Effect of Denervation on the Afferent Arteriole in the SHR

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SUMMARY

To study the role the renal nerves may play in the hypertension of the SHR, we conducted a morphometric study of the afferent arteriole of spontaneously hypertensive rats (SHR) and Wistar Kyoto rats (WKY) which were subjected to renal denervation (or sham-operation). Methacrylate casts were made of the renal vasculature after perfusion fixation with glutaraldehyde. These vascular casts were then examined and measurements made with the scanning electron microscope (SEM). Afferent arterioles of the denervated SHR were dilated in comparison to the sham-operated SHR, but there was no difference between the afferent arteriolar diameters of the 2 groups of WKYs. However, the afferent arteriolar diameters of the SHR (either group) were smaller than those of the WKY. Renal denervation caused a reduction in systolic blood pressure compared to sham-operated in both strains of rat. We concluded that the dilation changes of afferent arterioles of denervated SHRs may be related to renal autoregulation resulting from the decreased blood pressure. However, the effect of the loss of sympathetic innervation of the arterioles cannot be ruled out.

Additional Indexing Words:
SHR Renal denervation Afferent arteriole

IN spite of numerous suggestions that the autonomic nervous system and kidneys are important in the initiation and/or maintenance of hypertension in the spontaneously hypertensive rat (SHR), the role of the renal nerves has not been completely elucidated. The SHR has an increased sympathetic nerve activity,1,2) increased renal vascular resistance3) and smaller renal

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afferent arterioles at 6 and 12 weeks of age compared to Wistar Kyoto rats (WKY). It is conceivable that the decreased diameter of the resistance vessels may be related to the increased post-ganglionic sympathetic nerve activity. However, the precise role of the renal sympathetic nerves in producing these vascular alterations is unknown. The hypertension in this rat model can be prevented by neonatal sympathectomy, delayed and/or altered by bilateral renal denervation, and chronically attenuated by repeated renal denervations at 3 week intervals. Therefore the present study was designed to determine the effect of renal denervation on the renal microvasculature.

**METHODS**

Twelve-week-old male rats (SHR and WKY) from Harlan Sprague-Dawley Laboratories (Indianapolis, IN) were used in this study. Twelve-week-old rats were chosen because the SHR has significant hypertension and smaller afferent arteriolar diameters than the WKY at this age. SHR and WKY rats were divided into sham and denervated groups with 8 animals per group. Systolic blood pressures were measured in all animals by tail cuff (photoelectric sensor; IITC; Landing NJ) one day prior to anesthesia with sodium pentobarbital (0.01 mg/Kg) and surgery. The sham operation consisted of opening the left flank, exposing the renal artery by blunt dissection, and closing the flank in two layers. The same procedure was repeated on the opposite side. The denervation procedure consisted of opening the flank, isolating the renal artery, and stripping the nerves and perivascular sheath along the renal artery. A thin strip of Parafilm (American Can Company, Greenwich, CT) was placed beneath the artery to protect surrounding tissues and several drops of a solution of 10% phenol in 95% alcohol were placed directly on the artery. The excess phenol was blotted with cotton application sticks, the parafilm was removed, the flank was closed, and the procedure was repeated on the opposite side.

To determine the completeness of the renal denervation, 8 additional rats were denervated and sacrificed 7–10 days later. The kidneys were removed, frozen in isopentane cooled in liquid nitrogen and sectioned at 16 μm with a cryostat microtome at −30°C. Tissue sections were processed for localization of catecholamine containing cell processes and varicosities by the SPG method and examined with a Leitz Orthoplan microscope equipped with Ploem epi-illumination for histofluorescent visualization of catecholamines.

