Impaired Myogenic Responsiveness of Renal Microvessels in Dahl Salt-Sensitive Rats

Tsuneo Takenaka, Hayley Forster, Anna De Micheli, and Murray Epstein

The mechanisms mediating abnormal renal autoregulation in Dahl salt-sensitive (DS) rats have not been fully defined. In the present study, we assessed myogenic responsiveness of interlobular arteries (ILAs), afferent arterioles (AAs), and efferent arterioles in isolated perfused hydronephrotic Dahl rat kidneys. Dahl rats were divided into four groups according to strain (Dahl salt-resistant [DR] or DS rats) and dietary sodium manipulation (rats fed low or high salt diets). Systolic blood pressure was elevated only in DS rats fed the high salt diet (202±4 mm Hg, p<0.05). Myogenic responses were obtained by stepwise elevation of renal arterial pressure. Vessel diameters were determined by computer-assisted videomicroscopy. Preglomerular microvessels of DS and DR rats responded differently to changes in renal arterial pressure. AAs and ILAs manifested diminished myogenic responsiveness to increasing renal arterial pressure in DS rats compared with DR rats (p<0.05). Both AAs and ILAs in DS rats manifested a higher threshold pressure for eliciting myogenic responses and a decrease in maximal pressure-induced vasocostriction. The sensitivity of the AA myogenic response to nifedipine was enhanced in DS rats compared with DR rats (p<0.05). For rats fed the high salt diet, preglomerular vessels exhibited reduced myogenic responsiveness in both strains. In contrast to preglomerular microvessels, efferent arterioles from all four groups of rats failed to exhibit pressure-induced vasoconstriction. Our data suggest that diminished myogenic responsiveness of AAs and ILAs in DS rats contributes to impaired renal autoregulation in this strain. (Circulation Research 1992;71:471–480)

KEY WORDS • autoregulation • interlobular artery • afferent arterioles • sodium • membrane depolarization

The hemodynamic alterations that lead to the development of essential hypertension have not been fully determined. However, several lines of evidence strongly suggest that intrinsic renal abnormalities contribute to the sustained elevation of blood pressure seen in essential hypertension. Guyton et al1 have demonstrated that in the hypertensive state the renal capacity to excrete sodium and water is reduced, contributing to the development and maintenance of high blood pressure. Furthermore, transplantation of kidneys from normotensive donors to hypertensive patients normalizes the blood pressure of the recipients,2 indicating that renal abnormalities contribute substantially to the development of hypertension.

Recent studies1–5 have demonstrated that in Dahl salt-sensitive (DS) rats, a model of essential hypertension, autoregulation of glomerular filtration rate is reset toward a higher pressure and the pressure natriuretic response is impaired as compared with Dahl salt-resistant (DR) rats. Thus, at a given renal arterial pressure (RAP), decreased filtered load and/or increased tubular reabsorption of sodium and water seem to contribute to the development of hypertension in DS rats. We have also demonstrated that in DS rats an impairment of the renal prostaglandin system contributes to blunted sodium excretion but not to abnormal glomerular hemodynamics.5

To investigate further the mechanisms mediating abnormal glomerular circulatory behavior in DS rats, we carried out studies of renal microvascular responsiveness to increasing pressure in Dahl rats by using isolated perfused hydronephrotic kidneys. As detailed previously,6 this experimental model facilitates the examination of renal microvascular behavior in a controlled in vitro setting. Our current findings indicate that DS rats manifest blunted renal microvascular responsiveness to pressure when compared with DR rats, thereby contributing to the abnormal autoregulation of glomerular filtration rate observed in this strain.

Materials and Methods

General Procedure

Male and female Dahl rats were maintained on a low salt diet (0.2% NaCl, Teklad Premier Laboratory Diets, Madison, Wis.) and inbred in our animal facility. Experiments were carried out on 19 DR rats and 20 DS rats. After weaning, 4–5-week-old rats were used for induction of chronic hydronephrosis to permit direct visualization of the renal microcirculation. The right ureter was exposed by a small abdominal incision and ligated under methoxyflurane anesthesia (Metofane, Pittman-Moore, Mundelein, Ill.). Two or 3 weeks after surgery, seven DR and seven DS rats were placed on a

From the Nephrology Section, VA Medical Center, and the Department of Medicine, University of Miami, School of Medicine, Miami, Fla.

Supported by designated research funds from the Department of Veterans Affairs (Merit Review 2456–01).

Address for correspondence: Murray Epstein, MD, Nephrology Section, VA Medical Center, 1202 NW 16th Street, Miami, FL 33125.

