Hemodynamic Effects of Chronic Alteration in Hematocrit in Spontaneously Hypertensive Rats

DinkoSusic, M.D., Sc.D., Anil K. Mandal, M.D., and Dusan Kentera, M.D., Sc.D.

SUMMARY This study describes the effect of a chronic decrease in hematocrit on blood pressure, cardiac output (CO), total peripheral resistance (TPR), and plasma volume in spontaneously hypertensive rats (SHR), and all but plasma volume in normotensive Wistar rats (NWR). Hematocrit was decreased by treatment with either heparin, vitamin K inhibitor (pelentan), or by repeated blood letting (BL). The results show that in SHR, a decrease in hematocrit, regardless of how produced, was associated with a significant decrease (p < 0.01) in blood pressure. Prevention of heparin-induced decrease in hematocrit by repeated transfusions of red blood cells abolished the blood-pressure-lowering effect of heparin. By using combined data on hematocrit and systolic blood pressure in all five SHR groups, a significantly positive correlation and linear regression between hematocrit and blood pressure were obtained. When compared to control untreated SHR, heparin- or pelentan-treated SHR showed a significant (p < 0.001) decrease in TPR and a significant increase in CO, while in SHR BL, no difference in TPR or CO was found. Plasma or blood volume did not differ among the groups in NWR, heparin treatment resulted in significantly decreased hematocrit, decreased TPR, and increased CO compared to control normotensive rats. However, blood pressure did not change. Results confirming the authors' previous study and those of other investigators indicate a direct association between hematocrit and systemic hypertension. Lowering the hematocrit can effectively lower an elevated blood pressure. Moreover, the data suggest that heparin or pelentan induces a vasodilator effect that cannot be attributed to a decrease in hematocrit alone. (Hypertension 6:262–266, 1984)

Key Words: blood pressure • cardiac output • total peripheral resistance • plasma volume • spontaneously hypertensive rats

Classical studies of Whittaker and Winton have shown a direct relationship between hematocrit and apparent blood viscosity. Since, from Poiseuille's equation, blood pressure is directly proportional to viscosity, there is a theoretical possibility that an agent that lowers hematocrit also lowers blood viscosity and in this way induces a decrease in total peripheral resistance (TPR) and blood pressure. Studies relevant to this subject have yielded somewhat divergent results. Numerous studies in normotensive animals have shown that both acute and chronic wide-range changes in hematocrit do not influence blood pressure. In these studies, experimentally induced changes in blood viscosity, when either hematocrit or plasma viscosity was altered, were found to be associated with a quantitatively similar change in total peripheral resistance and a reciprocal change in venous return and cardiac output, but blood pressure remained unchanged. On the other hand, a number of studies in hypertensive humans and animals indicated that a direct relationship between blood viscosity and blood pressure may exist. In support of this idea are the findings that blood viscosity was increased in hypertensive subjects, that there was a direct relationship between pressure and viscosity in patients with essential hypertension, and that, parallel with the reduced blood pressure in hypertensive patients induced by either alpha methyldopa or prazosin, there was also a decrease in blood viscosity. Similarly, blood hyperviscosity has been found in rats with spontaneous hypertension. On the basis of these findings, it has been proposed that increased blood viscosity, resulting from increases in both hematocrit and plasma viscosity (due to increased plasma fibrinogen concentration), is partly responsible for the increase in blood pressure in hypertensive subjects. Thus, it appears possible that lowering the hematocrit in hypertensive subjects would also lower the blood pressure, to the extent that increased hematocrit participates in the genesis of increased blood viscosity and hence increased TPR and blood pressure.
Chronic heparin treatment has been shown to decrease blood pressure in several hypertensive animal models. In two of these studies, concomitant with heparin-induced decrease in blood pressure, a fall in hematocrit has also been observed. On the basis of these findings, we suggested that heparin-induced decreases in hematocrit and presumably in blood viscosity may account, at least in part, for the observed decrease in TPR and blood pressure in hypertensive rats treated with heparin.

This study was initiated to further examine the effect of a chronic decrease in hematocrit on blood pressure in spontaneously hypertensive rats (SHR). To this end, we studied the effect of several interventions, designed to decrease hematocrit on the following hemodynamic parameters: blood pressure, cardiac output (CO), TPR, and blood volume. These studies were performed in SHR and in normotensive Wistar rats (NWR).

