Cardiovascular Hypertrophy and "Waterlogging" in Coarctation Hypertension

Role of Sympathoadrenergic Influences and Pressure

HENRY W. OVERBECK, M.D., PH.D.

SUMMARY The abdominal aorta above the renal arteries was partially constricted or sham-constricted in rats age 6 weeks that had had adrenal demedullation at age 4 weeks and guanethidine injections over Weeks 2–4 after birth to produce peripheral sympathectomy (S rats), and in sham-sympathectomized sham-demedullated control rats (SS rats). Plasma norepinephrine and epinephrine levels were reduced by 94–96% in S rats. Arterial pressures were also reduced by 30% in S rats, which, nevertheless, had hearts and aortas of normal size respective to body weight. With aortic coarctation, femoral arterial pressures did not increase but carotid pressures rose by 18–25% (p < 0.01). Expressed in terms of body weight, heart weights increased 75% and 50% (p < 0.001) and weights of the thoracic portion of the aorta increased 58% and 37% (p < 0.05) with coarctation in SS and S rats, respectively. In S rats there was also a rise in weight (26%) of the normal pressure abdominal portion of the aorta (p < 0.01); in contrast, this was not true in S rats. In both SS and S rats, wall water contents of thoracic aorta, abdominal aorta, and thoracic vena cava increased by 3–8% with coarctation (p < 0.02). The normal size of heart and aorta in S rats, despite lower arterial pressures, suggests that factors other than pressure and sympathoadrenergic stimuli influence cardiovascular growth in young rats. The increased weight of the abdominal aorta in coarcted SS rats suggests that vascular wall hypertrophy in hypertension does not require elevated intravascular pressures. Increases in heart and thoracic arterial weight with coarctation in S rats suggest that cardiovascular hypertrophy may occur in the virtual absence of sympathoadrenergic stimuli. However, in the absence of both pressure and catecholamine influences, vascular hypertrophy apparently does not occur. Finally, these studies provide evidence for vascular wall "waterlogging" in coarctation hypertension in rats not attributable to elevated levels of intravascular pressure and occurring despite the virtual absence of sympathoadrenergic influences. These findings support the hypothesis that humoral factors are involved in vascular wall "waterlogging" in hypertension.

(Hypertension 1: 486–492, 1979)

KEY WORDS • cardiovascular growth • guanethidine • sympathectomy

In coarctation hypertension in rats, "waterlogging" occurs in the walls of the normotensive portion of the aorta downstream to the coarctation and also in veins. This finding strongly suggests that neurogenic or humoral factors, or both, must be involved in the pathogenesis of these compositional changes. Accompanying the increases in water and sodium content in normotensive vessels are rises in wall potassium content, suggesting the development of hypertrophy or hyperplasia of vascular smooth muscle. In the present experiments, changes in weight and composition of cardiovascular tissue with coarctation hypertension in rats were investigated. In addition, the role of neurogenic influences in these abnormalities was evaluated in rats with the sympathoadrenergic system ablated by guanethidine injections as newborns and surgical adrenal demedullation.

Methods

According to the protocol devised by Johnson et al., newborn male, Sprague-Dawley rats (obtained from Zivic-Miller Laboratories, Inc., Allison Park, PA) received guanethidine sulfate (Ismelin Sulfate, kindly supplied by Ciba-Geigy), 50 mg/kg/day i.p., 5 days per week for 3 weeks (age 7–27 days) for a total of 15 injections. At 28 days of age these rats un-
derwent bilateral surgical adrenal demedullation. These rats were designated "sympathectomized" rats. Control rats, designated "sham-sympathectomized" rats, received equal volumes of saline i.p. 5 days per week at ages 7–27 days and sham adrenal demedullation at age 28 days. All rats were maintained on standard rat chow, containing 25.4% protein, 5.3% fat, 0.53% sodium, and 1.44% potassium.

Fourteen sham-sympathectomized and eight sympathectomized rats, ages 10 to 17 weeks, were decapitated without anesthesia. Trunk blood was collected into heparinized tubes on ice. Blood was centrifuged at 3000 X g collected into heparinized tubes on ice. Blood was centrifuged at 3000 X g at 4°C. Plasma was stored at -20°C. Assays for epinephrine and norepinephrine were performed by a radioenzymatic thin-layer chromatographic procedure.