Received December 31, 1991; accepted March 31, 1992.
high salt diet (8% NaCl, Teklad), whereas the remaining rats were maintained on the low salt diet. The rat groups are designated as follows: DR-L, DR rats with low salt diet; DR-H, DR rats with high salt diet; DS-L, DS rats with low salt diet; DS-H, DS rats with high salt diet. Acute experiments were performed 3 weeks after the initiation of dietary sodium manipulation. At this point (i.e., 5–6 weeks of hydropnephrosis), renal tubular atrophy had progressed to a stage that allowed direct microscopic visualization of the renal microvessels.

For perfusion of the kidneys, animals were anesthetized with methoxyflurane. The renal artery of the hydropnephrotic kidney was cannulated by introducing a perfusion cannula through the aorta and into the mesenteric artery. Perfusion with warm, oxygenated medium (pH 7.4) was initiated during this cannulation procedure to avoid ischemia to the perfused kidney. The hydropnephrotic kidney was then excised and placed on the stage of an inverted microscope (model K, Nikon, Tokyo) modified to accommodate a heated chamber equipped with a thin glass viewing port on the bottom.

The perfusion medium consisted of Krebs-Ringer bicarbonate buffer containing 6.5 g/100 ml bovine serum albumin (Bovuminar, Intergen Co., Purchase, N.Y.), 5 mM D-glucose, and a complement of amino acids, as detailed previously. Perfusion was provided from a pressurized chamber. The driving pressure was maintained by the infusion of warm, hydrated gas of 95% \( \text{O}_2 \)-5% \( \text{CO}_2 \), which exited through an adjustable back-pressure regulator (model 10BP, Fairchild Industrial Products Co., Winston-Salem, N.C.). The perfusion pressure, monitored at the level of the renal artery, was altered by controlling the back-pressure regulator. Perfusion flow was monitored by an extracorporeal electromagnetic flow probe (model 300A, Carolina Medical Electronics, King, N.C.), which was placed in the perfusion line immediately proximal to the kidney, and recorded on a polygraph for later analysis (model 79, Grass Instrument Co., Quincy, Mass.). Renal vascular resistance (RVR) was calculated by dividing RAP by the perfusate flow (renal venous pressure was assumed to zero).

Kidneys were allowed to equilibrate for at least 30 minutes before basal measurements were obtained. Microvessels were selected on the basis of adequate perfusate flow. Flow was assessed by observing the rate of collapse and recovery of the vessels in response to a temporary (≈2 seconds) clamping of the perfusion line. Vessels that exhibited a sluggish or blunted response to this manipulation were considered to be perfused inadequately and were excluded from study.

Video images of renal microvessels were obtained with a video camera (model ITC-47, Ikegami, Tokyo) and recorded by a videocassette recorder (model NV-8950, Panasonic). To determine the vessel diameter, the video tape recording was transmitted to an IBM-AT computer equipped with a video acquisition and display board (model IVG-128, Datacube, Inc., Peabody, Mass.). Vessel diameters were estimated with an automated program custom designed to determine the mean distance between parallel edges of the selected vessels. A segment (=10 \( \mu \text{m} \) in length) of the interlobular artery or the afferent (AA) or efferent (EA) arteriole was scanned at 2–5-second intervals. Renal microvessel diameter was determined by averaging measurements obtained during the plateau of the response.

**Measurement of Systolic Blood Pressure**

On the day the kidneys were harvested, the systolic blood pressure (SBP) of the rat was determined by tail-cuff sphygmomanometry (model KN-210-1, Nantume, Tokyo). Before anesthesia SBP was calculated from the average of at least five measurements.

**Renal Microvascular Responses to Pressure**

Kidneys from DR (\( n=14 \)) and DS (\( n=14 \)) rats were used for investigating the effects of perfusion pressure on renal microvascular tone. Each strain of rats was divided into two groups according to dietary sodium intake (\( n=7 \) for each group). Initially, renal perfusion pressure was maintained at 80 mm Hg. The pressure was then raised in a stepwise manner by 20-mm Hg increments to 180 mm Hg, as described previously. The diameters of interlobular arteries and afferent and efferent arterioles were determined for 60 seconds at each level of renal perfusion pressure.

**The Effects of Nifedipine on Myogenic Responsiveness**

The effects of a calcium antagonist, nifedipine (provided by Pfizer Laboratories, New York), on myogenic AA constriction were examined in kidneys from DR-H, DR-L and DS-L rats (\( n=7 \) for each group). After basal myogenic AA responses were determined, increasing doses of nifedipine (from 1 nM to 1 \( \mu \text{M} \)) were added directly to the perfusate. At each concentration of nifedipine, AA responses to pressure were determined by observing the same regions of the vessel (i.e., as previously observed in the absence of nifedipine). Stock solutions of nifedipine were prepared freshly on the day of study in polyethylene glycol. A yellow filter (525-nm cutoff) and sodium lighting were used to avoid photodegradation of the dihydropyridine.

