Prevention of Cold-Induced Increase in Blood Pressure of Rats by Captopril

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To assess the possibility that the renin-angiotensin system may play a role in the development of cold-induced hypertension, three groups of rats were used. Two groups were exposed to cold (5±2°C) while the remaining group was kept at 26±2°C. One group of cold-treated rats received food into which captopril (0.06% by weight) had been thoroughly mixed. The remaining two groups received the same food but without captopril. Systolic blood pressure of the untreated, cold-exposed group increased significantly above that of the warm-adapted, control group within 4 weeks of exposure to cold. In contrast, chronic treatment with captopril prevented the elevation of blood pressure. Rats were killed after 4 months of exposure to cold. At death, the heart, kidneys, adrenal glands, and interscapular brown fat pad were removed and weighed. Although captopril prevented the elevation of blood pressure in cold-treated rats, it did not prevent hypertrophy of the kidneys, heart, and interstitial brown adipose tissue that characteristically accompanies exposure to cold. Thus, chronic treatment with captopril prevented the elevation of blood pressure when administered at the time exposure to cold was initiated. It also reduced the elevated blood pressure of cold-treated rats when administered after blood pressure became elevated. This suggests that the renin-angiotensin system may play a role in the elevation of blood pressure during exposure to cold. (Hypertension 1991;17:763–770)

When rats are exposed chronically to a cold environment, they increase their metabolic rate, circulating levels of catecholamines, and their metabolic responsiveness to norepinephrine and β-adrenergic agonists. Recent studies from this laboratory revealed a significant elevation in systolic, diastolic, and mean blood pressures, as well as hypertrophy of the heart and left ventricle, of rats exposed to cold for 4 weeks. These changes indicate that the syndrome of hypertension can be induced in rats by chronic exposure to cold. The possible mechanisms responsible for this phenomenon are not clearly understood. However, exposure to cold is known to induce an increase in the activity of the sympathetic nervous system. A consequence of this is an increased work load of the heart due to both the direct effect of norepinephrine on it and the increase in peripheral resistance that occurs during exposure to cold. Therefore, an increase in blood pressure and weight of the heart might be expected. Norepinephrine is also known to stimulate the production of renin, which contributes to the production of angiotensin II (Ang II), a potent vasoconstrictor agent. Thus, a possibility existed that Ang II might be involved in the induction of hypertension during exposure to cold.

To assess the possible role of the renin-angiotensin system in cold-induced hypertension, the effect of chronic administration of captopril, an angiotensin I (Ang I) converting enzyme inhibitor, was studied. To achieve this aim, captopril was administered before the development of cold-induced hypertension. In addition, the effect on blood pressure of administration and withdrawal of captopril was studied in cold-treated rats, as was the effect of administration of captopril on the persistent elevation of blood pressure of cold-treated rats after removal from cold.

Methods

All experiments were carried out using male rats of the Sprague-Dawley (Blue Spruce Farms) strain. In all experiments, the rats were housed individually in temperature-controlled rooms at either 26±2°C or 5±2°C. The rooms were illuminated from 7:00 AM to 7:00 PM daily. The rats were provided with finely powdered Purina Laboratory Chow (no. 5001, Ralston Purina Co., St. Louis, Mo.) and tap water ad libitum. Fluid containers consisted of infant nursing bottles with cast bronze drinking spouts. Food
Containers were spill-resistant and have been described previously.14

Systolic blood pressures of the rats in all experiments were measured weekly by the method of Fregly,15 using a polygraph (Narco BioSystems, Houston, Tex.). In addition, urinary concentrations of norepinephrine and epinephrine were measured by high-performance liquid chromatography (Bio-Rad Co., Rockville Center, N.Y.) with electrochemical detection (BAS Corp., West Lafayette, Ind.) as described earlier.16,17 Sodium and potassium concentrations in the urine were measured by flame photometry (Technicon Co., Tarrytown, N.Y.) using lithium as the internal standard.