At 6 weeks of age the remaining sympathectomized and sham-sympathectomized rats underwent laparotomy under ether anesthesia. To coarct the abdominal aorta, a silver clip, 0.813 mm inside diameter, (i.d.), was placed around the abdominal aorta upstream to the origin of both renal arteries. In other rats sham coarctation was created with a clip (i.d., 1.7 mm) too large to constrict the aorta. Tail systolic blood pressure in all rats was measured weekly without anesthesia by a tail cuff and optical sensor system (Natsume Rat Tail Manometer System, Model No. KN-0090). Four to 6 weeks after surgery in some rats, steady-state carotid and femoral arterial pressures were measured directly under light chloralose (50 mg/kg) anesthesia.

Four to 14 weeks after surgery the remaining rats were decapitated without anesthesia. Trunk blood was obtained from 14 sham-sympathectomized and eight sympathectomized rats for determination of serum sodium and potassium by flame photometry and creatinine by autoanalyzer. Then from 39 sham-sympathectomized and 24 sympathectomized rats, corresponding segments of descending thoracic aorta, abdominal aorta and the thoracic portion of the inferior vena cava were rapidly cleaned of adventitia and fat, excised and opened. In 30 of these sham-sympathectomized and 14 of these sympathectomized rats vascular segments were more rigidly standardized (the entire thoracic aorta downstream to the origin of the left subclavian artery, the entire abdominal aorta downstream to the left renal artery, and the entire thoracic portion of the inferior vena cava). In these rats the left ventricle (including septum) and right ventricle were also excised and opened. Placed on a glass plate, these specimens were blotted twice with filter paper to remove blood and surface fluid, and then weighed to the nearest 0.01 mg; this wet weight was recorded. The tissue was then oven dried at 100°C for 24 hours, and, when cooled to room temperature, reweighed and the dry weight recorded. The difference between the two weights was considered water content and was expressed as percent of wet weight.

Student's t test was used to compare values in sympathectomized rats with values in comparable sham-sympathectomized rats and values in rats with coarctation hypertension with those in sham-coarcted normotensive rats. When the alternative hypothesis predicted greater values in hypertensive rats, the one-tailed test was used. The null hypothesis was rejected at p < 0.05.

Results

Ptosis, enophthalmos, some diarrhea, and a modest growth defect were noted in the sympathectomized rats. At 10–11 weeks of age, body weights of sympathectomized rats averaged about 4–6% less than body weights of corresponding sham-sympathectomized rats (table 1). Between ages 10 and 20 weeks, this growth defect became greater, averaging about 20%.

In 14 sham-sympathectomized rats deliberately stressed by the decapitation procedure, plasma norepinephrine levels were 2870 ± 421 (SEM) pg/ml. Corresponding values in eight sympathectomized rats were 169 ± 23, representing a decrease of 94% (p < 0.001). In these sham-sympathectomized rats, plasma epinephrine levels were 4568 ± 660 pg/ml. Corresponding values in the sympathectomized rats were 170 ± 50, representing a decrease of 96% (p < 0.001). Mean (± SEM) body weights and ages of these sham-sympathectomized rats were 459 ± 26 g

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*Mean ± SEM; body weights and femoral and carotid pressures were measured 4–5 weeks after clipping; tail blood pressures were measured weekly over the 4–5 weeks after clipping and averaged.

†p <0.05.
‡p <0.01 for comparison of coarcted and sham-coarcted groups.
§No. = 13 sham-coarcted; 11 coarcted.
The body weights of coarcted rats 4–6 weeks after clipping (age 10–11 weeks) were not significantly less than body weights of corresponding sham-coarcted rats. However, between 6 and 14 weeks after clipping, coarcted rats were 5–12% lighter on the average than sham-coarcted rats. No other differences in health or in physical activity were noted between the two groups. Plasma Na, K, and creatinine levels were similar in coarcted and sham-coarcted rats, as has been previously reported.1 Autopsy of these rats, after cardiovascular tissue was obtained, verified aortic constriction by the clip in the absence of other gross abnormalities.

