Altered Venous Function in Hypertensive Rats

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SUMMARY The vascular beds of the upper or lower body of rats were perfused through the aorta with oxygenated Krebs-Ringer solution containing dextran (7 g/100 ml), at 37°C. Perfusion was stopped every 10 minutes, and the pressure rise in the jugular or the femoral vein was recorded during rapid infusion (15.3-90.0 ml/min) of Krebs-Ringer solution into the inferior vena cava. The following groups of rats were studied: (1) six male genetically hypertensive rats (GHR), 9-11 months old, New Zealand strain; (2) seven female GHR, 5-6 months old, New Zealand strain; (3) eight male spontaneously hypertensive rats (SHR), 4 months old, Okamoto strain; (4) five male rats with two-kidney Goldblatt hypertension (2-KGH), 40 days postclipping; (5) seven male 2-KGH rats, 65 days postclipping; (6) eight male rats with one-kidney Goldblatt hypertension (1-KGH), 40 days postclipping; (7) weight- and sex-matched normotensive control rats of the appropriate strain; and (8) weight- and sex-matched two-kidney and one-kidney, sham-clipped normotensive rats. Preliminary studies showed that rapid infusion into the venous circulation fills the veins, but there is no entry of fluid into the arterial side of the circulation. Compared to controls, the venous pressure-volume curves of GHR (male and female), SHR, 1-KGH rats and 2-KGH rats, 65 (but not 30) days postclipping, were shifted toward the pressure axis (P < 0.05). The shift of the venous pressure-volume curves persisted following the reduction of vasoconstrictor tone by killing the rats or by the administration of sodium nitroprusside (0.1 mg/ml perfusate), or by both. The findings suggest decreased venous capacity in hypertensive rats.

THERE IS evidence that venous compliance is decreased in dogs with one kidney and renovascular hypertension.1,2 The evidence for a reduction of venous compliance in rats and dogs with two kidneys and renovascular hypertension is inconclusive.3-5 Greenberg and Bohr4 observed a decreased passive extensibility of portal vein strips in spontaneously hypertensive rats. Since the veins are not exposed to the high pressure associated with arterial hypertension, decreased venous compliance in hypertension is probably not secondary to increased intraluminal pressure but must be the result of neurogenic or humoral vascular influences. In the case of the spontaneously hypertensive rats, the possibility of genetic differences in vascular wall anatomy also exists.

The purpose of this paper is to investigate further the venous function in rats with spontaneous and Goldblatt hypertension to see whether venous changes are a constant finding in the various forms of experimental hypertension.

Methods

Studies were performed in genetically hypertensive (GHR), spontaneously hypertensive (SHR), Goldblatt hypertensive, and normotensive control (NCR) rats (groups I-VI in Tables 1 and 2). The GHR and their controls were obtained by brother-to-sister inbreeding from the original Otago stock of albino rats.4 The National Institutes of Health was the source of the SHR and the Wistar-Kyoto NCR.5 Goldblatt hypertension was produced in male Sprague-Dawley rats (5-6 weeks old) by applying a silver clip with an inner diameter (i.d.) of 0.2 mm to the left renal artery, with or without removal of the right kidney 1 week later. Male Sprague-Dawley rats, age- and weight-matched, and sham-clipped (i.d. = 1.0 mm), with or without contralateral nephrectomy, served as controls for the Goldblatt hypertensive rats. The hypertensive and the normotensive rats were kept under the same conditions, fed regular rat chow and given water ad libitum. The rats were weighed, and their blood pressures were measured by tail plethysmography on the day of the hemodynamic studies.