Body weights, systolic blood pressures, food and water intakes, and urine output were analyzed by a repeated-measures, one-way analysis of variance (ANOVA). The dipsogenic responsiveness to Ang I, organ weights, and hormonal concentrations in the urine were analyzed by a one-way ANOVA. When a significant $F$ value ($p<0.05$) was obtained, a post hoc analysis using the Newman-Keuls procedure was performed.

Experiment 1: Effect of Administration of Captopril on Development of Cold-Induced Hypertension

Eighteen male rats weighing from 300 to 350 g were divided into three groups of six rats each. A 1-week control period preceded this experiment. During this time, the systolic blood pressures and body weights of each rat were measured weekly. At the end of this time, the rats were divided randomly into three equal groups. One group was kept at 26±2°C and served as warm-adapted controls. Two groups of rats were kept at 5±2°C. Six of the cold-treated rats served as controls and the other six were treated with captopril (Capotren, E.R. Squibb & Sons, Inc., Princeton, N.J.; 0.06% mixed thoroughly in their diet). Measurement of body weights and systolic blood pressures of all rats continued at weekly intervals. Daily food and water intakes, as well as urine outputs, were measured for 3 days during the fourth and 14th weeks of exposure to cold. Urinary concentrations of norepinephrine, epinephrine, sodium, and potassium were also measured.

The dipsogenic responsiveness to acute administration of Ang I (150 µg/kg s.c., Sigma Chemical Co., St. Louis, Mo.) was measured during day 45 of the experiment. At 9:00 AM each rat was weighed, administered Ang I, and placed alone in a metabolic cage at the temperature to which it was adapted. A preweighed bottle of tap water was placed on each cage. No food was available during the study. Water intake was measured gravimetrically at 0.5, 1.0, and 2.0 hours after treatment.

At the end of the experiment (112 days of exposure to cold), the rats were killed by decapitation. Blood was collected from the trunk into a beaker containing EDTA for subsequent analysis of plasma renin activity by radioimmunoassay (Baxter Laboratories, Cambridge, Mass.). The heart, kidneys, adrenal glands, and interscapular brown fat pad were removed, cleaned of extraneous tissue, and weighed on a torsion balance. The left ventricle was dissected from the heart and weighed on a torsion balance.

Experiment 2: Effect of Administration of Captopril on Blood Pressure of Rats Maintained at Normal Room Temperature

Another experiment was carried out parallel in time to experiment 1, the purpose of which was to assess the interaction of another compound (tryptophan) with captopril. The conditions under which the animals were kept and the route and dosage of captopril administered were identical to those of experiment 1. Systolic blood pressures were measured after 7 and 9 weeks of treatment with captopril. For purposes of comparison, the data of only two of the groups (control and captopril-treated) will be presented here.

Experiment 3: Effect of Administration of Captopril on Blood Pressure of Rats With Cold-Induced Hypertension

Twenty male rats (two groups of 10 rats each) weighing 280–320 g were exposed to cold (5±2°C) in a temperature-controlled room. Both groups received finely powdered Purina Laboratory Chow to eat. Two months after exposure to cold, and after the cold-induced elevation of blood pressure had persisted for 1 month (day 59), one of the groups was given the food containing captopril (0.06% by weight). Systolic blood pressures and body weights were measured weekly thereafter. On day 105 of the experiment, captopril was withdrawn from the diet of the treated group for 8 days. Captopril was then administered again (day 114) to the same group for 8 days (day 122), and subsequently withdrawn for 16 days (through day 138). Blood pressure was measured every 3–4 days to assess the time course of the effect of administration and withdrawal of captopril.

Experiment 4: Effect of Administration of Captopril on Persistent Elevation of Blood Pressure of Cold-Treated Rats After Removal From Cold

Twelve of the rats that were used in experiment 3 were used in this experiment. Captopril was removed from the diet (day 122) of the treated group for 16 days before the initiation of this experiment. At the end of this time (138 days of exposure), all rats were removed from cold. One of the groups (six rats) was given captopril (0.06% by weight in food) at the time of removal from cold. The second group (six rats) received the same food without captopril. Blood pressure was measured approximately once each week for the remaining 40 days of the experiment.