As may be seen from representative values in Table 1, tail systolic blood pressures averaged over the weeks following aortic coarctation were slightly lower in coarcted than in sham-coarcted rats. Tail pressures in sympathectomized rats were about 27% lower than pressures in the corresponding sham-sympathectomized group. Similarly, in the sympathectomized groups, femoral and carotid arterial pressures directly measured under light chloralose anesthesia 4–6 weeks after clipping averaged about 30% lower than pressures in the corresponding sham-sympathectomized group. In rats with coarctation, femoral arterial pressures did not significantly differ from values in sham-coarcted rats (p > 0.3). In contrast to femoral arterial pressures, carotid pressures in the rats with aortic coarctation were elevated by 18–25% (p < 0.01) over values in sham-coarcted rats. In the sympathectomized coarcted rats, the significant rise in carotid pressure (18 mm Hg) failed to bring this pressure up to the same level (133 mm Hg) as was observed in the sham-sympathectomized, sham-coarcted rats. In coarcted rats, the pressure gradient between carotid and femoral arteries averaged 34 and 27 mm Hg in the sham-sympathectomized and sympathectomized groups, respectively.

Figure 1 presents wet weights, wet weight-to-body weight ratio, and water content of left ventricles excised from these rats. One-tailed r test revealed highly significant (p < 0.001) increases in left ventricular weight-to-body weight ratio with coarctation, averaging 75% in sham-sympathectomized and 50% in sympathectomized rats. Changes in ventricular water content were small. Thus, increases in ventricular dry weights corresponded to increases in wet weights. Figure 2 presents similar observations in right ventricles, where wet weight-to-body weight ratio increased by 68% in sham-sympathectomized rats (p < 0.001). In sympathectomized rats there was a trend toward increase in weight of the right ventricle that did not reach statistical significance. Mean (± SEM) body weights and ages of these sham-sympathectomized rats were 468 ± 18 g and 12.1 ± 0.5 weeks (sham-coarcted, n = 14) and 418 ± 14 g and 11.6 ± 0.1 weeks (coarcted, n = 16), and of these sympathectomized rats were 373 ± 19 g and 12.0 ± 1.2 weeks (sham-coarcted, n = 6), and 381 ± 8 g and 11.2 ± 0.4 weeks (coarcted, n = 8).

Figure 3 presents wet weights of aortic and vena caval segments obtained from these same rats; because there were significant linear correlations (r = 0.694 and 0.733 for thoracic and abdominal aorta) between aortic and body weights, vessel weights are normalized to body weights in this figure. Wet weight of thoracic aorta increased with coarctation in both sham-sympathectomized and sympathectomized rats, whether or not expressed in terms of body weight. Standardized to body weight, wet weight increased 58% in sham-sympathectomized and 37% in sympathectomized rats (p < 0.02). In sham-sympathectomized rats with aortic coarctation, wet weight of the normotensive abdominal aorta, only when expressed in terms of body weight, also increased significantly (p < 0.01) but to a lesser degree (26%). This was not true in sympathectomized rats; although the number of observations was less, there was not even a trend for increased weight. Vena caval weight did not significantly increase with coarctation, although there was a trend in that direction. Similar calculations made using dry weights of aortic and vena caval segments resulted in similar conclusions.

Figure 4 represents water content of these vessels. With aortic coarctation, significant increases, ranging from 3% to 8%, were observed in all vessels studied (p < 0.02), whether the animals were sham-sympathectomized or sympathectomized. Mean (± SEM) body weights and ages of these sham-sympathectomized rats were 484 ± 18 g and 11.9 ± 0.3 weeks (sham-coarcted, n = 18) and 424 ± 13 g and 12.0 ± 0.2 weeks (coarcted, n = 21). In the sympathectomized rats corresponding weights and ages were 408 ± 18 g and 12.4 ± 0.6 weeks (sham-coarcted, n = 12) and 387 ± 7 g and 12.0 ± 0.4 weeks (coarcted, n = 12).

