Pressure-Independent Increases in Vascular Resistance in Hypertension: Role of Sympathoadrenergic Influences

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SUMMARY Experimental aortic coarctation in rats is accompanied by non-pressure-related increases in hindlimb total vascular resistance and its neurogenic and structural components. To investigate the role of the sympathoadrenergic system, we partially constricted or sham-constricted the abdominal aorta in rats age 6 weeks that had had adrenal demedullation and guanethidine injections to produce peripheral sympathectomy (SYMP rats, N = 13-coarcted, 14-sham-coarcted) and in sham-sympathectomized, sham-demedullated control rats (SHAM rats, n = 14-coarcted, 11-sham-coarcted). In both SHAM and SYMP rats with coarctation, tail and femoral arterial pressures did not increase but carotid pressures rose by 18–25% (p < 0.01), accompanied by significant increases in heart weight/body weight. However, arterial pressures in SYMP were 30% lower than those in SHAM rats (p < 0.005). In the pump-perfused (blood, 1ml/min), innervated, isolated hindlimbs of SYMP, compared to SHAM rats, the effect of acute section of local nerves on resistance was reduced and denervation hypersensitivity was documented. In contrast to SHAM, coarctation in SYMP rats was not accompanied by increases in total hindlimb resistance and its neurogenic component; there were, however, significant rises in the humoral-myogenic (p < 0.01) and structural (p < 0.05) components. Thus, the sympatho-adrenergic system influences arterial blood pressure and accounts for the elevated neurogenic component of peripheral vascular resistance in coarctation hypertension in rats, but does not account for the elevated structural component of resistance. An unknown humoral factor, or factors, may be incriminated in the latter. (Hypertension 2: 780–786, 1980)

KEY WORDS • neurogenic component of resistance • humoral-myogenic component of resistance • structural component of resistance • vascular wall-to-lumen ratio • guanethidine sympathectomy

In previous, related studies of the normotensive hindquarters vascular bed in rats with aortic coarctation hypertension, we observed several structural and functional abnormalities that could not be the result of local elevation of intravascular pressure. These abnormalities included an increased hindlimb vascular resistance, with a large contribution by an elevated neurogenic component and also a significant contribution by an elevated structural component. The purpose of the present experiments was to further explore the role of sympathoadrenergic influences in the underlying mechanisms of these abnormalities in resistance. Hindlimb resistance was studied in coarcted rats that had had the peripheral sympathoadrenergic system ablated by guanethidine injections as newborns and later had surgical adrenal demedullation.

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Methods

The protocols we used to ablate the peripheral sympathoadrenergic system and to coarct the aorta were identical to those previously reported. Briefly, newborn male, Sprague-Dawley rats (Zivic-Miller Labs, Inc.) received guanethidine sulfate (Ismelin Sulfate, kindly supplied by CIBA-GEIGY), 50 mg/kg/day i.p., 5 days per week for 3 weeks. At age 28 days these rats underwent bilateral surgical adrenal demedullation; they were designated "sympathectomized" rats. Control rats, designated "sham-sympathectomized" rats, received saline injections and sham adrenal demedullation. All rats were maintained on standard rat chow and water ad libitum.

When the rats were 6 weeks old, the abdominal aorta upstream to the renal arteries was coarcted (silver clip 0.813 mm i.d.) or sham-coarcted (clip 1.7 mm i.d.). Tail systolic blood pressure was measured weekly thereafter in unanesthetized rats (Natsume Tail Manometer System). Four to 6 weeks after coarctation, steady-state carotid and femoral arterial pressures were measured directly under light chloralose anesthesia; to produce approximately the same level of anesthesia, 37.5 mg/kg i.v. was given to sympathectomized and 50 mg/kg i.v. to sham-sympathectomized rats. Then, after administration of...
supplemental anesthesia (chloralose 37.5 or 50 mg/kg and pentobarbital 2.2-3 mg/kg i.v.), we used previously described techniques\(^4\) to measure perfusion pressure in the vascularly isolated, innervated, pump-perfused hindlimb vascular bed. Respiration was natural. The pump was primed with 2 ml of heparinized blood from a donor rat of the same strain. Then blood from the carotid artery of each rat was pumped at a constant rate of 1 ml/min into the femoral artery of its isolated hindlimb.

