-fed (n=10), Ang II–treated and salt-fed (n=6), and control rats (n=7) have been published previously.\textsuperscript{14}

### Results

By inspection, salt-fed and cold-stressed rats drank more water and urinated more than the other rats. Excessive drinking and urination were especially pronounced in the rats receiving the combined treatment of cold exposure and high-sodium diet. All rats remained healthy to the end of the study.

The tail systolic BPs of the 8 groups of rats are listed in Table 1. On the basis of these BPs, the BP load to which each rat and each group of rats was exposed during the study was calculated. During the 12-week study, the BP load of rats receiving the combined treatment of Ang II and high-sodium diet was increased and that of rats receiving the combined treatment of cold exposure and high-sodium diet was decreased (Table 2). When all the 3 treatments were applied to the same rats, the rats’ BP load was almost identical to that of control rats. The only change in directly measured MAPs, compared with those of control rats, was the increase in Ang II–treated and salt-fed rats (Table 2). In 6 cold-stressed and salt-fed rats, directly measured MAPs in the cold room were 131±1 mm Hg compared with 120±2 at room temperature (P<0.01, by paired Student’s t test). Thus, despite their low BP load at room temperature, these rats were mildly hypertensive in the cold.

The W/Ls of mesenteric arteries of control and treated rats are shown in Table 1. The most pronounced changes occurred in the W/L of intermediate-size and small resistance arteries among groups; there were no significant changes in the W/L of second-order mesenteric arteries. The W/L of intermediate-size and small resistance arteries was increased in Ang II–treated rats and in cold-stressed rats. The W/L of small resistance arteries of Ang II–treated (9.9±0.2%) and cold-stressed rats (9.9±0.3%) was increased to the same extent compared with controls (8.9±0.1%) (P<0.01 for both comparisons). The effect of Ang II treatment on the W/L of small resistance arteries was potentiated by the addition of high-sodium diet (9.9±0.2 versus 10.6±0.3%, P<0.05 by 1-factor ANOVA for multiple comparisons); the increase in the W/L of intermediate-size resistance arteries in Ang II–treated and salt-fed rats compared with that of rats receiving Ang II alone did not reach statistical significance (8.3±0.2 versus 7.8±0.3, P=0.16). No potentiation was detected between Ang II administration and cold stress or between cold stress and 2% NaCl diet. The latter combination actually resulted in the reduction of W/L of small resistance arteries (7.8±0.2%) compared with that of controls (8.9±0.1%) (P<0.01). The administration of all 3 treatments to the same rats had no effect on the W/L of mesenteric arteries.

Morphometric measurements did not reveal any changes in the dimensions of second-order mesenteric arteries (250 to 320 μm OD) in the 7 treatment groups (data not shown) compared with those of control rats, with one exception. The thickness of individual vascular smooth muscle cells in the arteries of rats exposed to cold and receiving a high-sodium diet (3.4±0.2 μm) was decreased compared with that of controls (3.9±0.1 μm) (P<0.05); vascular smooth muscle cell thickness in Ang II–treated and in Ang II–treated plus salt-fed rats was 4.2±0.2 and 4.2±0.2 μm, respectively (NS).

Categorization of resistance arteries into intermediate-size and small vessels precluded meaningful statistical analysis of vessel dimensions, as discussed above. For illustration purposes, the morphometric measurements of small resistance arteries, on which the calculations of W/Ls were based, are shown in Table 3.

### Discussion

The findings of this study confirm the potentiation of the hypertensinogenic and vascular trophic effects of Ang II by a high-sodium diet\textsuperscript{3,14} but did not show synergism between Ang II and sympathetic activation by cold exposure or between cold stress and a high-sodium diet. On the contrary, the combination of cold exposure and a high-sodium diet of rats resulted in daytime hypotension of rats and reduced the W/L of their small resistance arteries. This latter is a unique...
finding because there are few known nonpharmacological stimuli that result in relative thinning of small resistance arteries.\textsuperscript{7,20} The findings of this study are internally consistent. Changes in W/L of mesenteric arteries, when detected, were progressively more severe as the diameter of the vessel diminished. This was true for increases and reduction of W/L. A positive synergism was detected between the effects of Ang II administration and a high-sodium diet and a negative synergism between the effects of cold exposure and a high-sodium diet, but when the 3 stimuli were combined, there was no effect on either the BP or the mesenteric artery structure of rats.