Discussion

In animals with certain forms of experimental hypertension, "waterlogging" occurs in walls of vessels in apparently normotensive vascular beds, including both arteries1 and veins.1,7,9 These observations strongly suggest that intravascular pressure alone cannot account for these abnormalities and that neural or humoral factors, or both, must contribute. The experiments here reported were primarily addressed to the hypothesis that sympathoadrenergic influences may play a role in the pathogenesis of these abnormalities. In addition, the role of pressure and sympathoadrenergic influences on growth of cardiovascular tissues was investigated.

Adrenal demedullated rats that had been treated neonatally with guanethidine were studied. The efficacy of guanethidine peripheral sympathectomy of newborn rats has been well documented.2,10 In the present study plasma catecholamines obtained after the stress of decapitation were 94–96% depressed in the sympathectomized rats. In the perfused hindlimb preparation of these rats, the drop in resistance after acute section of the femoral and sciatic nerves was absent or minimal; there was also a prominent left shift.
Figure 1. Means ± SEM of wet weights, wet weight-to-body weight ratio, and water content of excised left ventricles. Values in rats with aortic coarctation are represented by stippled bars marked "C." Values in sham-coarcted rats are represented by clear bars marked "SC." Paired bars represent values in sham-sympathectomized (SHAM) and sympathectomized (SYMP) groups. Numbers of observations are identified in parentheses. *p* values are given for one-tailed *t* test comparing sham-coarcted and coarcted rats within each group.

Figure 2. Means ± SEM of wet weights, wet weight-to-body weight ratio, and water content of excised right ventricles. Labeled as in figure 1.
of the norepinephrine dose-response curve (Overbeck HW, unpublished data).

Despite the virtual absence in these rats of the peripheral sympathoadrenergic system since shortly after birth, and despite arterial pressures 30% lower than normal, cardiovascular development appears to be fairly normal; heart and aortic weight-to-body weight ratios were within normal limits. Thus, the stimulus for cardiovascular growth in these young rats appears to include factors other than afterload and sympathoadrenergic influences. Because heart rate may be decreased by about 10-20% in these sympathectomized rats (G. Simon, personal communication), it is possible that increased stroke volume may be involved. It is also possible, but not investigated in the present study, that increased cardiac output, humoral factors, or a combination, may play a role in cardiovascular development.

In these sympathectomized rats, certain forms of experimental hypertension apparently can develop. Douglas et al.\textsuperscript{10} report significant one-kidney Goldblatt hypertension in such rats. In the present study, it was clearly documented that coarctation hypertension also develops in the absence of an intact sympathoadrenergic system; carotid arterial pressures rose by 18%, and, although the magnitude of hypertension was attenuated, there was cardiac hypertrophy.

Thus, observations in the present study also support earlier reports that the development of cardiac hypertrophy in several forms of hypertension is not dependent on an intact sympathoadrenergic system.\textsuperscript{11, 12} The increases in ventricular weight in the coarcted sympathectomized rats were accompanied by significant increases in weight of the thoracic aorta, so arterial thickening in hypertensives also occurs in the virtual absence of an intact sympathoadrenergic system. It is noteworthy that in these sympathectomized rats left ventricular weight-to-body weight and thoracic aortic weight-to-body weight ratios increased with aortic coarctation, although carotid arterial pressures failed by 17 mm Hg to reach even levels that were normal in the sham-sympathectomized rats.

Aortic thickening in coarctation hypertension also appear to occur in the absence of local rises in arterial pressure. Significant increases, averaging 26%, were observed in the weight of the abdominal aorta in sham-sympathectomized coarcted rats, despite hindquarters arterial pressures that were never elevated when repeatedly measured; Nolla-Panades,\textsuperscript{13} Pamnani and Overbeck,\textsuperscript{1} and Bell and Overbeck\textsuperscript{14} also found no evidence for increases in hindquarters pressures in coarction hypertension in rats. Associated with this increase in weight of the normotensive aorta in coarcted rats are increases in hindlimb vascular resistance,\textsuperscript{14} impairment in maximal vasodilation,\textsuperscript{14} and vascular hyperresponsiveness to norepinephrine,\textsuperscript{13} suggesting increases in wall-to-lumen ratio of resistance vessels in the normotensive hindquarters vascular beds of these hypertensive animals. These changes in vascular growth and resistance are not attributable to elevated hindquarters blood flow,\textsuperscript{14} to hindquarters atrophy\textsuperscript{13, 14} or to any obvious decrease in physical activity in the rats with aortic coarctation.