The perfusion was continued for 20 minutes to establish a steady state. Perfusion pressure was monitored by a Statham P23Gb pressure transducer and a Hewlett Packard recorder. Perfusion pressure at 20 minutes was used to calculate total limb vascular resistance. Then the femoral and sciatic nerves to the perfused limb were severed. Ten minutes later the steady-state limb perfusion pressure was recorded and used to calculate resistance after nerve section. Then a supramaximal dose of sodium nitroprusside (0.15 mg/kg in 0.05-0.10 ml isotonic NaCl solution) was injected rapidly into the pump tubing upstream to the pump. Maximal vasodilation of the limb vascular bed was tested by successively doubling the dose. (In pilot experiments, no further vasodilation was elicited in three rats by papaverine injections). Perfusion pressure 4 minutes after the final nitroprusside injection was used to calculate resistance at maximal vasodilation.

Sodium nitroprusside was then infused into the pump tubing at 0.0153 mg in 0.0051 ml isotonic NaCl solution/min in all rats, a rate that maintained this maximal hindlimb vasodilation. With this infusion continuing, successively increasing doses of norepinephrine (Levarterenol Bitartrate, USP, Winthrop Laboratories, prepared in a saline solution with HCl added) were injected into the pump tubing over the range 0.0156-16 \(\mu\)g (each dose in 0.005 ml saline). Five minutes were allowed between each injection for resistance to return toward baseline. Response was calculated as increase in resistance over resistance at maximal vasodilation.

As previously,\(^2\) in all rats the perfusion pressure gradient across the outflow tubing and cannula was subtracted from the perfusion pressures used to calculate limb resistances, which were calculated as the ratio of perfusion pressure to limb blood flow and expressed in terms of hindlimb wet weight.

Four hindlimb resistances were calculated: 1) total resistance; 2) the "neurogenic component" of resistance, calculated as the difference between total resistance and resistance after acute nerve section. For data interpretation, the neurogenic component was also expressed as percentage of total resistance; 3) the "humoral-myogenic component" of resistance, calculated as the difference between resistance after acute nerve section and resistance at maximal vasodilation. Again, for data interpretation, the humoral-myogenic component was also expressed as percentage of total resistance; and 4) the "structural component" of resistance, the resistance at maximal vasodilation.

To assess the effect of the sodium-nitroprusside infusion on hindlimb responses, the procedures described above were used to create aortic coarctation (\(n = 4\)) or sham coarctation (\(n = 6\)) in intact Sprague-Dawley rats also obtained from Zivic-Miller Labs. Tail, carotid, and femoral pressures were measured, as described above, and 4 to 6 weeks after coarctation, hindlimb vascular responses to norepinephrine were measured, as described above, with the exception that sodium nitroprusside was not administered. Additionally, in two coarcted and three sham-coarcted rats, the femoral and sciatic nerves to the perfused limb were left intact.

After the perfusion to each rat all arterial blood samples were taken for measurement of hematocrit, plasma creatinine (autoanalyzer), and sodium and potassium concentrations (flame photometer). Each rat was autopsied to verify clip type, placement, and general health. The heart and the hindlimb were weighed.

Student's \(t\) test\(^5\) was used for comparisons between coarcted and sham-coarcted rats within the sympathectomized group and within the sham-sympathectomized group. For comparison of values in sympathectomized and sham-sympathectomized rats (across-group comparisons), analysis of variance, followed by mean comparison by treatment contrasts,\(^6\) was used. The null hypothesis was rejected at \(p \leq 0.05\).