The earliest structural vascular changes in Ang II–treated and cold-stressed rats appear to develop in the intermediate-size and small resistance arteries, some of which may be best characterized as arterioles (<80 \textmu m OD). These vessels, because of their relative inaccessibility and wide range of size, have been seldom investigated by researchers interested in the development of structural vascular changes in hypertension. Our findings are in good agreement with the investigations of Bohlen\textsuperscript{21} in spontaneously hypertensive rats (SHR). Bohlen found that the smallest arteries and the largest arterioles accounted for 50\% to 60\% of total vascular resistance in the mesenteric circulation of these rats. This was especially true of the developmental stages of hypertension; in the established phase of hypertension, the more proximal segments of the vascular tree contributed to total vascular resistance to a greater extent than in the early stages of hypertension. In 10- to 12-week-old SHR with established hypertension, Lee and coworkers\textsuperscript{22} found the same increase (percent) in W/L of large and small mesenteric arteries compared with Wistar-Kyoto rats, but the W/L of the superior mesenteric artery of SHR was unchanged. Postmortem specimens of patients with severe hypertension (average diastolic BP, 134 mm Hg) revealed more severe wall thickening of large than of small mesenteric arteries.\textsuperscript{23} As to why the earliest structural vascular changes predominate in small resistance arteries is not known. In a previous investigation that used higher doses of Ang II, we have provided evidence that the structural vascular changes induced by Ang II were due to hypertrophy of vascular muscle.\textsuperscript{14}

Cold stress is an accepted experimental model for the activation of the sympathetic nervous system.\textsuperscript{11,12} Sympathetic stimulation during cold exposure, as measured by urinary norepinephrine excretion, has been documented by Shechtman and coworkers\textsuperscript{14} and by us.\textsuperscript{24} Sympathetic stimulation endures as long as the cold stimulus is applied. The development of structural vascular changes in intermediate-size resistance arteries of cold-stressed rats was recently reported by us.\textsuperscript{24} In this study, we have extended these investigations to the smallest resistance arteries. Cold-stressed rats in the present study were intermittently hypertensive while being exposed to cold. Intermittent rise in BP may be sufficient to induce structural vascular changes; it is not necessary to invoke a direct trophic effect of sympathetic stimulation. This view is supported by the observation in our laboratory that long-term infusion of phenylephrine into rats did not raise BP or result in structural vascular changes.\textsuperscript{16} The structural vascular changes that developed in cold-stressed rats were not sufficient to produce hypertension while the rats were at room temperature. It appears that cold exposure induces additional hemodynamic or hormonal changes or both, which counteract the hypertensinogenic effect of structural vascular changes. The hemodynamic changes may be due in part to increased caloric intake of cold-stressed rats resulting in more frequent and prolonged increases in mesenteric blood flow than in control rats; how these changes may alter mesenteric artery structure independent of sympathetic activation is not known.

The investigation of synergism among the 3 stimuli chosen, Ang II, dietary sodium, and sympathetic stimulation, resulted in some unexpected findings. The potentiation of the hypertensinogenic and vascular trophic effects of Ang II by dietary sodium supplementation was confirmed even though a subpressor dose of the agonist was used; however, the anticipated synergism between cold stress and dietary sodium supplementation and between Ang II and cold stress did not occur. Renal sodium handling may in part explain the synergism between Ang II and dietary sodium and the lack of synergism between cold stress and high-sodium diet. Both Ang II and sympathetic stimulation cause renal vasoconstriction and sodium retention.\textsuperscript{3,25} In contrast, dietary sodium supplementation results in selective renal sympathectomy and natriuresis,\textsuperscript{26} which may counteract the effects of sympathetic stimulation by cold stress but do not reverse Ang II–induced vasoconstriction and sodium retention. That Ang II exacerbates sodium retention during dietary sodium supplementation has been documented by many experiments.\textsuperscript{3} We are not aware of studies that investigated the combined treatment of sympathetic stimulation and high-sodium diet on renal sodium handling. However, bilateral renal denervation has been shown to prevent the development of cold-induced hypertension.\textsuperscript{27}