In contrast to sham-sympathectomized rats, in the sympathectomized coarcted rats the weight of the abdominal aorta was not increased. This finding may indicate that the thickening of the abdominal aorta in the sham-sympathectomized coarcted rats is related to sympathoadrenergic influences. The observation also suggests that either an intact sympathoadrenergic system or a rise in intravascular pressure may be necessary for aortic thickening in coarctica hypertension in rats; in the absence of both (as in the coarcted, sympathectomized rats) no thickening occurs.

The increase in weight of cardiovascular tissues in coarctation hypertension in rats is apparently not accompanied by growth of other tissues. Body weights of these coarcted rats were slightly reduced. Also, in similar coarcted rats there were no increases in kidney or hindlimb weight; in fact, weight of the kidneys and hindlimbs actually decreased.\textsuperscript{14} However, the effect of coarctation on the weights of other organs, e.g., liver, spleen, was not studied.

In the present study, large increases in dry as well as wet weights of vascular tissues were observed with coarctation, suggesting hypertrophy and/or hyperplasia of vascular smooth muscle. Cellular hypertrophy or hyperplasia would explain the increases in potassium content in the aorta in coarctation hypertension observed by Pamnani and Overbeck.\textsuperscript{1} On the other hand, Bevan et al.,\textsuperscript{15} studying coarctation hypertension in rabbits, found no evidence for growth of the abdominal aorta. This group also found no changes in water and electrolyte content of arterial wall, not even in the hypertensive portions of the vascular bed,\textsuperscript{18} suggesting that basic disease mechanisms of coarctation hypertension may be different in rabbits and rats.

In the present study, increases in water content also occurred in vascular walls in the coarcted rats. These increases, although significant, accounted for only a small portion of the increases in vessel weight discussed above. Of special note, comparable increases in vascular wall water content occurred in the coarcted animals that had undergone guanethidine sympathetomy and adrenal demedullation. This was true in normotensive abdominal aortic and vena caval tissue as well as in the hypertensive thoracic aorta. Thus, these findings indicate that an intact sympathoadrenergic system is not necessary for the development of vascular wall "waterlogging" in coarctation hypertension in rats.

If "waterlogging" occurs despite normal levels of intravascular pressure and in the virtual absence of sympathoadrenergic influences, a humoral factor must be involved in its pathogenesis. This conclusion is supported by the finding of Simon\textsuperscript{a} that the vessels of a parabiont to a renal hypertensive rat develop "waterlogging." More recently Simon\textsuperscript{17} has shown that plasma from perinephritic hypertensive dogs causes "waterlogging" of rabbit aortic media explants in vitro tissue culture. The nature of the humoral
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THORACIC AORTA  |  ABDOMINAL AORTA  |  VENA CAVA

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FIGURE 4. Means ± SEM of water content, expressed as percent of wet weight, of walls of excised thoracic and abdominal aorta, and vena cava. Labeled as in figure 1.
factor is unknown, as is the mechanism by which it acts. Regarding mechanisms, it is noteworthy that Overbeck, Pamnnani, and Ku recently reported that the administration of digoxin to dogs for 4 weeks is associated with inhibition of the sodium pump of mesenteric arteries and with "waterlogging" of their walls; these dogs did not become hypertensive, but may well have had elevated peripheral vascular resistance. These findings would tend to associate the pathogenesis of vascular wall waterlogging with a plasma factor that inhibits the sodium pump of the vascular smooth muscle cell membrane. Some plasma factors that might be involved in this process were discussed in a recent review by Haddy and Overbeck.

Acknowledgments

Dr. Richard McCarty, Department of Pharmacology, University of Virginia, kindly performed the assays of plasma epinephrine and norepinephrine. The technical assistance of Mr. Jerome Little is appreciated.

References

Cardiovascular hypertrophy and "waterlogging" in coarctation hypertension. Role of sympathoadrenergic influences and pressure.

H W Overbeck

*Hypertension*. 1979;1:486-492
doi: 10.1161/01.HYP.1.5.486

*Hypertension* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1979 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

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