UPPER BODY VENOUS PRESSURE-VOLUME MEASUREMENTS

In these experiments, an in situ preparation was used in GHR and NCR (groups I and II in Table 1). Rats were anesthetized with ether, the right jugular vein was cannulated (PE 90), and heparin, 500 units, was given intravenously for anticoagulation. A midabdominal incision was made and the inferior vena cava (IVC) was cannulated (PE 240) above the left renal vein with the cannula pointing toward the heart. A 16-gauge needle was inserted into the abdominal aorta and the systemic vascular beds proximal to the renal arteries were perfused retrograde (Sigmamotor pump) with oxygenated (95% O2, 5% CO2) Krebs-Ringer solution containing dextran, 7 g/100 ml, at 37°C and at a flow rate of 3.8-5.4 ml/min. The perfusate drained from the cannulated IVC into a reservoir and was recirculated. Perfusion pressure, monitored through a T-tube arrangement between the pump and the aorta, was detected with a Statham P23Db pressure transducer and recorded on an oscillographic recorder. Mean perfusion pressure was kept at 40-50 mm Hg to minimize formation of edema. Spontaneous respiration of rats ceased 1-3 minutes after the start of perfusion.

For venous pressure-volume measurements, perfusion through the aorta was stopped and the perfusate was allowed to drain from the IVC catheter for 50 seconds. During this time, jugular venous pressure, measured with a Statham P23Db pressure transducer, fell to 0-2 mm Hg in every rat. Krebs-Ringer solution containing dextran at 37°C then was infused (Harvard infusion pump) through the IVC catheter.

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at 90.0 ml/min. A jugular venous pressure of 30–35 mm Hg was attained in 5–15 seconds. The infusion then was stopped, the IVC catheter was opened to the atmosphere, and perfusion through the aorta was restarted. Venous pressure-volume measurements were repeated at 10-minute intervals, until the pressure-volume curves were reproducible to within ±2 mm Hg at any given volume.

The average of two or three series of measurements was used to construct a venous pressure-volume curve for each rat. Using Student's t-test, volumes producing intravenous asphyxiation were compared separately. For purposes of intergroup comparison of hypertensive group with normotensive control group.

**LOWER BODY VENOUS PRESSURE-VOLUME MEASUREMENTS**

In these experiments an in vivo preparation was used in SHR, Goldblatt hypertensive, and normotensive control rats (groups III–VI in Tables 1 and 2). Pentobarbital-anesthetized (30 mg/kg, ip) rats were allowed to breathe spontaneously through a tracheal cannula. The left femoral vein was constricted (PE 50), and the femoral venous pressure was measured with a Statham P23Db pressure transducer. Asphyxiation was induced by hyperventilation. Heparin (500 units, iv) was given for anticoagulation. A midabdominal incision was made and the IVC and the aorta were cannulated below the left renal vein, the cannulas pointing toward the hindquarters. Mass ligatures were placed below the level of the kidneys to include the lumbar and abdominal musculature. The lower body vascular beds of rats were perfused at 1.4–2.6 ml/min through the aorta with Krebs-Ringer solution (see above), again keeping perfusion pressure at 40–50 mm Hg. Lower body venous pressure-volume curves were obtained similarly to the upper body pressure-volume curves, except that the rate of infusion was kept at 15.3 ml/min, reaching 25–30 mm Hg femoral venous pressure in 2–10 seconds.

After the third set of measurements the rats were killed by asphyxiation. Perfusion through the aorta was continued, but the perfusate no longer was recirculated. Additional pressure-volume curves were obtained at 10-minute intervals with or without perfusion of the hindquarters with sodium nitroprusside (0.1 mg/ml of perfusate) for 10 minutes. The lower body venous pressure-volume curves were obtained similarly to the upper body pressure-volume curves, except that the rate of infusion was kept at 15.3 ml/min, reaching 25–30 mm Hg femoral venous pressure in 2–10 seconds.
sons, the average of the second and third curves is reported. For purposes of intragroup comparisons, venous pressure-volume curves obtained before and after killing the rats were compared by the paired Student's t-test. Null hypotheses were rejected at \( P \leq 0.05 \).