Results

In the sham-sympathectomized groups, 11 sham-coarcted and 14 coarcted rats were studied. In the sympathectomized groups, 14 sham-coarcted and 13 coarcted rats were studied. At the time of the hindlimb perfusion studies, when the rats were 10 to 11 weeks old, body weights (fig. 1) of sympathectomized rats averaged 4-6% less than body weights of corresponding sham-sympathectomized rats. Body weights of coarcted rats did not significantly differ from those of sham-coarcted rats in the corresponding group (fig. 1). Hematocrits and plasma Na, K, and creatinine concentrations were similar and within normal ranges in all groups of rats, as previously.\(^1-4\) As previously reported,\(^2\) ptosis, enophthalmos, and some diarrhea were also observed in the sympathectomized rats, but otherwise all rats remained healthy with no evidence of cardiac or renal insufficiency or malignant hypertension at time of study. No differences in physical activity were noted among the groups. Autopsy of these rats verified aortic constriction (or sham-constriction) in the absence of other gross abnormalities.

Tail systolic blood pressures averaged over the weeks following aortic coarctation are presented in figure 2, along with femoral and carotid mean arterial pressures directly measured under light chloralose anesthesia 4-6 weeks after clipping. Tail systolic, femoral, and carotid pressures in sympathectomized rats averaged about 30% lower than pressures in the corresponding sham-sympathectomized group \((p < 0.005)\). In coarcted rats, as compared to the corresponding sham-coarcted group, tail pressures were,
FIGURE 1. Mean + SEM of body weight, heart weight/body weight, and hindlimb weight. 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 identified by "SHAM" and "SYMP" represent values in sham-sympathectomized and sympathectomized groups, respectively. Numbers of observations are identified in parentheses. The p values are given for comparison of sham-coarcted and coarcted rats within each group.

FIGURE 2. Mean + SEM of blood pressures. On the left of figure are indirect tail systolic pressures recorded at weekly intervals following clipping. On right are carotid (C) and femoral (F) mean blood pressures measured directly under light chloralose anesthesia 4 weeks after clipping. Groups and numbers of observations are identified as in figure 2. P values are given for comparison of sham-coarcted and coarcted rats within each group.
if anything, slightly lower, femoral arterial pressures were similar ($p > 0.3$), but carotid pressures were elevated by 18–25% ($p < 0.01$). In coarcted rats, the pressure gradient between the carotid and femoral arteries averaged 34 and 27 mm Hg in the sham-sympathectomized and sympathectomized groups, respectively.

Heart weight-to-body weight ratios (fig. 1) were elevated by 40–52% in the coarcted rats ($p < 0.001$), but their hindlimb weights did not significantly differ from weights in corresponding sham-coarcted groups.

Hindlimb vascular resistances in these rats, normalized for these hindlimb weights, are presented in figures 3 and 4. First, consider the sham-coarcted rats. To assess the influence of the sympathoadrenergic system on hindlimb resistance in these control rats, resistances in sympathectomized rats were compared to those in sham-sympathectomized rats. There was no significant difference ($p > 0.25$) in total hindlimb resistance (figure 3). However, the components of resistance, presented as clear bars in figure 4, differed greatly: Compared to sham-sympathectomized rats, sympathectomized rats had decreases in the neurogenic component averaging 52%, $p < 0.05$ (−58%, $p < 0.005$, if the component was expressed as percentage of total resistance), increases averaging 87% ($p < 0.01$) in the humoral-myogenic component (+56%, $p < 0.01$, if the component was expressed as percentage of total resistance), and increases averaging 35% of borderline significance ($p < 0.1$) in the structural component of resistance (residual resistance at maximal vasodilation).