**The Effects of KCl Depolarization and Nifedipine on Afferent Arterioles in Dahl Rats**

AA responsiveness to KCl depolarization and nifedipine was assessed in kidneys from DR-L rats (\( n=5 \)) and from DS-L and DS-H rats (\( n=5 \) for each group). After basal diameter was obtained, the concentration of potassium in the perfusate was isosmotically increased to 30 meq/l by adding media in which KCl was substituted for NaCl; Increasing doses of nifedipine (from 1 nM to 1 \( \mu \text{M} \)) were added directly to the perfusate. At each concentration of nifedipine, AA diameters were determined. The yellow filter and sodium lamp were also used for this study.

**Analysis of Data**

All data are expressed as mean±SEM. For comparison of the means between paired data within each group, data were analyzed by paired t test. For comparison of individual means between groups, data were initially subjected to analysis of variance (ANOVA) followed by the Newman-Keuls test at each level of pressure. When variances were heterogeneous, data were converted to natural logarithms and tested by Bartlett’s test to ensure homogeneity and then subjected to ANOVA. Values of \( p<0.05 \) were considered statistically significant.
Results

Measurement of Systolic Blood Pressure

SBP of DS-H rats (202±4 mm Hg, n=7) exceeded that of the other three groups of Dahl rats (p<0.05 versus each group). SBPs of the remaining three groups of Dahl rats did not differ from each other. Thus, SBP of DR-L rats (126±2 mm Hg, n=7) was not different from either that of DR-H rats (129±2 mm Hg, n=7, p=NS) or DS-L rats (130±3 mm Hg, n=7, NS).

Renal Microvascular Responses to Pressure

Interlobular arteries. Figure 1 summarizes the myogenic responses of interlobular arteries (ILAs) from Dahl rats. In DR-L rats (top left panel), the ILA is progressively constricted in response to increasing RAP. Thus, elevation of RAP from 80 to 100 mm Hg decreased the ILA diameter (from 30.6±1.9 to 29.3±1.7 µm, p<0.01, n=8). Further increases in RAP elicited further decrements in the diameter of the ILA (to 27.9±1.3, 26.6±1.4, 25.0±1.4, and 23.9±1.5 µm at 120, 140, 160, and 180 mm Hg, respectively; p<0.05 versus 80 mm Hg). The decrease in RAP to 80 mm Hg resulted in a prompt offset with a return of ILA diameter to baseline (to 30.4±2.0 µm, p>0.5 versus 80 mm Hg). In DR-H rats (bottom left panel), myogenic responses of the ILA were preserved. ILAs exhibited significant vasoconstriction at RAPs of 100 mm Hg and above (p<0.05 versus 80 mm Hg [30.5±2.1 µm], n=8).

In DS-L rats (Figure 1, top right panel), the ILA failed to constrict in response to elevation of RAP from...
80 to 100 or 120 mm Hg (p<0.10 versus 29.4±1.2 μm at 80 mm Hg, n=8). The threshold was shifted to 140 mm Hg, at which the ILA exhibited myogenic vasoconstriction (to 28.3±1.1, 27.6±0.9, and 26.7±0.9 μm at 140, 160, and 180 mm Hg, respectively; p<0.05 versus 80 mm Hg). In DS-H rats (bottom right panel), the ILA failed to constrict in response to all increments of RAP (from 100 to 180 mm Hg). Indeed, the ILA dilated when RAP was increased. Significant vasodilation was observed at 120 mm Hg and above (p<0.05 versus 26.4±1.0 at 80 mm Hg, n=8).

Figure 2 compares myogenic responsiveness of the ILA among the four groups of rats. All data are presented as percent changes in vessel diameter. In contrast to DR-L rats, DS-L rats manifested blunted myogenic responsiveness at RAPs of 100 mm Hg and above (p<0.05). At 180 mm Hg, DR-H rats, compared with DR-L rats, also exhibited diminished myogenic vasoconstriction (p<0.05). DS-H rats, compared with DS-L rats, showed different pressure-induced ILA responsiveness at 160 and 180 mm Hg (p<0.05). Furthermore, ILAs of DS-H and DR-H rats responded differently to pressure stimuli at 100 mm Hg and above (p<0.05).

