Cold Acclimation-Induced Increase of Systolic Blood Pressure in Rats Is Associated With Volume Expansion

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To investigate the mechanisms of cold-induced hypertension, the systolic blood pressure (SBP) and average daily water consumption were measured weekly in 6-month-old male Wistar rats; they were subsequently acclimated to thermoneutrality (26°C for 7 weeks), to cold temperature (6°C for 9 weeks), and then again reacclimated to 26°C for 5 weeks. Circulating plasma volume and whole blood viscosity were measured in subgroups of rats at the end of acclimation to 26°C after 2 days, after 1, 6, and 8 weeks of cold, and after 2 and 5 weeks of rewarming. The control values obtained at the end of thermoneutral period were: SBP = 130.8 ± 18.6 mm Hg, plasma volume = 41.9 ± 4.64 mL/kg, whole body viscosity at shear rate of 22.5 per sec = 6.7 ± 0.48 cps, and daily water consumption = 42.25 ± 16.81 mL. After 48 h of cold exposure there was almost a 50% increase in plasma volume that persisted to a lesser degree throughout the whole period of cold exposure (P < .05). After 2 weeks of cold exposure the daily water consumption increased and SBP began to increase. After 6 weeks of cold exposure the SBP was 30 mm Hg above that of the control level (P < .001) and was accompanied by a 25% increase in whole blood viscosity (P < .05). At the end of 8 weeks of cold exposure the plasma volume was 56.8 ± 9.51 mL/kg and the whole blood viscosity was 8.0 ± 1.79 cps at the 22.5 per sec shear rate. During the 5 weeks of rewarming the elevation of SBP and increased whole blood viscosity persisted, whereas the increased daily water consumption and expanded plasma volume returned to normal. Therefore, the acclimation to cold is accompanied by the development of a volume-associated hypertension, which is sustained after rewarming without volume expansion. Am J Hypertens 1999; 12:54–62 © 1999 American Journal of Hypertension, Ltd.

KEY WORDS: Plasma volume, blood volume, blood viscosity, ambient temperature, body temperature, cold acclimation.

There are a number of epidemiologic studies reporting seasonal variability of blood pressure. During colder seasons the arterial blood pressure is found to be elevated in both hypertensive patients and normotensive subjects. The relationship between ambient temperature and blood pressure is thought to be at least partly responsible for increased mortality from ischemic heart disease and stroke during cold seasons. The reasons for seasonal differences in blood pressure are not entirely clear.
poorly understood: colder ambient temperature was implicated in many studies, but compounding effects of behavioral and dietary changes could not be excluded. The only animal model of cold-induced hypertension was introduced and studied by Fregly et al. They found that when Sprague-Dawley rats were housed at 6°C, there was an elevation of arterial blood pressure, increased activity of sympathetic and renin-angiotensin-aldosterone system, increased food and water intake, and increased diuresis. Although Fregly et al classified the cold-induced elevation of blood pressure in rats as a mineralocorticoid model of hypertension, the associated changes in circulating plasma volume had never been assessed.

There is a controversy in the literature concerning cold-induced changes in plasma volume. A reduction of plasma volume is universally accepted as a consequence of short-term cold exposure. However, contradictory results (increase, decrease, or no change) with respect to plasma volume have been reported for long-term cold exposure. In most of the cases the plasma volume was not measured, and its change was either estimated on the basis of a change in hematocrit level or was calculated according to the equation of Dill and Costill, which assumes that the total amount of hemoglobin in blood does not change between the two compared conditions. These results therefore do not necessarily correctly reflect changes in plasma volume.

The purpose of this work was to further understand the mechanisms of cold-induced hypertension. To this effect we studied the time course of changes in systolic blood pressure during the acclimation to colder environmental temperature in Wistar rats and directly measured the changes in plasma volume as well as some associated rheologic changes.