Results

Experiment 1

Systolic blood pressures of the cold-treated control group first increased significantly ($p<0.05$) above those of the other two groups on day 28 of exposure.
Figure 1. Line graphs showing systolic blood pressures (panel A) and body weights (panel B) for cold-treated control, cold plus captopril–treated, and warm-adapted control groups during the course of the experiment. Groups are designated in the figure. One standard error is set off at each mean. Blood pressures of the cold-treated control group were significantly (p<0.01) greater than those of cold plus captopril–treated and warm-adapted control groups from day 28 throughout the remainder of the experiment.

to cold and remained elevated significantly (p<0.01) above those of the other two groups for the rest of the experiment (Figure 1A). The repeated-measures analysis of variance (ANOVA) revealed a significant [F(2, 12)=8.36; p<0.01] effect of treatment; a significant [F(13, 156)=15.02; p<0.01] effect of time, and a significant [F(26, 156)=127.05; p<0.01] group×time interaction. A post hoc analysis revealed no significant differences between the blood pressures of the warm-adapted and cold plus captopril–treated groups throughout the experiment (Figure 1A). Body weight of the warm-adapted group was significantly (p<0.01) greater than those of the other two groups beginning on the 42nd day and throughout the remainder of the experiment (Figure 1B). The repeated-measures ANOVA of body weight revealed a significant [F(2, 12)=7.43; p<0.01] effect of treatment, a significant [F(24, 288)=175.12; p<0.01] effect of time, and a significant [F(48, 288)=6.89; p<0.01] group×time interaction. The post hoc analysis revealed no significant differences between body weights of the two cold-treated groups.

Figure 2. Bar graphs showing water (panel A) and food (panel B) intakes, as well as urine (panel C) outputs, of the three groups for weeks 4 and 14 of the experiment. Groups are designated in the figure. One standard error is set off at each mean. **p<0.01, *p<0.05 compared with the warm-adapted control group.

Food intakes were measured during the fourth and 14th weeks of the experiment to determine the amount of captopril consumed daily (60 mg/kg body wt) by the treated group (Figure 2B). The two cold-treated groups ingested approximately twice (p<0.01) the amount of food ingested by the warm-adapted control group. Water intakes of the two cold-treated groups were significantly (p<0.05–<0.01) greater than that of the warm-adapted control group (Figure 2A). Daily urine output of the cold plus captopril–treated group was significantly (p<0.01) greater than those of the other two groups during the fourth week. However, during the 14th week, urine outputs of both cold-treated groups were significantly (p<0.01) greater than that of the warm-adapted control group (Figure 2C).
FIGURE 3. Bar graphs showing urinary outputs of sodium (panel A), potassium (panel B), and the sodium/potassium ratio (panel C) in urine of the three groups designated in the figure. One standard error is set off at each mean. **p<0.01 compared with the warm-adapted control group.

The concentrations of sodium, potassium, norepinephrine, and epinephrine were measured in the daily urine collected. Outputs of sodium and potassium by both cold-treated groups were significantly (p<0.01) greater than those of the warm-adapted control group during both weeks (Figures 3A and 3B). However, the ratio of sodium to potassium in urine did not differ significantly among the three groups during either weeks 4 or 14 (Figure 3C). The outputs of norepinephrine and epinephrine in the urines of both cold-treated groups were significantly (p<0.01) higher than those of the warm-adapted control group during both weeks 4 and 14 (Figures 4A and 4B).

The dipsogenic responsiveness to Ang I was measured to assess completeness of the blockade of Ang I converting enzyme by captopril. On day 45 of the experiment, the cold-treated control group consumed significantly (p<0.01) more water during the 2-hour study period than both the warm-adapted control and the cold plus captopril–treated groups in response to acute administration of Ang I (Figure 5). Thus, administration of captopril returned to control

FIGURE 4. Bar graphs showing urinary outputs of norepinephrine (panel A) and epinephrine (panel B) of the three groups designated in the figure. One standard error is set off at each mean. **p<0.01, *p<0.05 compared with the warm-adapted control group.