Materials and Methods

Experiments were performed in adult male SHR and NWR bred at the Institute for Medical Research, Beograd, Yugoslavia. The SHR used in this study were fourth generation descendents of breeders originally obtained through Taconic Farms, Germantown, New York. All animals were given a standard diet for laboratory rats (Veterinarski Zavod, Zemun) and tap water ad libitum, unless otherwise stated. Five groups of SHR were studied during a 4-week course.

Spontaneously Hypertensive Rats

SHR Controls

The following types of treatment were carried out. Control (C) SHR (28 animals) were not treated.

Heparin-Treated SHR (19 Animals)

Heparin (Galenika, 300 units) was given subcutaneously in two daily doses to SHR. In eight of the 19 heparin-treated (H) rats, bleeding time and platelet count were determined both before and 1 week after initiation of treatment.

Heparin-Treated SHR in which Hematocrit Was Kept at Control Level (14 Animals)

Repeated transfusions of homologous red blood cells were given twice a week to SHR H (300 units of heparin a day) intraperitoneally. 2 ml suspended in 1 ml of 0.9% NaCl solution. These rats have been designated as SHR HT.

SHR Treated with Vitamin K Inhibitor (Ethylbiscumacetate, Dicumarol [R] Pelentan, Krka) (15 Animals)

SHR in this group were given standard rat chow to which 50 mg of pelentan per 100 g of food was added (SHR P group). Calculated on a basis of average daily food intake per group, pelentan intake approximated 7 mg/day/rat. In preliminary experiments, this dose of pelentan has been shown to decrease the hematocrit to a level seen in the SHR H.

SHR in Which Hematocrit Was Decreased by Repeated Blood Letting (16 Animals)

Blood letting (BL) by removal of about 1 ml/100 g body wt twice a week by cardiac puncture has been shown in preliminary experiments to decrease hematocrit to a level seen in the SHR H.

Normotensive Wistar Rats

Normotensive Wistar Control Rats (10 Animals)

Control NWR were untreated.

Normotensive Wistar Rats Treated with Heparin (9 Animals)

NWR H were treated with heparin (300 units/day/rat). After initial determinations of body weight, systolic blood pressure (tail cuff), and hematocrit, rats were placed on their respective treatments. Measurements of blood pressure and hematocrit were made once a week during the 4-week course of the experiment. At the end, plasma volume was determined in seven randomly selected rats from each group of SHR using Evans blue as an indicator. Hematocrit was determined from samples of arterial blood. Blood volume was calculated from plasma volume and hematocrit. Hemodynamic variables, including blood pressure, CO, and TPR, were measured in all other rats. Animals were anesthetized with pentobarbital (35 mg/kg, i.p.), and a tracheal cannula was inserted. Blood pressure was measured directly through a femoral artery cannula (PE-50, Clay Adams Company, Parsippany, New Jersey), using a low volume displacement transducer (Model P23Db, Statham Laboratories, Hato Rey, Puerto Rico), and a direct recorder (Physiograph Four, Narco Bio System, Inc., Houston, Texas). Mean arterial pressure (MAP) was obtained by electronic integration. The CO was determined using a previously described modification of Coleman's application of the dye dilution technique. Indocyanine green (Hynson, Westcott and Dunning, Inc., Baltimore, Maryland) was used as an indicator, and a recording densitometer (Beckman Instruments, Fullerton, California) was used for detection of dye in the blood and registration of dilution curves. The TPR was calculated from the MAP and CO (assuming that mean right atrial pressure is 0). At the end of the experiment, rats were exsanguinated and their hearts removed. The atria were excised, the right ventricular lateral wall was separated from the left ventricle and septum, and their weights were immediately determined on an automatic analytic balance (Mettler). The ratio of left ventricular weight to body weight was determined.

Statistical analyses were done according to Steel and Torrie. Boniferroni's modification for multigroup comparison of Student's t test was used to test the significance of differences among the groups. The results are expressed as means ± 1 SEM.