MATERIALS AND METHODS

Subjects Seventy-four male, 6-month-old Wistar rats were obtained from the colony maintained at the Gerontology Research Center. Rats were housed individually in plastic boxes with sawdust bedding. The vivarium was maintained at 22 ± 1°C with a 12-h light:12-h dark cycle with the light off at 8 PM. The rats were provided with laboratory chow (NIH-7 formula: 23.5% protein, 50% carbohydrate, 4.4% fat, 3.4 Kcal/g) and tap water ad libitum. Average daily water consumption was determined by weekly weighing of drinking bottles.

Experimental Design

Experiment 1 Body weight, colonic temperature, and systolic blood pressure (SBP, tail-cuff technique) were measured weekly in 50 animals throughout the experiment. After rats became fully adapted to handling and their SBP reading remained stable during several consecutive measurements, they were moved into a climate-controlled room (Norlake Scientific, Hudson, WI), where they were maintained on the same food and light regimen as in the vivarium. During the initial 7 weeks in the climate-controlled room the air temperature was maintained at 26 ± 0.5°C, which is on the low end of thermoneutrality for Wistar rats. The temperature was then reduced to 6 ± 0.5°C for the subsequent 9 weeks. During the final 6 weeks of the experiment the temperature was once again set at 26°C (rewarming). Regardless of air temperature, the humidity was maintained at 65%.

At the end of the 26°C period, after 6 weeks, at the end (8 weeks) of the 6°C period, and after the second and fifth weeks of the rewarming period, groups of eight randomly selected rats were removed from the experiment to measure plasma volume, whole blood viscosity, hematocrit, and for tissue harvesting.

Experiment 2 To assess the changes in plasma volume and rheologic characteristics of blood during the early period of cold exposure, body mass, colonic temperature, and SBP were measured weekly in 24 additional rats of the same age. The animals had been acclimated to thermoneutrality for 6 weeks, followed by 6 weeks of cold (6°C). Groups of six rats were removed for measurements of plasma volume, whole blood viscosity, and hematocrit at the end of the thermoneutral period and after 2 days, 1 week, and 6 weeks of cold exposure. Because the weekly body mass, colonic temperature, and SBP changes were essentially the same in both experiments, as were the results of blood measurements at the end of the 26°C period and after 6 weeks of the 6°C period, it was considered justifiable to combine the results of blood measurements at different time points and to present them together with Experiment 1.

Measurement of Blood Pressure Systolic blood pressure was measured using the tail-cuff technique (Kent Scientific Corp., Litchfield, CT). Each rat was weighed and placed into a plastic restrainer, which prevented gross motor activity. After colonic temperature was measured using a RET-2 thermoprobe (BAT-10 thermometer, Physiotemp Instrument Inc., Clifton, NJ), the tail was placed under a heat lamp for 10 to 15 min to facilitate the measurement of blood pressure. The temperature under the heat lamp did not exceed 30°C. To protect the animal from overheating, the restrainer was covered by reflecting foil.

Blood Collection Each animal was anesthetized by a mixture of ketamine (50 mg/kg) and xylasine (7.5 mg/kg). An external jugular vein and right carotid artery were cannulated. The water solution of indo-
Measurement of Plasma Volume  The blood from the first vacutainer was used for the measurement of plasma volume. The blood was centrifuged (5000 rpm, 10 min, at 4°C) and the near-IR spectrum of the supernatant was measured from 700 nm to 850 nm using a Lambda 6 spectrophotometer (Perkin-Elmer Corporation, Norwalk, CT). The dye concentration in 1 mL of plasma was determined from the 750-nm to 850-nm region of the spectrum. The absorption was corrected for that of plasma in the spectral region and fit with the spectrum of a known concentration of dye. Total plasma volume was calculated from the amount of dye injected and the dilution of the injected dye in the plasma.