Afferent arterioles. As shown in Figure 3, myogenic responses of AAs from DR-L rats (top left panel) were well preserved. In analogy with the changes observed in the ILA, elevations of RAP from 80 to 100 mm Hg induced significant AA vasoconstriction (from 21.6±0.7 to 20.5±0.7 μm, p<0.001, n=13). Further increases in RAP produced greater decrements in AA diameters (to 19.3±0.8, 18.5±0.7, 17.5±0.7, and 16.8±0.7 μm at 120, 140, 160, and 180 mm Hg, respectively; p<0.001 versus 80 mm Hg). Reduction of RAP back to 80 mm Hg caused AA diameters to return close to control levels (20.9±0.9 μm, p>0.20 versus 80 mm Hg). In DR-H rats (bottom left panel), AAs exhibited a substantial reduction in diameter in response to increasing pressure. Thus, AAs manifested significant constriction even when RAP was increased to 100 mm Hg (from 20.6±0.8

**Figure 3.** Graphs showing myogenic response of afferent arteriole (AA) in Dahl rats. DR-L, DR-H, DS-L, and DS-H indicate Dahl salt-resistant rats with low salt diet, Dahl salt-resistant rats with high salt diet, Dahl salt-sensitive rats with low salt diet, and Dahl salt-sensitive rats with high salt diet, respectively. *Significant difference from control values (i.e., at 80 mm Hg).
to $19.8 \pm 0.8 \mu m, p < 0.005, n = 13$). AA diameter progressively decreased while RAP was further increased ($p < 0.001$ versus $80$ mm Hg).

In contrast to DR kidneys, AAs of DS-L rats (Figure 3, top right panel) failed to constrict in response to a pressure stimulus of $100$ mm Hg ($p > 0.50, n = 13$). At pressures of $120$ mm Hg and above, however, AAs constricted progressively as pressure was increased (to $20.4 \pm 0.7, 19.9 \pm 0.6, 19.0 \pm 0.6$, and $18.3 \pm 0.6 \mu m$ at $120, 140, 160$, and $180$ mm Hg, respectively; $p < 0.005$ versus $21.3 \pm 0.7 \mu m$ at $80$ mm Hg). In striking contrast to the above groups, within the examined pressure range, pressure-induced AA vasoconstriction was abolished in DS-H rats. Indeed, AAs dilated in response to increasing RAP to $100$ mm Hg ($p < 0.025, n = 13$). No measurable changes in AA diameter were observed at the subsequent RAPs of $120$–$180$ mm Hg ($p > 0.10$ versus $19.4 \pm 0.9 \mu m$ at $80$ mm Hg).

Figure 4 summarizes AA myogenic responsiveness from the four groups of rats with the data plotted as percent changes of vessel diameter. As compared with DR-L rats, DR-H rats manifested blunted AA myogenic responsiveness in the range of RAPs from $120$ to $180$ mm Hg ($p < 0.05$). DS-L kidneys, compared with DR-L kidneys, also exhibited diminished AA myogenic responsiveness at $100$ mm Hg and above ($p < 0.05$). Pressure-induced changes in AA diameter of DS-H rats differed from those of DS-L rats at RAPs between $120$ and $180$ mm Hg ($p < 0.05$) and differed from those of DR-H rats at pressures from $100$ to $180$ mm Hg ($p < 0.05$).

**Efferent arterioles.** Figure 5 summarizes the effects of increasing pressure on EAs. In DR-L rats, basal EA diameter was $20.0 \pm 1.0 \mu m$ (at $80$ mm Hg, $n = 8$). Increasing RAP from $80$ to $180$ mm Hg failed to elicit changes in EA diameter ($p > 0.20$ versus $80$ mm Hg). In DR-H rats, EAs tended to dilate in response to increasing RAP. EAs vasodilated significantly at $140$ mm Hg and above (to $19.6 \pm 1.3, 19.6 \pm 1.1$, and $20.0 \pm 1.3 \mu m$ at $140, 160$, and $180$ mm Hg, respectively; $p < 0.05$ versus $19.2 \pm 1.2 \mu m$ at $80$ mm Hg; $n = 8$).

Similarly, AAs of DS-L rats vasodilated at $180$ mm Hg (to $20.4 \pm 1.1 \mu m, p < 0.05$ versus $19.2 \pm 1.3 \mu m$ at $80$ mm Hg, $n = 8$). EAs of DS-H rats also tended to manifest pressure-induced vasodilation with EA diameters exceeding the diameter at $80$ mm Hg at RAPs from $140$ to $180$ mm Hg ($p < 0.05$ versus $20.5 \pm 1.1 \mu m$ at $80$ mm Hg, $n = 8$).

In summary, increasing pressure failed to induce vasoconstriction in EAs of all four study groups.

**Renal vascular resistance.** Figure 6 summarizes pressure-induced changes in RVR. Under basal conditions...
myogenic vasoconstriction with low salt against nifedipine

**FIGURE 8.** Graph comparing the effects of nifedipine on afferent arteriolar myogenic responses in Dahl rats. Data (same data as figure 7) are expressed as percent inhibition of myogenic vasoconstriction (at 180 mm Hg) and plotted against nifedipine concentrations. ○, Dahl salt-resistant rats with low salt diet; ■, Dahl salt-resistant rats with high salt diet; △, Dahl salt-sensitive rats with low salt diet.

