Prenatal and Postnatal Hydralazine Treatment Does Not Prevent Renal Vessel Wall Thickening in SHR Despite the Absence of Hypertension

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Previous studies in our laboratory have shown that the renal blood vessels of 21-week-old Wistar-Kyoto spontaneously hypertensive rats exhibited thicker vascular walls than age matched Wistar-Kyoto normotensive rats. Morphometric analysis of the relaxed renovascularure revealed an increase in the cross-sectional area of the media, which in most cases was associated with an increase in the number of smooth muscle cell layers. To test if these structural changes occur in the absence of raised blood pressure, hydralazine was administered to spontaneously hypertensive rats and normotensive controls prior to and during pregnancy (100 ml/l drinking water), and to the newborn males up to 21 weeks of age (16.9 mg/kg/day by gavage until weaning followed by 100 mg/l in the drinking water). Treated animals were compared with untreated rats. Treatment prevented hypertension development in spontaneously hypertensive rats but did not alter the structural changes found in untreated animals with hypertension. At 21 weeks of age, hydralazine-treated spontaneously hypertensive rats had similar wall-to-lumen area ratios, medial cross-sectional areas and numbers of medial smooth muscle layers as untreated hypertensive rats while these parameters were greater in treated and untreated spontaneously hypertensive rats than in either treated or untreated normotensive controls. Withdrawal of hydralazine from 26-week-old spontaneously hypertensive rats that had been treated in utero and postnatally and had normal blood pressures throughout life resulted in the rapid onset of hypertension. Our results show that renal vascular wall thickening in spontaneously hypertensive rats occurs in the absence of high blood pressure and therefore is not a secondary effect of raised blood pressure. (Circulation Research 1988;63:534–542)
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FIGURE 1. Systolic blood pressure versus age profiles of untreated male Wistar-Kyoto spontaneously hypertensive rats (SHR) and Wistar-Kyoto normotensive rats (WKY), and male SHR and WKY treated in utero and postnatally with hydralazine. SHR and WKY females were treated with 100 mg hydralazine/l drinking water. Once blood pressure was normalized in SHR, a male rat was introduced to inseminate the female. Hydralazine treatment was continued during pregnancy. Newborn pups were fed 16.9 mg hydralazine/day/kg rat weight by gavage up to weaning (3–4 weeks old). Subsequently, 100 mg hydralazine/l was placed in the drinking water up to 21 weeks of age.

Materials and Methods

SHR and WKY were obtained from a colony maintained at McMaster University, Hamilton, Ontario, Canada. The systolic blood pressure of the animals was measured using a tail-cuff compression method.

Treatment Protocol

Female SHR were treated with 100 mg/l hydralazine (in the drinking water) until the blood pressure was lowered to the same level as that present in age- and sex-matched normotensive WKY. A male SHR was then introduced into the cage to inseminate the female. Hydralazine treatment was continued throughout the pregnancy. In humans, hydralazine is known to cross the placental barrier and has been found in the fetal circulation at concentrations comparable to that present in the maternal blood. Therefore, the antihypertensive effects of the drug experienced by the fetus in utero should be the same as those observed in the maternal circulation. However, since little active drug is present in breast milk of hydralazine-treated subjects, newborn SHR prior to weaning were tube fed with hydralazine at a concentration of 16.9 mg/day/kg. This dose was determined by a drinking rate study. It was observed that when 100 mg hydralazine/l was introduced into the drinking water of 21-week-old male SHR (with established hypertension), the blood pressure of the animals was normalized after 1 week of treatment. During such treatment, the drinking rate was monitored for 5 days/week for 1 month using calibrated spill-resistant water bottles. The mean±SEM dose of ingested drug was 14.5±1.3 mg/day/kg. The highest rate of hydralazine ingestion observed in the drinking rate study, which normalized the blood pressure of SHR with established hypertension, was 16.9 mg/day/kg. After weaning (at 4 weeks of age), SHR and WKY were given 100 mg hydralazine/l drinking water. Treated SHR were compared with identically treated WKY and with untreated SHR and WKY.