The dye used in this study for the measurements of circulating plasma volume, indocyanine green, while it binds firmly to plasma proteins, is rapidly eliminated from blood through the liver and has a half life of less than 10 min. The accurate estimation of plasma volume with the use of this dye normally requires repeated sampling and plotting of the elimination curve on a logarithmic scale with projection of the concentration of the dye at time zero. Because repeated sampling in rats would also affect the accuracy of plasma volume estimation, and as we were mainly interested in relative plasma volume values at different conditions, we settled on the one-sample technique. We took care to collect blood precisely 60 sec after the dye injection. In this case the calculated values have to be somewhat overestimated due to a partial loss of dye through liver elimination, but they were close to values determined by others with the use of different dyes. For instance, circulating plasma volume estimated with the Evans blue dye dilution technique in Sprague-Dawley rats was reported as 39.6 ± 1.3 mL/kg, only 5.5% less than the value estimated for our control group (the end of the thermoneutral period in the Results section), 41.9 ± 4.64 mL/kg. In any case, the degree of overestimation must be similar across all experimental conditions. It is noteworthy that a one-sampling technique usually employed with the use of Evans Blue — a dye traditionally used for estimation of plasma volume — also results in an overestimation.

Measurement of Whole Blood Viscosity  Whole blood viscosity was measured in triplicate using the second vacutainer within 1 h from the time of blood collection (DV-III, Beta 1.3 rheometer with circulating CP-44y cup and No.40 spindle, Brookfield Engineering Laboratories, Inc., Stoughton, MA). Before viscosity determination the rheometer cap was preheated to 37°C for 1 h (EX100, Constant Temperature Circulating Bath, Brookfield Engineering Laboratories, Inc.). The rheometer was preset for measurements at 60, 30, 12, 6, and 3 rpm with respective shear rates of 450, 225, 90, 45, and 22.5 sec⁻¹. The rheometer was cleaned and recalibrated for each sample. The level of viscosity for each shear rate was defined as the average of three measurements.

Hematocrit Measurement  Three heparinized microhematocrit tubes (ID = 0.56 mm; Clay Adams, Becton, Dickinson & Co, Parsippany, NJ) were filled from the second vacutainer of blood. They were centrifuged for 4 min in a hematocrit centrifuge (IEG MB Centrifuge, Damon/IEG Division. Needham, MA). Hematocrit was measured with a Micro-Capillary Reader (IEG MB Centrifuge, Damon/IEG Division, Needham, MA). The level of hematocrit was determined from the average measurements obtained from three tubes.

Statistical Analyses  Every weekly measured parameter was averaged for the last 3 weeks of the 7-week-long thermoneutral period. These averages were accepted as control levels and were compared with all other weekly measurements using ANOVA and a pairwise t test, with Bonferroni correction. The relative roles of different variables as predictors of weekly blood pressure were assessed using stepwise regression analysis. Total blood volume was calculated on the basis of plasma volume and hematocrit. Because we were interested in relative changes only, the red cell volume was not corrected for trapped plasma. Hematologic parameters measured in different groups of rats killed at different time points of the experiment were compared with a group measured at the end of the thermoneutral period using a t test. Whole blood viscosity was compared at different shear rates separately and also was evaluated using ANOVA for repeated measurement with different shear rates as five within-factors.

In the text all parameters are presented as mean ± SD and in the figures as mean ± SE. Statistical significance is accepted at $P < .05$. According to Bonferroni correction for 18 pairwise comparisons the significance at the .05 level indicates that actual $P < .002778$.

RESULTS

The average SBP (Figure 1) during the last 3 weeks of the thermoneutral period (control) was 130.8 ± 18.6 mm Hg. At the end of the first and second weeks of cold exposure the increase of SBP was statistically nonsignificant compared with the control level (134.5 ± 18.4 and 137.2 ± 16.4 mm Hg, respectively; $P > .05$), but at the end of the third week the SBP
increase became statistically significant \((P < .001)\) and SBP continued to increase, reaching its maximum level at the end of the fifth week \((159.9 \pm 15.1\) mm Hg; \(P < .001)\).