**FIGURE 9.** Graphs showing the effects of nifedipine on KCl-induced afferent arteriolar vasoconstriction in Dahl rats. ○, Dahl salt-resistant rats with low salt diet; ■, Dahl salt-sensitive rats with low salt diet; △, Dahl salt-sensitive rats with high salt diet. *Significant difference from the control values.

(i.e., at 80 mm Hg), RVR of DR-L, DR-H, DS-L, and DS-H rats was 5.0±0.5, 6.0±1.8, 6.4±0.8, and 12.0±2.7 mm Hg/(ml/min), respectively (n=7 for each group). Basal RVR of DS-H rats was slightly higher than that of the other three groups (p<0.05). The increase in RAP from 80 to 180 mm Hg elevated RVR in DR-L rats (by 11.8±5.0%, p<0.05 versus 80 mm Hg). In DR-H and DS-L rats, elevation of RAP failed to increase total RVR (by 3.6±3.5% for DR-H rats, p>0.20 versus 80 mm Hg; by 0.9±2.8% for DS-L rats, p>0.50 versus 80 mm Hg). In contrast, DS-H rats reduced their total RVR when RAP was increased (~25.3±4.7%, p<0.005 versus 80 mm Hg). This reduction in RVR to pressure in DS-H rats differed significantly from the responses of the other three groups (p<0.05).

**FIGURE 7.** Graphs showing the effects of nifedipine on afferent arteriolar (AA) myogenic responsiveness in Dahl rats. DR-L, DR-H, and DS-L indicate Dahl salt-resistant rats with low salt diet, Dahl salt-resistant rats with high salt diet, and Dahl salt-sensitive rats with low salt diet, respectively. ○, Without nifedipine; ■, with 1 nM nifedipine; △, with 10 nM nifedipine; ▲, with 100 nM nifedipine; □, with 1 μM nifedipine.

Effects of nifedipine on myogenic responsiveness. The effects of nifedipine on myogenic responses are summarized in Figure 7. In DR-L rats (left panel), the addition of increasing doses of nifedipine did not alter the basal AA diameter (NS versus without nifedipine [22.1±0.7

---

*Note: The original text contains graphs and figures that are not easily transcribed into a text format. The description provided here is a conceptual representation of the content. For a detailed and accurate transcription, visual inspection of the original content is required.*
μM), n=11). Nifedipine, however, inhibited myogenic responses in a dose-dependent manner. Thus, at 1 μM nifedipine, myogenic response of AA was completely abolished (p<0.20 versus 80 mm Hg).

In the absence of nifedipine, myogenic responsiveness of DR-H rats (Figure 7, middle panel) was blunted as compared with that of DR-L rats. The administration of increasing doses of nifedipine did not change basal AA diameter (from 20.6±0.8 μm [without nifedipine] to 20.6±0.8, 20.7±0.7, 20.5±0.7, and 20.9±0.7 μm at 10⁻⁹, 10⁻⁸, 10⁻⁷, and 10⁻⁶ M, respectively; NS; n=13) but substantially attenuated the myogenic responses. Even though myogenic responsiveness to increasing RAP was blunted in these rats, 1 μM nifedipine was necessary to block myogenic response completely (p<0.05 for all pressure versus 80 mm Hg).

DS-L rats (Figure 7, right panel) manifested blunted myogenic responses as compared with DR-L rats. Nifedipine failed to alter basal AA diameter at all concentrations examined (from 21.3±0.7 μm [control] to 21.4±0.7, 21.5±0.6, and 21.7±0.5 μm at 10⁻⁹, 10⁻⁸, 10⁻⁷, and 10⁻⁶ M, respectively; NS; n=13). At concentrations as low as 100 nM, nifedipine abolished myogenic responses in this strain (p<0.05 for all pressure versus 80 mm Hg).

To facilitate comparisons among groups, the data from Figure 7 were expressed as percent inhibition of vasoconstriction at 180 mm Hg and plotted against nifedipine concentrations (Figure 8). Nifedipine was equally efficacious in inhibiting AA myogenic responses of DR-L and DR-H rats. Half-maximal inhibitory concentration (IC₅₀) for nifedipine of DR-H rats (207±24 nM, n=13) was nearly identical to that of DR-L rats (258±21 nM, n=11). In DS-L rats, the sensitivity of myogenic response to nifedipine was enhanced. The IC₅₀ of DS-L rats (26±1 nM, n=11, p<0.05) was substantially less than that of DR-L or DR-H rats.

Effects of KCl depolarization and nifedipine on afferent arterioles in Dahl rats. Figure 9 summarizes the effects of KCl depolarization and nifedipine on AA responsiveness. In DR-L rats (top panel), KCl-induced (29.6±0.8 meq/l) membrane depolarization stimuli decreased AA diameter by 35±2% (from 20.2±0.4 to 13.1±0.5 μm, n=12, p<0.001). The subsequent addition of increasing doses of nifedipine antagonized this AA constriction in a dose-dependent manner. At 1 μM, nifedipine completely reversed the decrements in AA diameter elicited by KCl (to 20.1±0.5 μm, p>0.50 versus basal values).