Sampling Protocol

The blood pressure of treated and untreated SHR and WKY was measured using a tail-cuff compression method. At 21 weeks of age, the left kidney was exposed and fixed for light microscopy by vascular perfusion. The main renal, interlobar, arcuate, and interlobular arteries were sampled and a detailed morphometric analysis of all vascular components was carried out using methods previously described.2
The renal vascular bed was perfused under fully relaxed conditions at a constant flow of 0.82 ml/min and was subsequently perfusion fixed with PO₄ buffered 2.5% glutaraldehyde (400 mosm pH 7.4). Vascular segments were subsequently cut out of the kidney and processed with cacodylate-buffered 1% OsO₄ (pH 7.4), 0.5% uranyl acetate, and dehydrated with alcohol. Previous studies have outlined the fixative compositions and their preparation in detail and have shown that such fixation does not alter the volume of cultured aortic smooth muscle cells (SMC). Tissue samples were embedded in Spurr's resin and 1-μm thick sections stained with Azure II-methylene blue were cut and mounted on glass slides. All morphometric dimensions measured from vessel cross sections on light micrographs were corrected for section angle (see Smeda et al). The number of SMC layers in the media was counted using phase contrast illumination. Cortical arteries were separated into arcuate and interlobular branches as described by Fourman and Moffat. The mean lumen diameter of arcuate and interlobular arteries of treated and untreated SHR and WKY were not significantly different in the four groups studied. Since the range of lumen diameters was large for cortical vessels (20–175 μm), these arteries were further subdivided according to the internal diameter (DI) between the internal elastic lamina (IEL). The cross-sectional area of intima + IEL, the media, and the number of SMC layers were compared for each DI interval in the groups studied.

Hydralazine Withdrawal Study

The effects of hydralazine withdrawal were studied in SHR that had been treated in utero and postnatally up to 26 weeks of age. The blood pressure of the treated SHR was measured before and subsequently 2, 6, and 14 days after drug withdrawal.

Statistical Analysis

An analysis of variance (ANOVA) was carried out among the four study groups. An unpaired Student's t test was subsequently used to determine which groups significantly (p<0.05) differed from each other. All results are presented as the mean ± SEM.

Results

Systolic Blood Pressure in Treated and Untreated SHR and WKY

Figure 1 shows the blood pressure profiles of SHR and WKY treated in utero and postnatally with hydralazine, as well as untreated SHR and WKY. Hydralazine reduced the blood pressure of SHR to normal levels. The blood pressures of the treated SHR were significantly lower than the untreated SHR at all ages greater than 6 weeks. At all ages studied, the treated SHR had the same blood pressures as untreated WKY. However, it should be noted that hydralazine also significantly reduced the blood pressure of WKY animals at 9, 11, 13, 14, and 21 weeks of age. Treated SHR generally had slightly higher blood pressures than treated WKY.

Physical Characteristics of Rats Used in Morphometric Study

The physical characteristics of the treated and untreated SHR and WKY used for morphometric study at the time of sampling (21 weeks of age) are detailed in Table 1. The hydralazine-treated SHR and WKY had lower body weights than their non-
treated counterparts. Within the hydralazine-treated group, SHR were heavier than WKY. The blood pressures of treated SHR and WKY groups were, respectively, lower than the untreated SHR and WKY groups. Within the hydralazine-treated group, the mean systolic blood pressure was 20 mm Hg higher in SHR than in WKY, and 63 mm Hg higher in the untreated groups. The kidney-to-body weight ratio was increased in both treated and nontreated SHR when compared with WKY. Hydralazine treatment did not alter kidney-to-body weight ratio in either SHR or WKY. Renal vascular resistance was significantly lower in hydralazine treated SHR and WKY than nontreated SHR and WKY, but there was no difference between SHR and WKY in either treated or untreated groups.

**Morphometric Analysis of the Renal Arteries**

Figures 2–6 outline the structural alterations in the renal vasculature of 21-week-old control SHR and WKY raised on tap water throughout their life (respectively, SHRc and WKYc) and age-matched SHR and WKY treated in utero and postnatally with hydralazine (respectively, SHR, and WKY,).

**Alterations in Lumen Diameter**

Figure 2 summarizes the lumen diameters of the renal arteries in the various treatment groups. The lumen diameter of the main renal artery of SHR, and SHR, was larger than that present in, respectively, WKYc and WKY,. In spite of the fact that blood pressure was normal in SHR,, the lumen diameter of the main renal artery was similar to that of SHR,, with high blood pressure and also to that of untreated WKYc. The lumen diameter of the main renal artery of WKY,, however, was significantly smaller than that of the other groups.