**VALIDATION OF METHOD**

An additional 30 pentobarbital-anesthetized (30 mg/kg, ip) male and female Sprague-Dawley rats, 4–10 months of age, were studied. Venous pressure-volume curves were obtained (1) with the aortic perfusion needle open and closed to the atmosphere, and (2) before and after perfusion of the lower and the upper body vascular beds with norepinephrine \((10 \mu g/ml \text{ of perfusate})\) or epinephrine \((10 \mu g/ml \text{ of perfusate})\) for 10 minutes. The effect of reduction of vasoconstrictor tone on the lower body pressure-volume measurements was investigated by killing the rat or by perfusion of the lower body vascular beds with sodium nitroprusside \((0.1 \text{ mg/ml})\) for 10 minutes, or by both procedures. Lower body venous pressure-volume measurements also were performed during asphyxia for 3 minutes and before and after ligation of the major tributaries (femoral and lumbar veins) of the IVC. In six rats, Evans blue dye in Krebs-Ringer solution was infused rapidly \((15.3 \text{ ml/min})\) through the IVC catheter into the lower body venous beds (see above), and the distribution of the dye was viewed under a dissecting microscope. Finally, in one male Goldblatt hypertensive rat and in one male normotensive Sprague-Dawley rat, rapid sequence (exposures at 1-second intervals) venography was performed during rapid infusion of equal volumes of metrizoate sodium (Isoopaque 440, Winthrop Laboratories) and Krebs-Ringer solution into the lower body venous beds.

**Results**

**VALIDATION OF METHOD**

The upper and the lower body venous pressure-volume curves in the same rat were reproducible to within ±2 mm Hg at any given infused volume, with the exception of the first curve which was displaced toward the pressure axis. Opening or closing the arterial side of the circulation to the atmosphere had no effect on the shape or the position of the pressure-volume curves \( (n = 10) \). Infusion of fluid into the lower body venous beds did not increase pressure on the arterial side of the circulation; this was a constant finding in every rat of which the lower body venous pressure-volume curves are reported in this paper. A shift of the venous pressure-volume curves toward the pressure axis, suggesting venoconstriction, was noted after perfusion of the vascular beds with norepinephrine and epinephrine \( (n = 6) \). In the studies of the lower body vascular beds, aortic perfusion pressure at constant flow rose sharply, while femoral vein pressure increased slightly or in some rats remained un-
changed during the first 2–5 minutes of asphyxia (Fig. 1). Thereafter, as the rat died of asphyxiation, aortic perfusion pressure returned to a level lower than that prior to the clamping of the tracheal cannula. Similar changes were demonstrated in every rat subjected to the lower body venous pressure-volume measurements, indicating that the vasomotor innervation to the lower body vascular beds was intact and that killing the rats reduced the vascular resistance. Lower body venous pressure-volume curves obtained during the peak rise of aortic perfusion pressure were shifted toward the pressure axis ($P < 0.001$ by paired Student's t-test) (Fig. 1).

Reduction of vasoconstrictor tone by killing the rat or by the administration of sodium nitroprusside, or both, shifted the lower body pressure-volume curves towards the volume axis. This finding is illustrated in Figure 2B, showing these responses in hypertensive and normotensive control rats. Tying off the major tributaries (femoral and lumbar veins) of the IVC in eight rats did not change the shape or position of the lower body venous pressure-volume curves in the pressure range of 0–10 mm Hg. In the higher pressure ranges (10–25 mm Hg), there was a small (1–3 mm Hg at a given volume) shift of the pressure-volume curves toward the pressure axis.