At 12–14 days after operation, all rats were sacrificed for casting of the renal vasculature. The perfusion fixation and casting methods as well as their validating support were as described in detail previously. The sacrifice
procedure was as follows: after pentobarbital anesthesia, the abdominal aorta was exposed through a midline incision and cannulated. A ligature was loosely placed around the aorta superior to the renal arteries. As 150 mls of fixative, 2.5% glutaraldehyde in 0.1 N cacodylate buffer (pH 7.4, 37°C, 380 mOs m) was perfused through the cannula, the renal vein was cut and the superior aortic ligature was tightened allowing an in vivo perfusion fixation of the kidneys at the systolic pressure of that animal. Following perfusion, Batson’s #17 methacrylate (Polysciences, Inc) was infused into the kidney and allowed to set overnight. Thereafter, the renal parenchyma was digested in 30% KOH at 85°C. The vascular cast was dissected and processed for examination with an AMR 1000A scanning electron microscope.

The diameters of the afferent arterioles were measured directly on the visual CRT of the SEM at a constant magnification (450×) from the coded specimens as previously described. A keyboard data and measuring system on the SEM served as a reference for, and assisted in, the collection of the morphometric data. Measurements of both proximal and distal afferent arteriolar regions were made. There is variability in the lengths of the afferent arterioles at all levels of the cortex, but the afferent arterioles could be broadly grouped as long (>100 μm) or short (<100 μm). For the long vessels, the proximal afferent diameter (PAD) was measured 90 to 150 μm from the glomerulus while, for the short afferents, the PAD was measured 15 μm from the interlobular artery. The distal afferent arteriolar diameter (DAD) was measured at the narrowed point of the afferent arteriole within 20 μm of the glomerulus. The location of each glomerulus was noted and recorded as either juxtamedullary inner mid- or outer cortex. The data from juxtamedullary and inner cortical glomeruli were combined as the inner group while measurements from mid- and outer cortical groups were combined as the outer group. The cortical regions were separated since the distribution of post-glomerular blood flow differs between these regions, i.e. vasa rectae for inner cortical glomeruli and peritubular capillaries for outer cortical glomeruli. For each animal, a minimum of 25 afferent arterioles were measured. The means of the measurements, i.e., blood pressure, PAD, and DAD, per glomerular group from each animal, were analyzed by three-way analysis of variance.

**Results**

The effectiveness of the renal denervations is shown in Figs. 1 and 2. Fine, varicose, linear fluorescent profiles are clearly seen in the (sham) kidneys (Fig. 1) while there is an obvious lack of such profiles in the denervated
Fig. 1. Fluorescence photomicrograph of the fluorescent nerve plexus (arrow) surrounding a small artery (A) within the kidney.

Fig. 2. Fluorescence photomicrograph of a small artery (A) within the kidney of a rat 10 days after renal denervation. Note the lack of a fluorescent nerve plexus as seen in Fig. 1.

kidneys (Fig. 2). Periarterial histofluorescence is evident in large arteries, medium sized arteries, and in arterioles of the control kidney sections while comparable vessels from denervated kidneys display no fluorescence. An occasional section from the denervated kidneys show diffuse histofluorescent fibers in scattered areas. Even in these areas, the intensity of the fluorescence is greatly reduced.

The diameter of the afferent arterioles of the sham-operated SHR (Fig. 3) were smaller than those of the denervated SHR (Fig. 4). Quantitative measurements of the proximal afferent diameter (PAD) and distal afferent diameter (DAD) are shown in Table I. Analysis of variance showed the afferent arteriolar diameter of the SHR to be smaller than that of the WKY (p<0.001), regardless of the experimental condition. Furthermore, the afferent diameter data showed a two-way interaction (p<0.05) between rat strain (SHR vs WKY) and operation (sham vs denervation) indicating that
the rat strains reacted differently to renal denervation. Post hoc analysis using Student’s t-test suggests that the major effect of the denervation is an increase in diameter of the afferent arteriole in the SHR. The afferent arterioles from denervated WKY showed no change in diameters when compared with sham WKY. The systolic blood pressures (Table II) decreased ($p<0.05$) in the SHR after renal denervation. The post-operative blood pressure in the denervated WKY is also decreased ($p<0.05$) compared to the sham-operated WKY (Table II).