FIGURE 5. Bar graph showing dipsogenic responsiveness to angiotensin I during 2 hours after treatment for the three groups designated in the figure. Angiotensin I (150 μg/kg s.c.) was administered on day 45 of the experiment. One standard error is set off at each mean. AI, angiotensin I. *p<0.05 compared with warm-adapted control group; **p<0.05 compared with cold plus captopril–treated group.
level the increased dipsogenic responsiveness of the cold-treated control group.

All of the rats in each group were killed on day 112 of exposure to cold. The weights of the heart, left ventricle, kidneys, and brown fat of the warm-adapted group were significantly less than those of the cold-treated and cold plus captopril-treated groups (Figures 6A and 6B). There were no significant differences between cold-treated and cold plus captopril-treated groups in the weights of any of these organs excepting brown adipose tissue. The weight of the brown adipose tissue of the cold plus captopril-treated group was significantly (p<0.05) less than that of the cold-treated control group. Adrenal weights did not differ significantly among the three groups and are not shown in Figure 6.

PRA values for the warm-adapted, cold-treated, and cold plus captopril-treated groups were 2.2±0.2 (SEM), 1.9±0.4, and 7.8±2.5 ng/ml/hr, respectively. There was no significant difference between the warm-adapted and cold-treated groups. There were significant (p<0.01) differences between the cold plus captopril-treated group and the other two groups.

**Experiment 2**

After either 7 or 9 weeks of treatment with captopril, there was no significant difference between blood pressures of treated and control groups (7 weeks, 106±6 and 114±4 mm Hg; 9 weeks, 107±7 and 111±3 [SEM] mm Hg, respectively). Others have also reported failure of chronic treatment with converting enzyme inhibitors to affect the blood pressure of normotensive rats.18-20

**Experiment 3**

Two months after initial exposure to cold, systolic blood pressures of both groups of cold-treated rats averaged approximately 153±5 mm Hg. There were no significant differences between groups. At this time (59 days of exposure to cold, designated as 0 in Figure 7), one group of rats received captopril (0.06%) in their diet. Within 10 days after administration of captopril, systolic blood pressure (128±1 mm Hg) was significantly (p<0.01) reduced compared either with that of the untreated control group measured at the same time or with blood pressure before treatment (Figure 7). Blood pressures remained at a level of about 125 mm Hg during treatment. When captopril was removed from the diet (day 105 of exposure to cold and designated as day 48 in Figure 7) of this group, blood pressure increased within 3 days to reach the level of the untreated control group (approximately 150 mm Hg). To confirm the decrease in blood pressure induced by captopril, the experiment was repeated. The group that had been treated previously with captopril was given captopril again (day 114 of exposure to cold, designated as day 55 in Figure 7). Blood pressure decreased to levels significantly lower than that of controls within 5 days of treatment. Body weights of the two groups did not differ significantly and are therefore not shown. Thus, the effect of captopril on systolic blood pressures of rats exposed to cold is exerted rather rapidly (i.e., within 5 days). Removal of captopril from the diet is followed by an increase
Chronic exposure to cold characteristically increases food intake and urine output of rats. Treatment with captopril had no significant effect on these. In addition, chronic exposure to cold was accompanied by increased urinary outputs of sodium and potassium. Treatment with captopril also failed to affect these. The greater outputs of sodium and potassium in the urine of cold-treated rats compared with the warm-adapted controls are most likely the result of the increased food intake of these groups. The similarity of the urinary sodium/potassium ratios in the cold-treated and warm-adapted control groups supports this suggestion.

Chronic treatment with captopril did not affect the increased urinary output of norepinephrine and epinephrine characteristic of chronic exposure to cold. At first glance, this might suggest that increased production of these catecholamines does not contribute to the development of cold-induced hypertension since the outputs of both of these hormones were similar in cold plus captopril-treated and cold-treated control groups, while their blood pressures were significantly different. However, other properties of captopril may complicate interpretation of its antihypertensive effect, including the fact that captopril does have α-adrenergic inhibitory activity. Further, inhibition of the Ang I converting enzyme increases the half-life of bradykinin, a peptide known to decrease vascular resistance. In addition, captopril has been reported to inhibit plasma enkephalinase, a peptidase capable of degrading enkephalins. The relative contribution of each of these to the reduction in blood pressure cannot be stated at present and remains for further study.