Rewarming resulted in a sharp decline of SBP to the control level at the end of week 2 \((132.8 \pm 14.3\) mm Hg; \(P < .05)\), but after that SBP increased again and remained significantly greater than the control level through the end of the fifth week of this period, when the experiment was terminated.

Cold exposure (Figure 2) resulted in a sharp elevation of colonic temperature \((37.59 \pm 0.6°C\) after the first week of exposure in comparison with \(36.96 \pm 0.26°C\) in control; \(P < .001)\), but during the subsequent weeks body temperature fell progressively so that elevation became statistically nonsignificant starting with the sixth week \((P > .05)\) and became similar to the control level during the last 2 weeks of cold exposure and during the entire rewarming period \((P > .05)\).

Exposure to cold resulted in a sharp reduction in body weight (Figure 3), which became statistically significant at the end of the second week \((584.4 \pm 50.4\) g and \(561.4 \pm 56.4\) g vs \(603.8 \pm 42.1\) g at the control level; \(P > .05\) and \(P < .01\) for the first and second week of cold exposure, respectively). The body weight remained below control level during subsequent weeks \((P < .001)\), but showed a tendency to increase during the last 2 weeks of cold exposure so that during the last week it became not statistically different from control \((P > .05)\). During the rewarming period the body weight continued to return to the control level.

During the first 2 weeks of cold temperature, the daily water consumption (Figure 4) was reduced, compared with thermoneutrality \((35.3 \pm 14.4\) and \(37.2 \pm 8.8\ mL/day vs \(42.25 \pm 16.81; P < .05)\). However, beginning at week 3 the water consumption increased above the level of thermoneutrality and reached its maximum during the fourth week \((60.46 \pm 5.42; P < .05)\). During the first week of rewarming the water consumption increased markedly, to \(83.5 \pm 25.3\ mL/day (P < .01); it then decreased and reached the control level during the third week \((P > .05)\).

Stepwise regression analysis indicated that among multiple variables changing in the course of the experiment, only air temperature closely correlated with SBP \((R = 0.427)\). Body weight, colonic temperature, and daily water consumption did not contribute significantly as predictors of SBP \((F = 3.9)\).

After the first 2 days of cold exposure, the average plasma volume corrected for body weight (Table 1) increased almost 50% in comparison with control level \((P < .01)\) and remained increased at all tested time points of cold exposure, ie, the end of weeks 1, 6, and 8 \((P < .05)\). Plasma volume was not different from control level at both time points of the rewarming period \((P > .05)\).

The hematocrit (Table 1) was significantly lower after 2 days and after 1 week in the cold than in thermoneutrality \((P < .05)\), but was not different from control levels at all other time points, with the exception of the end of the second week of the rewarming period, when it was sharply elevated \((P < .01)\).

The calculated blood volume corrected for body
weight (Table 1) was significantly higher compared with control level after 2 days and after 1, 6, and 8 weeks of cold exposure ($P < .05$). At both time points during the rewarming period blood volume was not different from that obtained at thermoneutrality ($P > .05$).

Whole blood viscosity (Table 2) did not change after 2 days and after 1 week of cold exposure, but became markedly elevated at the end of the sixth and eighth weeks of cold exposure ($F(4,51) = 31.65, P < .001$ and $F(4,56) = 5.37, P < .001$, as revealed by ANOVA for

**FIGURE 2.** The effects of cold ambient temperature (6°C) and rewarming on weekly values of colonic temperature in Wistar rats (means ± SE).

**FIGURE 3.** The effects of cold ambient temperature (6°C) and rewarming on weekly values of body mass in Wistar rats (means ± SE).
repeated measurements). Blood viscosity remained elevated during the entire rewarming period ($F(4,44) = 9.24, P < .001$ and $F(4,56) = 8.0, P < .001$ for the second and fifth weeks of rewarming). When the elevations of whole blood viscosity were compared at different individual shear rates, the changes were not statistically significant at a shear rate of 450 per sec, but became significant at all lower shear rates. The increase of whole blood viscosity became progressively greater as the shear rate was reduced. For example, when the increases in blood viscosity between thermoneutrality and the sixth week of cold exposure were compared, the viscosity was 10% higher at a shear rate of 450 per sec and 20% higher at a shear rate of 22.5 per sec.