The lumen diameters of the interlobar arteries of treated SHR, were larger than those present in either WKYc, or WKY,, and comparable in diameter to the arteries sampled from control SHRc. There was no difference between the lumen diameters of interlobar arteries of control SHRc and WKYc.

The lumen diameters of arcuate and interlobular arteries were not different between treated and nontreated groups of animals.

**Alterations in Cross-Sectional Areas of Intima (Endothelium, Subendothelial Space, IEL)**

Figure 3 outlines the cross-sectional area of the intima present in the blood vessel wall of the various treatment groups.

Qualitatively similar alterations were observed when the SHRc and SHR, were compared with WKYc and WKY,, respectively. The cross-sectional area of intima was greater in SHRc than WKYc in renal arteries with a DI >95 μm, whereas arteries from SHRc with a DI >30 μm had a larger intima than WKYc. Hydralazine treatment increased the cross-sectional area of the intima, particularly in the larger renal arteries. SHR, were found to have larger intima than SHRc and WKYc in all classes of arteries studied and in arteries with a DI >120 μm, the intima of WKYc was larger than WKYc.

**Alterations in the Cross-Sectional Area of Media**

Figure 4 outlines the cross-sectional area of the media in the various blood vessels of treated and nontreated animals.

The in utero and postnatal normalization of blood pressure in SHRc had little effect on the cross-sectional area of arterial media present in SHR. In all the classes of arteries studied both SHRc and SHR, had increased quantities of media when compared with WKYc and WKY,, respectively. Despite the absence of increased blood pressure in SHR, when this group was compared with SHRc, similar quantities of media were present in all the arterial groups, with the exception of those having a DI between 95 and 120 μm. Furthermore, in all arterial classes, except those having a DI between 70 and 95 μm, greater quantities of media were present in SHRc than WKYc. These findings indicate that SHR...
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Intimal cross sectional area, μm²

Figure 3. Cross-sectional area quantities of intima present in renal arteries of control (SHRc, WKYc) and hydralazine-treated (SHRt, WKYt) SHR and WKY (mean ± SEM; ANOVA test, all arterial groups were significant at p<0.05 or p<0.01). MRA, main renal artery; D₀, internal diameter.

In summary, the normalization of blood pressure did not alter the increased numbers of SMC layers observed in SHR.

When compared with WKYc, the WKYt group was found to have a significantly decreased number of SMC layers in the media of the main renal, interlobar, and cortical arteries with a DI between 70 and 95 μm. On the other hand, in cortical arteries with a DI between 30 and 70 μm, the number of SMC layers was significantly increased in WKYt when compared with WKYc.

Alterations in Wall-to-Lumen Ratio

Figure 6 summarizes the alterations in the wall-to-lumen area ratio in the various treatment groups. The wall-to-lumen ratio is the combined area of the intima, IEL, and media divided by the area of the lumen.

ANOVA testing of the wall-to-lumen ratio in the main renal and interlobar arteries indicated that no significant alteration was present between the test groups. In arteries with a DI <175 μm, both SHRc and SHRt exhibited a significant increase in the wall-to-lumen ratio over WKYc and WKYt, respectively. Within these same classes of arteries, the ratio was also elevated in SHR, over WKYc.

When WKY, were compared with WKYc, the wall-to-lumen ratios of the cortical arteries with a DI between 95 and 120 μm and 30 and 70 μm were not significantly altered. However, hydralazine treatment of WKY did reduce the wall-to-lumen ratio in cortical arteries with a DI between 120 and 175 μm and 70 and 95 μm.

Hydralazine Withdrawal Experiment

Five male SHR treated in utero and postnatally with hydralazine were used in this experiment. The
Discussion

The renal vascular wall of SHR was thickened mainly due to an increase in the cross-sectional area of the media. This structural change occurred to an almost identical degree in hydralazine-treated SHR with normal blood pressure as in untreated SHR with high blood pressure. The cross-sectional area of media, the wall (intima + media)-to-lumen ratio, and the number of SMC layers in the media of renal vessels were similar in both treated and untreated SHR groups but greater than that present in either treated or untreated WKY. These results indicate that in the renovascularure of SHR, medial thickening and increases in the number of SMC layers occur in the absence of an increased blood pressure and support the conclusion that high blood pressure is not the cause of such structural changes.