Rapid infusion of Evans blue dye in Krebs-Ringer solution, producing pressures up to 40 mm Hg, resulted in the filling with dye of the small veins (0.01–0.05 mm) surrounding the intestines, bladder, and testes in the six rats tested. There was, however, no staining of the wall of the intestines, the muscles of the thigh and back, or the parenchyma of the testes as viewed under a dissecting microscope. Rapid sequence venography in two rats confirmed the findings of the Evans blue dye infusion (Fig. 3). At 10–15 mm Hg intravenous pressure the large veins and their major branches were visualized. At 20–25 mm Hg pressure there was filling of the smaller veins and of the venous plexus surrounding the epididymis and the vas deferens without evidence for capillary filling ("capillary blush"). Evidence for capillary filling was still lacking when intravenous pressure was raised to 40–45 mm Hg.
VENOUS PRESSURE-VOLUME MEASUREMENTS

Six groups of hypertensive and normotensive control rats, were studied (Tables 1 and 2). The hypertensive and normotensive control rats were matched with respect to body weight. The blood pressure in each group of hypertensive rats was significantly higher than that of controls. The femoral venous pressures of SHR and of two- and one-kidney Goldblatt hypertensive rats was $1.6 \pm 0.28$ (±SE) mm Hg ($n = 5$), $2.0 \pm 0.15$ ($n = 6$), and $1.9 \pm 0.25$ ($n = 8$), respectively. These pressures were similar ($P > 0.5$) to those of controls, $1.8 \pm 0.28$ ($n = 5$), $1.8 \pm 0.29$ ($n = 8$), and $1.9 \pm 0.16$ ($n = 8$).

Figures 2 and 4 illustrate venous pressure-volume curves in GHR, SHR, and their controls. Compared to values in normotensive control rats, there is a statistically significant ($P < 0.05$ by Student’s $t$-test) shift of the upper body venous pressure-volume curve in male GHR (group I, Table 1) in the direction of the pressure axis. Profile analysis corroborated this finding ($P < 0.01$ for parallelism and $P < 0.001$ for lack of strata differences). The shift of the pressure-volume curve of the hypertensive group is statistically significant when either pressure or volume is taken as the independent variable (Fig. 4A). The same observation applies to the analysis of the other venous pressure-volume curves. In the female GHR (group II, Table 1) the shift of the upper body venous pressure-volume curve is statistically significant ($P < 0.05$ by Student’s $t$-test) in the low and intermediate pressure ranges but not at 25 mm Hg pressure; this is due to the greater experimental variation in the higher pressure range (20–30 mm Hg) (Fig. 4B). However, profile analysis in the pressure range of 0–30 mm Hg showed both a shift ($P < 0.05$) and a lack of parallelism ($P < 0.05$ for parallelism) between the pressure-volume curves of GHR and NCR.

In SHR (group III, Table 1), there is a statistically significant shift ($P < 0.001$ by Student’s $t$-test; $P < 0.01$ for parallelism and $P < 0.01$ for lack of strata differences) of the lower body pressure-volume curve toward the pressure axis at all pressures examined (Fig. 2A). In both SHR and their controls, the lower body venous pressure-volume curve is shifted away from the pressure axis following reduction of vasoconstrictor tone by killing the rat or by the administration of sodium nitroprusside, or both (Fig. 2B). However, the shift of the venous pressure-volume curve of hypertensive rats toward the pressure axis was not abolished.

At 30 days after clipping and sham-clipping, the lower body venous pressure-volume curves of two-kidney Goldblatt hypertensive rats and their controls (group IV, Table 2) overlapped the entire pressure range (0–25 mm Hg) (data not shown). At 65 days postclipping there was a statistically significant shift ($P < 0.05$ by Student’s $t$-test; $P < 0.01$ for...
parallelism and lack of strata differences) of the lower body venous pressure-volume curve of two-kidney Goldblatt hypertensives rats (group V, Table 2), toward the pressure axis (Fig. 5). In one-kidney Goldblatt hypertensive rats (group VI, Table 2) the shift of the venous pressure-volume curve toward the pressure axis \(P < 0.05\) by Student's t-test; \(P < 0.01\) for parallelism; and \(P < 0.001\) for lack of strata differences) was apparent by 40 days postclipping (Fig. 5). The shift of the venous pressure-volume curves of two- and one-kidney Goldblatt hypertensive rats persisted following the reduction of vasoconstrictor tone by killing the rat or by the administration of sodium nitroprusside, or both (data not shown).