**DISCUSSION**

We have shown that in Wistar rats the chronic exposure to cold temperature ($6^\circ$C) resulted in the slow development of an increased SBP, accompanied by increased daily water consumption. The elevation of SBP and water consumption started 3 weeks after the beginning of cold exposure and lasted throughout all 9 weeks of cold exposure. Systolic BP remained elevated during all 5 weeks of rewarming, whereas water consumption normalized. These findings agree with observations reported previously for Sprague-Dawley rats.

The main new finding of the present study is a marked, almost 50%, increase of the plasma volume and 30% increase in calculated total blood volume at the beginning of the cold exposure (48 h). Despite the reduction in the hematocrit, the calculated cell volume also increased. Plasma and blood volumes remained elevated throughout 8 weeks of cold exposure, although to a lesser degree. During the rewarming period plasma and blood volumes returned to the control level, while SBP remained elevated.

There is general consensus in the literature, cover-
TABLE 2. WHOLE BLOOD VISCOSITY AT DIFFERENT SHEAR RATES IN DIFFERENT GROUPS OF RATS AT THE END OF ACCLIMATION TO 26°C AND AT DIFFERENT TIME POINTS OF COLD (6°C) AND REWARMING (26°C) PERIODS (MEANS ± SD)

<table>
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<tr>
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<th>26°C (2 days)</th>
<th>6°C (1 week)</th>
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<th>6°C (8 weeks)</th>
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<td>450 (1/sec)</td>
<td>3.6 ± 0.26</td>
<td>3.5 ± 0.47</td>
<td>3.7 ± 0.13</td>
<td>3.9 ± 0.37</td>
<td>3.9 ± 0.50</td>
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<td>225 (1/sec)</td>
<td>3.9 ± 0.27</td>
<td>3.9 ± 0.32</td>
<td>4.1 ± 0.19</td>
<td>4.5 ± 0.55*</td>
<td>4.5 ± 0.72*</td>
<td>4.7 ± 0.31*</td>
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<td>90 (1/sec)</td>
<td>4.9 ± 0.37</td>
<td>4.9 ± 0.56</td>
<td>5.1 ± 0.21</td>
<td>5.9 ± 0.57*</td>
<td>5.7 ± 1.02*</td>
<td>5.8 ± 0.66*</td>
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<td>45 (1/sec)</td>
<td>5.7 ± 0.40</td>
<td>5.5 ± 0.64</td>
<td>5.9 ± 0.31</td>
<td>6.9 ± 0.75*</td>
<td>6.7 ± 1.33*</td>
<td>7.0 ± 1.04*</td>
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<tr>
<td>22.5 (1/sec)</td>
<td>6.7 ± 0.48</td>
<td>6.4 ± 0.80</td>
<td>6.7 ± 0.40</td>
<td>8.4 ± 0.95*</td>
<td>8.0 ± 1.79*</td>
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* Significantly (P < .05) different from the values at 26°C.

ing more than half a century, that cold exposure results in a reduction of body water, especially in the vascular compartment, which our findings clearly contradict. For instance, during short-term cold exposure in humans estimated plasma volume was reduced by 15%. In most of the experiments reporting cold-related reduction of plasma volume the conclusion was drawn on the basis of changes in hematocrit or hematocrit and hemoglobin (after the formula of Dill and Costill was introduced in 1974). In the only two studies in which the plasma volume was directly measured rather than estimated, a cold-associated increase of plasma volume was reported. It now seems obvious that the formula designed to estimate the level of dehydration that assumes an unchanged level of total hemoglobin is unacceptable in the cases of cold exposure in which activation of the sympathetic nervous system might lead to an immediate release of concentrated red blood from the spleen. In our experiment, 48 h after the beginning of cold exposure, despite the decline of hematocrit, the calculated cell volume and therefore hemoglobin was higher than at thermoneutrality (22.85 mL v 19.3 mL).