Next, consider the sham-sympathectomized group. As indicated in figure 3, aortic coarctation was accompanied by a 51% elevation in total resistance (total hindlimb resistance, 1902 ± 177 and 2875 ± 149 mm Hg/ml min$^{-1}g^{-1}$ in sham-coarcted and coarcted rats, respectively, $p < 0.01$). Resistance fell with nerve section in all rats. The neurogenic component of resistance (fig. 4) was 81% greater ($p < 0.01$) in the coarcted than in the sham-coarcted rats. With the neurogenic component expressed as percentage of total resistance, this rise was 27% (41.6 ± 4.7 and 52.8 ± 1.5% of total resistance in sham-coarcted and coarcted rats, respectively, $p < 0.02$). In all rats resistance fell further following intrafemoral arterial injection of supramaximal doses of sodium nitroprusside. The humoral-myogenic component of resistance (fig. 4) differed only if expressed as percentage of total resistance (38.6 ± 3.6 and 29.3 ± 1.5% of total resistance in sham-coarcted and coarcted rats, respectively, $p < 0.02$, a fall in the coarcted rats averaging 24%). Again, residual resistance at maximal vasodilation (the structural component of resistance — fig. 4) remained higher in the coarcted rats by an average of 41% (357 ± 20 and 502 ± 41 mm Hg/ml min$^{-1}g^{-1}$ in sham-coarcted and coarcted rats, respectively, $p < 0.01$).

In contrast to these sham-sympathectomized rats, in which results were similar to those we have previously reported,⁴ in the sympathectomized, adrenal-demedullated rats, no significant elevation of total resistance (fig. 3) occurred with coarctation (total hindlimb resistance 2182 ± 120 and 2460 ± 215 mm Hg/ml min$^{-1}g^{-1}$ in sham-coarcted and coarcted rats respectively, $p > 0.2$) and there were absent or small drops in limb resistance with acute nerve section. In these sympathectomized rats, the calculated neurogenic component of resistance (fig. 4) was significantly smaller in coarcted rats if expressed as percentage of total resistance (17.6 ± 3.0 and 6.5 ± 2.4% of total resistance in sham-coarcted and coarcted rats respectively, $p < 0.01$). There was a drop in resistance in each rat in response to in-

![Figure 3](http://hyper.ahajournals.org/)
trafemoral arterial injection of supramaximal doses of sodium nitroprusside. The calculated humoral-myogenic component of resistance in sympathectomized coarcted rats (fig. 4) was 36% greater ($p < 0.01$) than that in sympathectomized sham-coarcted rats. (If expressed as percentage of total resistance, this component was 24% greater in coarcted rats, $p < 0.01$: 60.2 ± 2.9 and 74.6 ± 3.9% of total resistance in sham-coarcted and coarcted rats, respectively). Again, residual resistance at maximal vasodilation (the structural component of resistance) was higher (fig. 4) in the sympathectomized coarcted rats (612 ± 56 mm Hg/ml min⁻¹g⁻¹) than in the sympathectomized sham-coarcted rats (480 ± 19 mm Hg/ml min⁻¹g⁻¹) by an average of 28% ($p < 0.05$).

Figure 5 presents norepinephrine dose-response relationships in the hindlimb vascular beds of the four groups of rats. It may be clearly seen that these curves are displaced to the left in the sympathectomized rats with reductions in $ED_{50}$. However, curves in corresponding coarcted and sham-coarcted groups appear similar.

Finally, figure 6 presents norepinephrine dose-response relationships in the intact rats not receiving sodium nitroprusside. Mean values in coarcted and sham-coarcted rats are identified for groups with intact or acutely sectioned hindlimb nerves. There was again no trend for steepened curves in the coarcted rats.

**Discussion**

In the present study, the sham-sympathectomized rats were similar to those we have previously studied. The results of the present study in these control rats

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**Figure 4.** Means ± SEM of components of total hindlimb vascular resistance: 1) neurogenic; 2) humoral-myogenic; and 3) structural. Derived from data presented in figure 3. Groups and numbers of observations are identified as in figure 1. The $p$ values are given for comparison of sham-coarcted and coarcted rats within each group.