**Discussion**

This study describes a difference in the way the renal afferent arterioles of SHR and WKY react to renal denervation. The diameter of renal afferent arterioles of denervated SHRs was larger than that of afferent arterioles of sham-operated SHR, while no difference in arteriolar diameters was detected between the WKY groups. In addition, afferent arteriolar diameters in both groups of SHR were smaller than those of WKY.
Renal denervation ameliorates hypertension in several genetic and experimental models of the disease. Renal denervation in prehypertensive SHR, delays and in young hypertensive SHR alleviates the hypertension. These effects have been attributed to an interruption of renal efferent (sympathetic) fibers. The efferent fibers are described as playing a similar role in DOCA-salt hypertensive rats. The renal afferent nerves have also been described as playing a role in the pathogenesis of hypertension in Goldblatt and coarctation models of hypertension. The afferent nerves may affect the blood pressure by inducing secondary changes in efferent sympathetic activity. Such an afferent-efferent nerve interaction is inferred from the recent work of Kruegger et al in which they describe bilateral renal denervation of the SHR causing, not only a dilation of renal vasculature, but a shift in cardiac output from muscle toward splanchnic organs as well.

The larger diameter of the afferent arterioles in the denervated SHR of the present study suggests that a renal autoregulatory response to decreased blood pressure occurred. Reductions of renal perfusion pressure in the SHR

Fig. 4. Scanning electron micrograph of renal vascular cast from a denervated SHR showing the afferent arteriole (arrow), glomerulus and efferent arteriole (arrowhead), 490×.
**Table I. Afferent Arteriolar Diameters of Denervated and Sham Denervated SHR and WKY (mean in µm±SEM)**

* p<0.001 for difference between SHR and WKY for both PAD and DAD.
** p<0.05 for difference between sham and denervated.
PAD (proximal afferent diameter) and DAD (distal afferent diameter) are defined in the Methods section.

**Table II. Effect of Renal Denervation on Systolic Blood Pressure of SHR and WKY (mean systolic blood pressure in mmHg±SEM)**

* Post-Op B.P. was taken 11–14 days after the operation. Blood pressures were significantly different for each rat strain between sham and denervated groups (p<0.05).
** Blood pressure significantly different between pre-op and post-op with paired difference t test (p<0.05).

... elicit an autoregulatory response during which glomerular capillary pressure is maintained. Our observed changes in afferent arteriolar diameter are consistent with such a response, but since we did not measure renal blood flow in the present experiment, the presence of autoregulation of blood flow cannot be assumed.

In the present study, blood pressures of sham-operated WKY rose significantly (Table II), possibly as a result of operative stress, while the blood pressures of denervated WKY remained unchanged. The reasons for these responses in the WKY are unclear, but the data from one study suggests a similar response in sham-operated WKY, 1 and 2 weeks after operation. However, several studies have described no change in blood pressure...
or renal function in response to renal denervation in WKY. Nevertheless, in
spite of differences in the blood pressures between sham and denervated WKY,
no difference in the afferent arteriolar diameters were observed.

The lack of afferent arteriolar diameter differences in the WKY groups
suggests that there was not an appropriate autoregulatory response in the
sham-operated WKY or, alternatively, that a diameter change necessary for
autoregulation was not large enough for us to detect. An autoregulatory
curve for the WKY at renal perfusion pressures similar to those that were ob-
served in our study has been described previously. However, carotid oc-
closure was used in that study to elevate renal perfusion pressure above the
normal range. Such a maneuver increases sympathetic activity. Thus, the
reported autoregulatory curves may not accurately reflect the autoregulatory
capabilities of the WKY. While all animals were treated similarly, never-
theless, it is possible that the extraneous stimuli (blood pressure measurement,
anesthesia, surgery, fixative, etc) may have affected the vasculature of one
strain (SHR or WKY) or operative group (denervated or sham-operated)
more. These differences, if present, could be a function of the disease or
state of innervation. There is no way at present to determine an alteration in
microvascular dimension which took place as the result of extraneous stimuli
rather than changes caused by the denervation.

In conclusion, the denervation-induced blood pressure changes noted
in SHR are associated with an apparant dilation of renal afferent arterioles.
These renal vascular changes are consisnt with an appropriate autoregula-
tory response to the decreased blood pressure, but the extent to which de-
creased sympathetic tone may also have affected vessel diameter remains to
be determined.

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