The combined stimulus of cold stress and dietary sodium supplementation instead of increasing the W/L of small resistance arteries, as expected, reduced it. This finding is of considerable theoretical significance because it suggests that there are as-yet unidentified stimuli that may potentially reverse or prevent the structural vascular changes induced by pressor stimuli. Although we do not recommend cold exposure and a high-sodium diet for the treatment of hypertension, further investigation of the “hypotrophic” effect of cold and salt combined may reveal mechanisms with therapeutic implications. One possible mechanism that comes to mind is the combined dipsogenic effect of the two stimuli resulting in sustained diuresis and, possibly, natriuresis.\textsuperscript{11,28} This mechanism may have been operative in rats treated simultaneously with Ang II, cold exposure, and a high-sodium diet. In these rats, BP and mesenteric artery structure were unaltered compared with controls. In effect, the results in rats receiving the triple treatment were the arithmetic mean of the results obtained in Ang II–treated and salt-treated and in cold-exposed and salt-treated rats, providing internal consistency to the observations made in this study.

The lack of synergism between Ang II administration and sympathetic activation by cold stress is contrary to previous reports of synergism between the two pressor systems.\textsuperscript{4,5} On the one hand, there have been reports that Ang II stimulates
sympathetic outflow from the central nervous system, facilitates neurotransmission in the periphery by altering the uptake and release of norepinephrine at nerve terminals, and enhances vascular smooth muscle responses to norepinephrine. On the other hand, sympathectomy by various means may prevent the full expression of Ang II–induced hypertension.\(^3\) However, much of the evidence supporting the interaction between the renin-angiotensin system and the sympathetic nervous system is based on short-term experiments with pharmaceutical doses of Ang II. Chemical sympathectomy by induction of hypotension may produce hemodynamic and hormonal changes that are not relevant to the normal physiological state. If we accept that cold stress is a physiologically meaningful model of sympathetic stimulation, then the present findings are evidence against an interaction between the renin-angiotensin system and the sympathetic nervous system in the pathogenesis of hypertension. Alternatively, it is possible that the same compensatory mechanisms that prevent cold-stressed rats from being hypertensive while at room temperature despite the development of structural vascular changes (see above) also oppose synergism between Ang II–induced and cold-induced sympathetic activation.

By design, these studies have been descriptive in nature. We aimed to detect the earliest changes in vascular structure during the development of hypertension. To mimic the development of essential hypertension, we investigated the long-term interaction of initially subpressor stimuli in producing hypertension and structural vascular changes. The magnitude of changes that we have detected and the long time frame required for their development will make the investigations of mechanisms difficult. The importance of the present study lies in the long-term in vivo administration of physiologically meaningful stimuli. Only in vivo can one determine whether or not a proposed stimulus plays a role in the gradual development of hypertension.

In summary, the findings of this study confirm the potential of the hypertensinogenic and vascular trophic effects of Ang II by a high-sodium diet but do not provide evidence for synergism between the pressor action of Ang II and of cold exposure. The combination of cold stress and dietary sodium supplementation resulted in hypotension at room temperature and structural vascular changes suggesting hypotrophy. This finding is of considerable theoretical importance because there are few known nonpharmacological vascular hypotrophic stimuli. The ultimate test of the hypertensinogenic potential of known pressor stimuli alone or in combination is their long-term administration in physiologically meaningful doses to experimental animals.

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**References**

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