The apparent differences in the lower body venous pressure-volume curves of control rats (Figs. 2 and 5) reflect differences in strain (Wistar-Kyoto vs. Sprague-Dawley), age, and body weight (Tables 1 and 2).

Discussion

Disturbance of venous function in experimental hypertension has been suggested by several investigators. Direct evidence that venous function is altered in experimental hypertension comes from the work of Overbeck and of Simon et al., who measured pressure-volume relationships in collateral-free segments of femoral and mesenteric veins from one- and two-kidney perinephritic hypertensive dogs. In one-kidney perinephritic hypertensive dogs, the pressure-volume curves of the femoral and mesenteric veins were shifted in the direction of the pressure axis, indicating a decreased venous compliance. In dogs with early (less than 2 weeks) two-kidney perinephritic hypertension, femoral and mesenteric vein compliances were unchanged. More recently, Greenberg and Bohr demonstrated a decreased passive extensibility of portal vein strips in vitro from spontaneously hypertensive rats (Okumoto strain).

The experimental method used in the present study was an adaptation of Alexander's technique for measuring mesenteric venous distensibility in dogs. Alexander's technique consisted of rapid retrograde infusion of fluid into the venous side of a collateral-free mesenteric vascular arcade, after arterial inflow had been temporarily occluded, and the continuous measurement of the resulting venous pressure. The preliminary experiments of the present study indicate that the venous beds of rats respond to pharmacological and neural stimuli in a manner similar to the response of the mesentric veins of dogs. Venoconstrictor drugs (norepinephrine and epinephrine) and sympathetic nervous system discharge (asphyxia) shift the venous pressure-volume curves toward the pressure axis. The venodilator agent, sodium nitroprusside, and the reduction of neural vasoconstrictor tone by killing the rat shift the curves toward the volume axis.

Our preliminary experiments also indicate that in the venous pressure range of 0-10 mm Hg, rapid infusion of fluid results in the filling of the large veins primarily. In the higher pressure range (10-25 mm Hg), there is also filling of the smaller veins. The infused fluid does not enter the arterial side of the circulation; therefore, arterial compliance does not contribute to the shape or the position of the venous pressure-volume curves. The limitations of the present method are similar to those of Alexander's technique. The initial unstressed volume, although probably small because of the collapse of veins during draining, is unknown. Venous compliance, \(\Delta V/\Delta P\), cannot be calculated. The contribution of branching of veins to the pattern of pressure-volume curves is uncertain.

The findings of this study indicate that the venous pressure-volume curves in genetically hypertensive, spontaneously hypertensive, and two-kidney and one-kidney Goldblatt hypertensive rats are shifted toward the pressure axis. The shift of the pressure-volume curves persists following the reduction of vasoconstrictor tone, suggesting that factors other than active venoconstriction account, at least in part, for the shift.

In view of decreased intravascular volumes in genetically hypertensive rats and normal intravascular volumes in one-kidney Goldblatt hypertensive rats at 4 weeks postclipping, increased initial unstressed volume of veins in hypertension is an unlikely explanation for the findings of this study. The shift of the venous pressure-volume curves in a low pressure range (5-10 mm Hg) is evidence against the possibility that the differences between hypertensive and normotensive rats were due to a more rapid capillary filling in normal rats. Furthermore, capillary filling could not be demonstrated following addition of a dye or a radiographic contrast material to the infusate, even if venous pressure was raised to 40-45 mm Hg. Capillary filling during the rapid infusion must be small and due mainly to the displacement of residual perfusate from the veins into the capillaries. A genetically determined abnormality of venous anatomy, such as a reduction in the number of veins, in rats with spontaneous hypertension is also unlikely to account for the differences between hypertensive and normotensive rats, because similar shifts of the venous pressure-volume curves were produced in normal rats by constricting one renal artery.