In DS-L rats (Figure 9, middle panel), KCl-induced depolarization (30.3±0.5 meq/l) constricted AAs by 22±2% (from 21.8±1.1 to 17.2±1.1 μm, n=12, p<0.001). The subsequent addition of nifedipine reversed this vasoconstriction in a dose-dependent manner. At 1 μM nifedipine, AA diameter returned to the control levels (to 22.0±1.1 μm, p>0.50 versus basal values).

In DS-H rats (Figure 9, bottom panel), KCl-induced depolarization (29.4±0.4 meq/l) elicited AA vasoconstriction of 18±3% (from 18.7±0.9 to 15.4±1.1 μm, n=12, p<0.001). At concentrations of 100 nM and 1 μM, nifedipine returned AA diameters back to baseline values (to 18.5±1.0 and 18.9±0.9 μm, respectively; p>0.20 versus the control value).

Discussion

Kidneys exhibit a remarkable capacity to maintain blood flow and glomerular filtration rate relatively constant in the face of marked variations in blood pressure. Sustained hypertension substantially modifies the renal response to alterations in renal perfusion pressure, shifting the threshold pressure of both autoregulation of renal blood flow and pressure-induced natriuresis. This resetting of the renal response to pressure represents a renal adaptation to hypertension. Previous studies have demonstrated that autoregulation of renal blood flow is impaired in hypertensive animals, including spontaneously hypertensive rats (SHR), Goldblatt hypertensive rats, and deoxycorticosterone acetate–salt hypertensive dogs.12,14,15 We and other investigators have demonstrated that the autoregulation of glomerular filtration rate is impaired in DS rats compared with DR rats. The threshold of renal blood flow autoregulation was also reported to be shifted to higher pressures in DS rats. These findings prompted our current examination of myogenic responsiveness to changes in perfusion pressure in the renal microvasculature of DS rats to determine whether altered renal microvascular responsiveness contributes to the derangements of renal autoregulation found in this model.

Pressure-induced vasoconstriction of renal microvessels constitutes an important determinant of renal autoregulation. It is extremely difficult to assess directly the renal microvascular response to pressure in situ. Furthermore, when arterial pressure is altered in vivo, concomitant changes in neural and humoral determinants of renal microvascular tone tend to counter the induced changes. The isolated perfused hydrenephrotic kidney is uniquely suited to the study of renal microvascular responsiveness in face of alterations in perfusion pressure. In this model, renal microvessels can be visualized in an intact in situ setting, yet under in vitro conditions in which RAP is under direct experimental control. Extrarenal neural and humoral influences on renal vascular tone are thereby eliminated. Furthermore, since hydrenephrosis induces tubular atrophy, tubuloglomerular feedback is absent.

Altered Pressure Responsiveness of Interlobular Arteries

In the present study using the isolated perfused hydrenephrotic kidney, we provide the first evidence that the renal microvascular responsiveness to pressure (in both ILA and AA) is diminished in DS rats. DS rats manifested decreased myogenic responsiveness of the ILA as compared with DR rats. Whereas DR-L rats manifested a 21% reduction in ILA diameter at 180 mm Hg, ILAs from DS-L rats exhibited only a 12% decrease. Similarly, at pressures from 100 to 160 mm Hg, myogenic responsiveness of ILAs of DS-L rats was also diminished when compared with that of DR-L rats. The threshold of myogenic responsiveness also differed in the two strains. In DR-L rats, ILAs constricted at pressures of 100 mm Hg, whereas in DS-L rats, ILAs did not constrict until a pressure of 140 mm Hg was attained. Conceivably, this altered threshold of myogenic responsiveness may contribute to the shift of threshold of autoregulation in glomerular filtration rate observed in DS rats.3,4
Altered Pressure Responsiveness of Afferent Arterioles

The extent of AA myogenic responsiveness also varied between the two Dahl strains in analogy with the changes seen in ILAs. AA myogenic responsiveness was blunted in DS-L rats as compared with DR-L rats. Maximal decrements of AA diameter were less in DS rats than in DR rats. The threshold of myogenic responses was also shifted to higher pressures in DS rats. These observations are in accord with recent in vivo studies indicating that the autoregulatory index of renal blood flow is higher in DS rats than in DR rats, especially at lower perfusion pressures. Azar et al. also reported that hypertensive DS-H rats manifested reduced preglo merular vascular resistance as compared with DR-H rats. These findings are in accord with our current demonstration that, when rats are fed a high sodium diet, AA myogenic vasoconstriction is absent or possibly reset toward a higher pressure in DS rats, whereas the capability for pressure-induced vasoconstriction is retained by DR rats within the examined pressure range. Our data indicate that myogenic responsiveness to increasing pressure (from 80 to 180 mm Hg) is diminished in both preglo merular vessels (i.e., ILA and AA) of DS rats.