Results

Spontaneously Hypertensive Rats

There was no difference found in body weight or heart rate among the groups, either at the beginning or at the end of the study (Table 1).
TABLE 1. Body Weight (BW), Heart Rate (HR), and Left Ventricular Weight/BODY Weight Ratio (LV/BW) in Differently Treated SHR

<table>
<thead>
<tr>
<th>Variable</th>
<th>Rat group</th>
<th>BW (g)</th>
<th>HR (bpm)</th>
<th>LV/BW</th>
</tr>
</thead>
<tbody>
<tr>
<td>C (28)</td>
<td>303 ± 7</td>
<td>399 ± 4</td>
<td>0.320 ± 0.003</td>
<td></td>
</tr>
<tr>
<td>H (19)</td>
<td>304 ± 6</td>
<td>394 ± 7</td>
<td>0.285 ± 0.003*</td>
<td></td>
</tr>
<tr>
<td>HT (14)</td>
<td>307 ± 6</td>
<td>420 ± 10</td>
<td>0.298 ± 0.008*</td>
<td></td>
</tr>
<tr>
<td>P (15)</td>
<td>295 ± 7</td>
<td>397 ± 12</td>
<td>0.296 ± 0.002*</td>
<td></td>
</tr>
<tr>
<td>BL (16)</td>
<td>299 ± 6</td>
<td>418 ± 9</td>
<td>0.318 ± 0.004</td>
<td></td>
</tr>
</tbody>
</table>

C = control untreated rats; H = rats treated with heparin; HT = rats treated with heparin and given repeated transfusions of red blood cells; P = rats treated with pelentan; BL = rats in which hematocrit was decreased by means of blood letting. Values are expressed as means ± 1 SEM. Numbers of rats in each group are given in parentheses.

*p < 0.05 when compared to control rats.

The systolic blood pressure and hematocrit changes before and during 4 weeks of treatments are shown in Figure 1. Parallel with the reduction in hematocrit, induced by either heparin or pelentan or repeated blood letting, a significant decrease (p < 0.01) in systolic blood pressure was observed. On the other hand, blood pressure remained at control level in H rats in which hematocrit was kept at a level similar to control rats by repeated transfusions. When combined data for all five groups were used, a significant positive correlation (r = 0.854, t = 864, p < 0.001) and linear regression between hematocrit and systolic blood pressure were observed. The results in MAP, hematocrit values, CO, and TPR obtained at the end of the 4-week experimental period are shown in Figure 2. From Figure 2, it is evident that rats in which hematocrit was decreased by either heparin, pelentan, or blood letting had significantly lower (p < 0.05) MAP, while MAP in SHR H in which hematocrit was kept at control level by repeated transfusions did not differ from control animals. A significant increase in CO and a significant decrease in TPR were observed in SHR H and SHR P. On the other hand, neither CO nor TPR in SHR BL was different from that in control SHR.

Left ventricular weight/body weight ratio was found to be significantly (p < 0.05) reduced in H and P rats (Table 1). Despite a significant decrease of MAP in SHR BL, the left ventricular/body weight ratio was not different from that in control SHR. Plasma or blood volume did not differ among the groups.

Normotensive Wistar Rats

Chronic heparin treatment significantly decreased hematocrit in NWR but had no effect on blood pressure (Figure 3). A significant decrease in TPR was also observed in NWR H, as in SHR H.

Coagulation Studies

Chronic heparin treatment induced a significant (p < 0.05) prolongation of bleeding time (from 162 ± 11 to 251 ± 38 seconds) and a significant (p < 0.01) decrease in platelet number (from 1004 ± 24 × 10⁹ platelets to 760 ± 52 × 10⁹ platelets). A significant (p < 0.01) prolongation of prothrombin time was observed in NWR P rats (from 15.4 ± 0.5 seconds before treatment to 58.3 ± 9.5 seconds at 1 week after start of treatment).

Discussion

Results of this study confirm previous observations that chronic heparin treatment reduces blood pressure in hypertensive rats. Furthermore, our data show that a heparin-induced decrease in hematocrit accounts for the antihypertensive effect of heparin. Thus, a similar reduction in hematocrit produced in SHR by means other than heparin, that is, by treatment with vitamin K inhibitor or by repeated blood letting, was associated with a quantitatively similar decrease in blood pressure as in SHR H. On the other hand, prevention of the heparin-induced decrease in hematocrit by repeated
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transfusions abolished the antihypertensive action of heparin.