Various studies have been performed to determine the effect of antihypertensive drug treatment on the vascular structure of SHR. Mulvany et al7 used 6-hydroxydopamine to chemically sympathectomize SHR and WKY from birth. At 24 weeks of age, the sympathectomized SHR had slightly (but significantly) higher mean arterial pressure (mean difference 12 mm Hg) than similarly treated WKY. It was found that the lowering of blood pressure had no effect in reducing the wall thickness of small mesenteric vessels in SHR. In studies performed by Scott and Pang,8 2-day-old SHR and WKY were injected with capsaicin. At 12 weeks of age, the mean arterial pressure of previously treated SHR and WKY was reduced by approximately 32 mm Hg over that present in untreated SHR and WKY. Although treated SHR and untreated WKY had similar mean arterial pressures (respectively, 101 ± 5 versus 91 ± 5 mm Hg), the vascular wall of premucosal jejunal arteries from treated SHR was 55% thicker than in untreated WKY. In a carefully performed study, Jespersen et al9 treated SHR and WKY with hydralazine from 4 weeks of age and subsequently studied the mesenteric vascular bed at 12-14 and 22-27 weeks of age. Despite the fact that throughout the treatment period hydralazine normalized the blood pressure of SHR, it prevented the reduction in lumen diameter but not the increase in wall thickness observed in the mesenteric arteries of young SHR. When compared with WKY, the older group of SHR also exhibited a reduced diameter and a thicker vascular wall. However, at this age, hydralazine treatment significantly increased

rats were 26 weeks of age and weighed 302 ± 5 g at the time of drug withdrawal. The effect on blood pressure of substituting tap water for 100 mg/l hydralazine is shown in Figure 7. Two days after withdrawal, the blood pressure had increased from a mean value of 120 ± 5 mm Hg to 192 ± 11 mm Hg. Over the next 12 days, the blood pressure increased still further to 210 ± 6.71 mm Hg and was not different from that present in age-matched nontreated SHR.
the lumen diameter of arteries in SHR but not WKY and slightly decreased the arterial wall thickness in SHR and increased the wall thickness of the mesenteric arteries of WKY.

Studies using other forms of antihypertensive treatment are less clear about the role high blood pressure plays in wall thickening. In two studies performed by Mulvany and his colleagues, felodipine was used to reduce the blood pressure of SHR. In one study, the SHR were treated between 6 and 12 weeks of age, whereas in the second study, the treatment was extended from 6 to 14 weeks of age. In the initial study, drug treatment reduced the difference in wall thickness of mesenteric arteries between SHR and WKY. However, upon examining the data, it appears that antihypertensive treatment did not decrease the vascular wall thickness in SHR but increased the vascular wall thickness in WKY. In the second study, felodipine treatment had no significant effect on the mesenteric wall-to-lumen ratio in either SHR or WKY.

In general, the above studies involving the mesenteric arteries of SHR support the findings of the present study in that factors other than the elevation of blood pressure are important in promoting vascular wall thickening in SHR. However, some studies of the mesenteric vasculature have shown that the vascular wall is already thicker in SHR over WKY at 3–4 weeks of age, while studies by Gray have shown that at least in some animal colonies, SHR are born with an elevated blood pressure and a thicker carotid artery wall. Hence, it is possible that the previously mentioned antihypertensive treatment studies may reflect the inability of drug treatment to produce a regression of structural changes in the vascular wall of SHR instead of preventing such changes from occurring. In the present study there is reason to believe that blood pressure was in fact normalized in SHR in utero and from birth onward. Therefore, there is more ample reason to believe that vascular wall thickening in the renal vasculature of SHR over WKY is not dependent on the elevation of blood pressure.

In perfusion studies, an elevation in the amplitude of vascular resistance change from maximal relaxation to maximal contraction in SHR compared with WKY has been used as a functional indicator of the presence of a thickened vascular wall in SHR. Folkow et al have shown that immunosympathectomized SHR and WKY had a lower reactivity than that present in untreated SHR. Thus, it appeared that the lowering of blood pressure partly attenuated the exaggerated response in SHR thought to be produced by structural adaptations. Weiss and Lundgren, using similar techniques, studied the effects of the antihypertensive drugs metabolites.