The myogenic responsiveness in preglo merular vessels to increasing pressure seems to contribute to the changes in RVR in response to increasing RAP. In DR-L rats, in response to elevation of RAP, RVR was increased concomitantly with a decrease in the diameters of preglo merular vessels. In contrast, in response to increasing RAP, DS-H rats manifested a decrease in RVR with no preglo merular vasoconstriction. These findings support the concept that myogenic vasoconstriction of renal microvessels constitutes an important determinant of renal autoregulatory changes and suggest that blunted preglo merular myogenic responsiveness of DS rats contributes to impaired autoregulatory responses in this strain of rats.

Despite substantial vasoconstriction of preglo merular vessels of DR-H and DS-L rats, the concomitant increase in RVR did not retain statistical significance. Although the reasons for this discrepancy are not fully established, they may relate to the experimental conditions of this study. Vascular resistance is related to both vessel diameter as well as the viscosity of the fluid within the vessel. We perfused the hydronephrotic kidney with cell-free medium, which possesses a markedly lower viscosity than blood. As discussed previously, the reduced viscosity of the cell-free perfusate greatly reduces both the basal resistance and changes in resistance associated with decreased vessel diameter. The discrepancy of the responses between RVR and preglo merular vasoconstriction to increasing pressure may relate to the low viscosity of the perfusate.

Under identical experimental conditions, we have previously demonstrated that, in response to a pressure stimulus of 180 mm Hg, AAs of kidneys of SHR and Wistar-Kyoto rats were constricted to an identical degree, although the pressure–diameter curve for SHR was shifted to the right. In the present study, we have provided the first direct evidence indicating that, in comparison with DR rats, DS rats manifest both a shift in the threshold pressure of myogenic response and a decrease in maximal vasoconstriction. This impairment of myogenic responsiveness in DS rats could account for the failure to sufficiently increase preglo merular vascular resistance, thereby allowing direct transmission of systemic blood pressure to the glomeruli. Thus, our current observations are consistent with previous micro puncture studies indicating that glomerular capillary pressure is maintained in SHR and elevated in DS rats.

Dietary Sodium as a Determinant of Myogenic Responsiveness

In the present study, alterations of dietary sodium intake also affected myogenic responsiveness of preglo merular microvessels. Although the SBP of DR-H rats was identical to that of DR-L rats, AAs of DR-H rats exhibited blunted myogenic responsiveness as compared with AAs of DR-L rats. At 180 mm Hg, ILAs from DR-H rats also manifested less myogenic responsiveness than ILAs from DR-L rats (−12±3% [n=8, DR-H rats] versus −21±4% [n=8, DR-L rats], p<0.05). These findings are in accord with previous studies. Schor et al. reported that a high salt intake increased glomerular capillary plasma flow rate in Munich-Wistar rats. Kaloyanides et al. also demonstrated that autoregulatory responses of renal blood flow in dogs were impaired by high salt feeding, sufficient to suppress renin secretion. The precise mechanisms for the blunted myogenic response in DR-H rats are not readily apparent. However, we have demonstrated that the sensitivity of AA myogenic response to nifedipine was identical between DR-L and DR-H rats. Thus, it is probable that the mechanisms, whereby a high salt intake attenuated myogenic responsiveness, are mediated independent of alterations in voltage-dependent calcium channels. Our current observations support and extend the abovementioned studies and suggest that alterations in salt intake may affect renal autoregulation by modifying myogenic responsiveness of preglo merular vessels.

ILAs of DS-H rats exhibited no myogenic vasoconstriction within the range of RAPs examined, whereas DS-L rats retained the capability for myogenic constriction in the ILA. Thus, myogenic responsiveness of the ILA was blunted to a greater extent in DS-H rats than in DS-L rats. The AAs of DS-H rats also exhibited blunted myogenic responsiveness as compared with the AAs of DS-L rats. Thus, in analogy with DR rats, the increase in salt intake appeared to alter myogenic responsiveness of preglo merular vessels from DS rat kidneys. Despite the elevation of SBP in DS-H rats, it is unlikely that the absence of myogenic vasoconstriction of preglo merular microvessels was attributable to remodeling of these arteriolar structures and development of arteriolar sclerosis. The AAs of DS-L rats (22±2%, n=12) and DS-H rats (18±3%, n=12, NS versus DS-L rats) constricted identically in response to KCl-induced depolarization. These observations favor a functional rather than anatomic basis for the absence of myogenic vasoconstriction in DS-H rats.