The present results also support the theory that increased blood viscosity participates in the genesis of high blood pressure. Thus, a direct relationship between hematocrit level and blood pressure was found in SHR in this study. Moreover, acute hemodilution, produced in SHR by dextran infusion, has also been found to lower blood pressure, suggesting that further supports the same idea. Apparently, the decrease in hematocrit lowers blood pressure only to the extent to which increased hematocrit participates in the genesis of hypertension. Thus, heparin-induced lowering of hematocrit to a level far below that found in our study evoked quantitatively the same decrease in blood pressure in stroke-prone SHR.

Additionally, a slight but nonsignificant decrease in blood volume found in the heparin (7.21 ± 0.04 ml/100 g), pelentan (7.36 ± 0.31 ml/100 g), and bloodletting (7.36 ± 0.21 ml/100 g) groups, compared to the control group (7.82 ± 0.42 ml/100 g), might contribute to a decrease in blood pressure seen in the treated groups.

In normotensive rats, heparin treatment reduced hematocrit but did not affect blood pressure, a finding well in agreement with previous reports on the relationship between hematocrit and blood pressure in normotensive animals. Hemodynamically, this was characterized by a decrease in TPR and a reciprocal increase in CO. In other words, a decrease in TPR, presumably resulting from a decrease in hematocrit and hence blood viscosity, was fully compensated for by an increase in CO, so that blood pressure did not change. On the other hand, the decrease in TPR produced in SHR by a decrease in hematocrit was proportionally greater than the increase in CO, so that the net result was a decrease in blood pressure.

One more finding deserves comment. Hemodynamic variables such as CO or TPR showed significant changes in SHR H and SHR P. These hemodynamic variables did not change in SHR BL despite a similar decrease in hematocrit in all three groups. This disparity raises the possibility that heparin and pelentan have an additional action, such as a vasodilator effect, which cannot be attributed to a decrease in hematocrit alone.

Whether vasodilation and decreased hematocrit are effected via a common pathway, such as release of prostaglandins and inhibition of splenic contraction (as
suggested in our previous study\textsuperscript{17} and shown in Figure 4) remains to be determined. However, in support of the proposed hypothesis, studies have shown that increased blood viscosity decreases blood flow through peritubular capillaries and, indirectly, through oncotic pressure, enhancing sodium and water reabsorption by the renal tubules.\textsuperscript{24} Conversely, a fall in hematocrit has been associated with a decrease in filtration fraction (glomerular filtrate rate/renal plasma flow). Thus, a smaller percentage of protein-free ultrafiltrate formation occurred at the glomerulus, so that the normal rise in postglomerular protein concentration was less; and a fall in hematocrit indirectly lowered postglomerular oncotic pressure. Such a decrease in peritubular capillary oncotic pressure may cause a decrease in tubular sodium reabsorption, and natriuresis and diuresis.\textsuperscript{25} Also, it has been shown that a decrease in peritubular capillary blood flow diminishes total blood flow to the medullae and papillae and may have a deleterious effect on the synthesis of prostaglandin E\textsubscript{2} by the medullary tubules and papillary interstitial cells.\textsuperscript{26} Thus, it is reasonable to conclude that some effect of the changes in hematocrit and blood viscosity in blood pressure regulation seems to be mediated by the kidneys.

Acknowledgments

The authors are grateful to Mirjana Zdravkovic, B.Sc., and Dr. Pavle Mitenkov, Beograd, Yugoslavia, for determining the platelet count and prothrombin time. The expert technical assistance of Gordana Funduk and Zaga Jovanovic, Beograd, Yugoslavia, is also acknowledged. The authors thank Donna Herndon, Department of Physiology, Medical College of Georgia, Augusta, Georgia, for preparing the manuscript. Pelentan was kindly given by Dr. Azra Tasic of Krka, Novo Mesta, Yugoslavia.

References


Figure 4. Possible mechanism of the antihypertensive action of heparin.
Hypertensive rats.

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Hypertension. 1984;6:262-266
doi: 10.1161/01.HYP.6.2.262

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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