**Figure 5.** Norepinephrine dose-response curves in pump-perfused hindlimbs infused with sodium nitroprusside. Vertical axis represents increment evoked in resistance over resistance at maximal vasodilation. Solid symbols represent means ± SEM in sham-sympathectomized and open symbols means ± SEM in sympathectomized groups. $ED_{50}$ identified by arrows.
confirm previous observations by us\(^1\) and by others\(^7\) that peripheral resistance rises in normotensive vascular beds of rats with aortic coarctation. Again we observed that aortic coarctation was accompanied by increases in hindlimb total vascular resistance with prominent contributions by the neurogenic component of resistance. By “the neurogenic component of resistance” we refer to that component of total resistance that is related to neural influences; however, it cannot be said whether changes in this component are the result of changes in nerve traffic, modulation of nerve traffic, response of the vessel to nerve traffic (on a structural and/or functional basis), or a combination. In previous\(^4\) and present studies, this increased total hindlimb resistance also included a lesser, but significant, contribution by the structural component of resistance, as measured by maximal vasodilation.

Because these resistance changes with coarctation cannot be attributed to increases in hindlimb intravascular pressures,\(^6\) the primary purpose of the present study was to attempt to assess the role of sympathoadrenergic influences. In this regard, there is evidence for important participation of the sympathetic nervous system in the mechanisms of several other forms of hypertension. These include spontaneously hypertensive rats of the Japanese strain,\(^8\) genetically hypertensive rats of the New Zealand strain,\(^9\) salt-sensitive Dahl rats,\(^10\) and DOCA-salt hypertension.\(^9\) In these previous studies, the contribution of the sympathetic nervous system was assessed before and after chemical or immunological ablation. There is also evidence that adrenergic neurons have trophic effects on vascular smooth muscle.\(^11\) Thus, altered sympathoadrenergic influences in coarctation hypertension might account for the non-pressure-related increases we had observed in both the neurogenic and the structural components of peripheral vascular resistance.

The effectiveness of ablation of the peripheral sympathoadrenergic system we produced with guanethidine injections and adrenal demedullation has been previously documented.\(^4\)-\(^13\) In brief, in rats similarly treated, plasma catecholamines at age 10–20 weeks are depressed by 94–96%.\(^9\) Furthermore, Johnson and his co-investigators' report that rats so treated have undetectable levels of tyrosine hydroxylase activity in superior cervical ganglia, and light microscopic examination of these ganglia reveals that sympathetic neurons are virtually absent. Norepinephrine levels in peripheral tissues (spleen, heart, mesenteric vasculature) are markedly reduced at both 9 and 16 weeks of age.\(^1\) Yet there are no decreases in norepinephrine in whole brain, indicating that the abnormalities produced exclude the central nervous system. Physiological testing indicates functional denervation of the vasculature; stimulation of vasomotor outflow after pithing does not increase blood pressure in treated rats at 9 or 26 weeks of age;\(^4\) tyramine vasoconstriction is markedly reduced;\(^13\) there is also a marked insensitivity to pentolinium vasodepression and a pressor, rather than depressor, response to phenolamine.\(^13\) The present results further indicate that acute section of the femoral and sciatic nerves in these treated rats had only minimal effects on hindlimb vascular resistance; therefore, the neurogenic component of hindlimb vascular resistance was greatly reduced by this treatment. Furthermore, in the present study denervation hypersensitivity of hindlimb vasculature to norepinephrine was clearly documented. Although catecholamines were not measured in vascular wall in these rats, the above is ample evidence for nearly complete denervation of the peripheral vessels in the sympathectomized rats at the time when hindlimb resistance was measured in this study.

The normal levels of serum sodium and potassium found in the sympathectomized rats is evidence that adrenal demedullation did not destroy the adrenal cortex with reduction of plasma corticosterone, although levels of this hormone were not measured. It is possible, however, that the adrenal demedullation did result in some alteration in blood steroid levels.