**Figure 5.** Numbers of medial smooth muscle cell (SMC) layers present in renal arteries of control (SHR, WKY) and hydralazine-treated (SHR, and WKY). SHR and WKY (mean±SEM; ANOVA test, all arterial groups significant, p<0.01). MRA, main renal; D, internal diameter.
prolol, hydralazine, propranolol, and hydralazine + guanethidine on the hind limb vasculature. SHR were treated continuously from weaning to 10 months of age. Each of the drugs studied reduced the blood pressure of SHR, but the blood pressure in treated SHR was still elevated over untreated WKY. Although the hind limb vascular reactivity decreased in treated SHR, it was still significantly elevated over that present in WKY. Both Folkow et al\textsuperscript{13} and Weiss and Lundgren\textsuperscript{14} have suggested that SHR resistance vessels might, for genetic reasons, be more prone to adapt structurally to smaller pressure loads than those of WKY, that is, a very small elevation in blood pressure could cause an exaggerated structural alteration. Hamilton,\textsuperscript{15} on the other hand, found that lowering of blood pressure in SHR between 4 and 16 weeks of age using a combination of reserpine, hydrochlorothiazide and hydralazine eliminated the changes in reactivity to norepinephrine and serotonin in the mesenteric vasculature. However, in this study the treated SHR were compared with treated Sprague-Dawley normotensive control animals and not WKY.

Unlike the present study in which the vasculature wall was directly measured, the above perfusion studies provided indirect evidence for the presence of structural alterations. The validity of perfusion experiments depends on the ability of the vasculature to attain maximal contraction. It seems possible that prolonged drug treatment or immunosympathectomy could cause a functional alteration where maximal pressor responses are reduced or enhanced. If such a situation existed, alterations in vascular reactivity may not reflect the presence of structural alterations.

An area of concern in the present study was the possibility that increased sympathetic nerve activity during hydralazine treatment could exert a trophic influence on the renal vasculature. Studies have shown that sympathectomy of vascular regions produces a thinning of the vascular wall\textsuperscript{16,17} that is associated with a reduction in \textsuperscript{3}H-thymidine uptake into SMC nuclei.\textsuperscript{17} If such is the case, an argument could be made that a potential decrease in vascular wall thickening produced by the normalization of high blood pressure with hydralazine treatment might be counteracted by a tendency of the vascular wall to thicken due to the presence of an overactive sympathetic nervous system associated with baroreceptor reflexes apposing the fall in blood pressure. However, this hypothesis does not fit the observations made with regards to the renal vasculature of WKY. In this group, hydralazine-treated WKY had either an unaltered or thinner renal vascular wall than untreated WKY. Furthermore, studies involving conscious SHR\textsuperscript{18} have indicated that baroreceptor reflexes that appose the early fall in blood pressure and peripheral resistance during hydralazine treatment rapidly adapt to the prevailing lower blood pressure. This latter finding is consistent with the observations made by Tsoporis...
and Leenen\textsuperscript{19} who observed that in renal hypertensive rats treated with hydralazine sympathetic activity returns to normal within the first 2 days of chronic hydralazine treatment. Therefore, it appears that a potential thickening of the renal vascular wall by the trophic influence of an overactive nervous system is not a serious concern in the present study.

At the present time, the underlying mechanisms producing blood vessel wall thickening in SHR are unknown. However, it appears that a significant degree of wall thickening in SHR may be genetically programmed to occur in these animals. Such changes may serve an adaptive role enabling the arteries of SHR to better handle the greater circumferential tensions produced by an elevated blood pressure, or alternatively, be primarily involved in initiating hypertension in SHR. If the presence of a thickened vascular wall is necessary for the maintenance of high blood pressure, it is possible that during hydralazine treatment the effects of these structural alterations were masked or attenuated by the vasodilating effects of hydralazine. The rapid onset of hypertension after the withdrawal of hydralazine from in utero and postnatally treated SHR is consistent with this view since the reversal of vasodilation would result in the reestablishment of vascular hyperreactivity and hypertension in SHR.

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References


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