Absence of Myogenic Vasoconstriction in Efferent Arterioles

In the present study, the postglomerular vessels (EAs) of all four groups of Dahl rats failed to constrict in response to increasing RAP. We have previously demonstrated that KCl depolarization preferentially
constricts AAs but not EAs of Sprague-Dawley rats, suggesting that voltage-dependent calcium channels are sparingly distributed or physiologically silent in the EA. Myogenic responses are thought to be mediated, at least in part, by membrane depolarization and activation of voltage-dependent calcium channels. As we have postulated previously, the absence of myogenic responsiveness of the EA may be attributable to the minimal responsiveness of this microvessel to depolarization-induced vasoconstrictor stimuli.

**Altered Vasoconstrictor Responsiveness to Membrane Depolarization**

Several lines of evidence indicate that the mechanisms mediating myogenic responsiveness involve membrane depolarization. Harder et al. have reported that membrane depolarization of the vascular smooth muscle cell is involved in pressure-induced vasoconstriction in diverse experimental preparations, including isolated dog ILA and cat middle cerebral artery. To further investigate the mechanisms that mediate the blunted myogenic responsiveness of preglomerular vessels in DR rats, we examined the effects of KCl-induced depolarization on AAs in DS rats. As depicted in Figure 10, in DR-L rats, increasing perfusion pressure to 180 mm Hg constricted AAs by 22±3%. In striking contrast, despite an identical low salt diet and similar blood pressure, AAs from DS-L rats manifested diminished myogenic vasoconstriction (−16±2% at 180 mm Hg) as compared with AAs from DR-L rats (p<0.05). KCl depolarization induced a 35±2% decrement in AA diameter of DR-L rats. In contrast, AAs from DS-L rat kidneys manifested a diminished constrictor response to KCl depolarization (22±2%) as compared with AAs from DR-L rat kidneys (p<0.05). These data suggest that reduced vasoconstrictor responsiveness to membrane depolarization contributes to the blunted myogenic responsiveness of AAs in DS rats.

**Enhanced Sensitivity of Myogenic Response to Calcium Antagonists**

Since voltage-dependent calcium channels participate in mediating myogenic responses, we further characterized the role of these channels by assessing the effects of nifedipine on the myogenic response of Dahl rats. DS-H rats were not included in this study, because AAs of these rats had failed to constrict in response to increments of perfusion pressure. The concentration for nifedipine, at which half-maximal inhibition of myogenic response was observed, was greater in DR rats (258 nM [low salt], 207 nM [high salt]) than in DS rats (26 nM). Thus, the sensitivity of myogenic responses to nifedipine, a calcium antagonist, was enhanced in DS rats. Our findings are in accord with the observations of Steele and Challoner-Hue. Using isolated perfused kidneys preconstricted with norepinephrine, they demonstrated that in response to superimposition of calcium antagonists, DS rats, as compared with DR rats, manifested an exaggerated increase in glomerular filtration rate.

Although the reasons for the enhanced sensitivity of myogenic response to nifedipine in DS rats are not readily apparent, they may relate to calcium channel abnormalities on the AA. In contrast, Abel et al. reported that vascular smooth muscle cells from DS rats did not manifest abnormal resting potential, suggesting that DS rats possess no abnormality in ionic channels. On the other hand, Cravan et al. have recently reported that DS rats exhibit decreased intracellular calcium concentration and phospholipase activity in renomedullary interstitial cells. They have suggested that DS rats may possess abnormalities in diffusible calcium, calcium pump, or calcium binding protein. If similar derangements occur in AAs of DS rat kidneys, these alterations in cellular calcium handling might also contribute to increased sensitivity of myogenic responsiveness to nifedipine in DS rats. Additional studies are required to elucidate the precise mechanisms that mediate enhanced sensitivity of AA myogenic responsiveness to calcium antagonists in DS rats.

In conclusion, we have demonstrated that preglomerular microvessels of DS and DR rats respond differently to changes in RAP. In DS rats, both ILAs and AAs manifest diminished myogenic responsiveness to increasing pressure. The blunted myogenic responsiveness of these preglomerular vessels in DS rats may contribute to the deranged autoregulation of glomerular filtration rate in this strain. Furthermore, variations in salt intake are capable of influencing myogenic responsiveness. During high salt feeding, preglomerular vessels exhibit reduced myogenic responsiveness. Finally, our current data suggest that an altered linkage between membrane depolarization and AA vasoconstriction may contribute to impaired myogenic responsiveness of this vessel in DS rats.

**Acknowledgment**

The authors thank Maritza G. Laguna for her excellent preparation of the artwork.

**References**

Impaired myogenic responsiveness of renal microvessels in Dahl salt-sensitive rats.
T Takenaka, H Forster, A De Micheli and M Epstein

Circ Res. 1992;71:471-480
doi: 10.1161/01.RES.71.2.471

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1992 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/71/2/471

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org/subscriptions/