In sympathectomized, sham-coarcted rats of the present study, despite a considerably lower contribution by the neurogenic component to hindlimb resistance, resting hindlimb resistance did not differ significantly from that in sham-sympathectomized, sham-coarcted rats. A large increase in the humoral-myogenic component of resistance, coupled with a small increase of borderline significance in the structural component, apparently serve to maintain hindlimb resistance at normal levels.

Results in these sympathectomized, adrenal-demedullated rats that underwent coarctation extend our previous observations of peripheral vascular resistance in this form of experimental hypertension. In contrast to intact rats, in sympathectomized rats aortic coarctation is no longer accompanied by
elevations in hindlimb vascular resistance and its neurogenic component. Also, although coarctation hypertension with cardiac hypertrophy does develop in these sympathectomized rats, its severity is much attenuated; carotid arterial pressures, although rising significantly, do not even reach levels that are normal in rats with an intact sympathoadrenergic system. These findings illustrate the important role played by the sympathoadrenergic system in the regulation of peripheral vascular resistance and arterial blood pressure in this form of hypertension. An intact system appears to be necessary for full expression of both systemic and local hemodynamic changes in coarctation hypertension, as well as in the other forms of hypertension noted above.

The experiments presented here were not addressed to the mechanism of the rise in blood pressure, albeit attenuated, that occurred in the sympathectomized coarcted rats. We may only say that it appears that the vascular bed of the hindquarters does not participate in the hypertension. It may be that resistance was elevated in other vascular beds in these rats or that their cardiac outputs were increased, or both.

With aortic coarctation in these sympathectomized rats in the present study, there were significant rises in the humoral-myogenic component of hindlimb resistance. It is possible, but not investigated in the present study, that these represented rises in plasma angiotensin levels greater than those we had previously observed in intact coarcted rats. Nevertheless, these rises in the humoral-myogenic component of resistance were insufficient to significantly elevate total hindlimb resistance in the sympathectomized, coarcted rats.

With aortic coarctation in these sympathectomized rats, there were also small, but significant, elevations in hindlimb residual resistance at maximal vasodilation. Thus, neither elevated intraarterial pressure nor an intact sympathoadrenergic system is necessary for the development of impaired maximal vasodilation in coarctation hypertension. Residual resistance at maximal vasodilation, with the smooth muscle entirely relaxed, is determined by the geometry of the vessel wall and represents the structural component of resistance. Impairment of maximal vasodilation, then, could be the result of fewer vessels, of increases in vessel wall-to-lumen ratio, or a combination. Our previous experiments in rats with hindquarters atrophy due to infrarenal aortic coarctation indicate that it is unlikely that rarification of the vascular bed accounted for impaired maximal vasodilation in the coarcted rats in this study. Increases in vessel wall-to-lumen ratio would enhance vascular responses to norepinephrine, and, in fact, Nolla-Panades observed significant vascular hyperresponsiveness in the solution-perfused hindquarters of rats with coarctation hypertension. In the present study, in blood-perfused hindlimbs infused with sodium nitroprusside, we failed to find such hyperresponsiveness. This failure was apparently not attributable to effects of the sodium nitroprusside. It may be that a lower sensitivity of our assay procedure or a smaller increase in the structural component of resistance in our coarcted rats, or both, accounted for this difference in results.

Thus, we consider the combination of the impaired maximal hindlimb vasodilation we observed with the vascular hyperresponsiveness observed by Nolla-Panades as good evidence that non-pressure-related increases occur in the wall-to-lumen ratio of resistance vessels in coarctation hypertension. Clearly, these structural vascular changes cannot be attributed to intravascular pressure, to genetic predisposition, or to sympathoadrenergic influences. Thus, we feel that an unknown humoral factor, or factors, may be implicated in the pathogenesis of structural changes thickening walls of resistance vessels in hypertension. The relationship of vascular wall "waterlogging," which also have attributed to humoral factors, to these changes in structural resistance in coarctation hypertension requires further investigation.

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