Assuming that the initial unstressed volume of veins in hypertensive and control normotensive rats was similar, the shift of the venous pressure-volume curves toward the pressure axis in hypertensive rats suggests decreased venous capacity. Venous capacity is defined in this paper as the amount of fluid on the postcapillary or venous side of the circulation at a given pressure.

In this study, the mechanisms responsible for the venous changes in hypertensive rats were not investigated. In this regard, Lucas and Floyer found increased interstitial tissue pressure and unaltered interstitial fluid volume in one-kidney Goldblatt hypertensive rats at 60 days postclipping, suggesting a decreased interstitial space compliance. Decreased interstitial space compliance may in turn reduce the venous capacity and shift the venous pressure-volume curves toward the pressure axis. Pamnani and Overbeck demonstrated increased water, sodium, and potassium contents of veins in rats with coarctation and two- and one-kidney Goldblatt hypertension. The same authors found increased water content in veins of genetically hypertensive rats. Venous "water logging" or increases in ion-binding cellular and paracellular matrix, or both, may account for the abnormal venous pressure-volume relationships in hypertensive rats.

In view of unchanged venous pressure in hypertensive rats, the venous changes do not seem to be adaptive or
secondary to increased intraluminal pressure. The postmortem venous pressure-volume measurements in rats and the use of an artificial solution to perfuse the vascular beds of rats eliminate the possibility that neural or humoral factors are immediately responsible for the venous changes. The possibility that the venous changes were the residual effect of neural or humoral vascular stimuli was not investigated.

References

Effects of Acetylsalicylic Acid on the Ductus Arteriosus and Circulation in Fetal Lambs in Utero

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SUMMARY Intra-arterial and intravenous catheters were inserted in six fetal lambs at 125–130 days of gestation. On the following day, fetal arterial pressures and blood gases were monitored and fetal cardiac output and its distribution were measured by injection of radiolabelled microspheres 15 μm in diameter. Acetylsalicylic acid, 55–90 mg/kg of estimated fetal weight, then was administered into the fetal stomach. Fetal pulmonary arterial pressure rose significantly after an average of 58 minutes, increasing the pressure difference between the pulmonary artery and the aorta from 2 ± 0.3 (SEM) mm Hg during control to 11.2 ± 1.6 mm Hg. Resistance across the ductus arteriosus rose from 4.2 ± 0.5 (SEM) to 27.4 ± 4.01 units, and flow fell from 495 ± 44 (SEM) to 409 ± 20 ml/minute. The proportion of combined ventricular output distributed to the placenta, adrenals, heart, and lungs increased, whereas the proportion of combined ventricular output distributed to the brain, liver, intestine, kidneys, and upper and lower body fell. In two fetuses infusion of prostaglandin E1 reversed the pulmonary hypertension. Inhibition of prostaglandin synthesis in fetal lambs produced constriction of the ductus arteriosus and redistribution of cardiac output. It is probable that prostaglandins, particularly E1, are involved in regulation of blood flow through the ductus arteriosus and various vascular beds in the normal resting fetus.

glandins in controlling the fetal circulation we studied the effects of inhibition of prostaglandin synthesis in fetal lambs in utero. This inhibition was produced by the administration of acetylsalicylic acid, which blocks prostaglandin synthetase, an enzyme system essential for production of prostaglandins.

Methods

We studied six pregnant ewes with time-dated gestational periods of 125–130 days. Under epidural anesthesia with tetracaine HCl, 20 mg, polyvinyl catheters were inserted into the maternal femoral artery and vein. The uterus was exposed through a midline incision and a small hysterotomy was performed. Catheters were inserted into a fetal hindlimb vein and artery and passed centrally into the inferior vena cava and abdominal aorta, respectively. An incision was made in the fetal neck through a second hysterotomy, and a
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