The cold-induced elevation of SBP was declared to be a mineralocorticoid model of hypertension involving activation of the renin-angiotensin-aldosterone system. The key evidence was found in the experiment with spironolactone, because the blockade of the mineralocorticoid receptors reduced water intake and prevented the development of hypertension. In our experiment, however, the increase of daily water consumption started only after 2 to 3 weeks of cold exposure, whereas the elevation of plasma volume started not later than 48 h after the onset of cold. Although the understanding of mechanisms of early cold-induced plasma expansion requires additional experiments, it is clear that increased water intake does not initiate it. At this point we can only guess that plasma expansion at the early stage of chronic cold exposure probably depends on increased blood osmolarity due to accumulation of the metabolites related to shivering. The time course of changes in colonic temperature and SBP during cold exposure also suggests the functional relationship between acclimation to cold and development of increased blood pressure. In any case, the increased plasma volume associated with cold-induced hypertension supports the idea of its mineralocorticoid origin.

One of the most interesting findings of this study is an elevation of whole blood viscosity, which accompanies the increased SBP during cold exposure. Moreover, the increased blood viscosity persisted during the rewarming period even after the volume expansion was normalized. This elevation of whole blood viscosity during cold exposure cannot be attributed to the elevated hematocrit, which is believed to be a common cause of elevated blood viscosity. With the exception of the second week of the rewarming period the level of hematocrit was not different from the control period even though blood viscosity was increased. Though additional experiments are needed to identify the basis for the higher blood viscosity, it is reasonable to assume that the increased blood viscosity might contribute to the elevation of the SBP during cold exposure, especially during rewarming.

In our experiment, as well as in experiments of others using the tail-cuff technique, SBP elevation did not occur earlier than after 2 to 3 weeks of cold exposure. However, in the only experiment in which cold-induced elevation of SBP was measured through an implanted catheter in rats, the rise in SBP was detected during the first 5 h of cold exposure. The explanation offered in the paper for a greater sensitivity of the direct measurement is difficult to accept. Indeed the tail-cuff technique is notorious for being capricious and imprecise; however, the presence of a systematic error responsible for elevated SBP measured by the tail-cuff method can be excluded in a carefully designed experiment. Moreover, the elevation of SBP during cold acclimation was verified by direct measurements conducted by others and in our experiments (unpublished observations).
experiments with human subjects exposed to cold. Blood pressure was also measured indirectly and elevated SBP was reported during the first 2 h of exposure. It is clear that the time course of SBP changes after the onset of cold has to be studied further using implantable devices and telemetry. It is quite conceivable that earlier (measured in hours) elevation of SBP due to massive sympathetic activation is suppressed by different compensatory mechanisms and reappears later (in 2 to 3 weeks) as a consequence of a prolonged volume expansion.

The shear number and redundancy of regulators of water and sodium balance and their interplay (for comprehensive review see reference 42) make it difficult to identify at this time any particular mechanism or mechanisms involved in the development of cold-induced hypertension, although the mineralocorticoid interpretation remains preferable. Nevertheless, the fact that elevation of SBP was preceded by sustained elevation of plasma volume and that the SBP remained elevated while plasma volume returned to normal during rewarming fit the general description of hypertension as an “inevitable long-term consequence of reduced renal excretory capacity with associated long-term blood volume expansion, characterized by an elevation in total peripheral resistance.” We believe that in this model, the long-term cold-induced blood volume expansion led to morphologic vascular changes responsible for an elevation in total peripheral resistance that continued to support increased blood pressure in the absence of volume expansion after rewarming. This model is especially valuable because it is the only model (with the exclusion of genetic models) that does not require a permanent artificial damage of renal function or a massive hormonal imbalance.

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