Chronic administration of captopril did not affect the cardiac and left ventricular hypertrophy characteristic of exposure to cold. This may suggest a role for catecholamines in cardiac hypertrophy since urinary output of norepinephrine is increased during chronic exposure to cold, and chronic treatment of rats with β-adrenergic agonists is known to induce hypertrophy of the heart. Thus, cardiac hypertrophy in response to chronic exposure to cold may represent a direct effect of excessive production of norepinephrine. It must also be recognized that structural and morphological changes may occur in the heart that are not readily reversible after removal from cold, even after the increased secretion of norepinephrine has returned to control level. Thus, in an earlier study, it was reported that cardiac hypertrophy occurring in rats after 5 weeks of exposure to cold did not regress until approximately 4 weeks after removal from cold. In contrast, the increased urinary excretion of norepinephrine occurring during exposure to cold regressed to the level of the control group within 3 days after removal from cold.

Hypertrophy of the heart is also reported in hypertensive humans and laboratory animals. Although it has been shown in vitro that thyroxine can alter myocardial mechanics directly and indepen-
dently of catecholamines, it is most likely that a significant portion of its effect on the heart is associated with catecholamines. Thus, epidural block and treatment with \( \beta \)-adrenergic blockers can reduce heart rate and cardiac output in many hyperthyroid patients.\(^{33} \) In the case of the cold-exposed rat, a recent review of the literature revealed that the consensus of the studies to date shows that the concentrations of protein-bound iodine and thyroxine in serum are unchanged or possibly decreased during exposure to cold, whereas the concentrations of thyroid-stimulating hormone and triiodothyronine (T\(_3\)) are increased.\(^{34} \) Thus, it is possible that catecholamines may interact with T\(_3\) to induce cardiac hypertrophy in rats chronically exposed to cold.\(^{34} \) It is unlikely, however, that thyroid hormones mediate the hypertension induced by exposure to cold since their major effects on the cardiovascular system are to increase cardiac output and systemic blood pressure while diastolic blood pressure is reduced and mean blood pressure is unchanged.\(^{33} \) In contrast to hyperthyroidism, chronic exposure to cold is accompanied by increases in systemic, diastolic, and mean blood pressures.\(^{9} \)

An increase in the weight of the kidneys of cold-treated rats has often been reported.\(^{11,12} \) The increased weight of the kidneys under these conditions has been attributed to the increased food intake accompanying exposure to cold and its consequent increase in excretion of metabolites and electrolytes. In the present study, chronic treatment with captopril did not affect this response. The increase in the weight of interscapular brown adipose tissue of the cold-exposed groups is observed characteristically in rats exposed chronically to cold.\(^{36} \) Brown adipose tissue is important for nonshivering thermogenesis during exposure to cold. Chronic treatment of cold-exposed rats with captopril attenuated the increase in the weight of brown adipose tissue. Reasons for this are not clear and will require further study. It may be speculated at present that the sequence of events that occurs to initiate cold-induced hypertension may be an increase in secretion of catecholamines, particularly norepinephrine, immediately on exposure to cold and continuing for the duration of exposure to cold.\(^{4,16} \) The elevated levels of norepinephrine in the plasma exert numerous effects, including vasoconstriction of peripheral vessels, inotropic and chronotropic effects on the heart, and an increase in metabolic rate. Another important effect of the increased concentration of norepinephrine in plasma is to increase the release of renin from the juxtaglomerular apparatus of the kidneys, with subsequent increased formation of Ang II, a potent vasoconstrictor.\(^{12} \) Although plasma renin activity of the chronically cold-treated control group was not different from that of the warm-adapted group, the present results suggest a role for the renin-angiotensin system in the development and maintenance of cold-induced hypertension. A possibility exists that exposure to cold may upregulate receptors for Ang II and thereby contribute to the role played by the renin-angiotensin system in the development and maintenance of a cold-induced elevation of blood pressure. Additional studies will be required to define the role of the renin-angiotensin system more precisely.

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