Renal Mechanoreceptor Dysfunction
An Intermediate Phenotype in Spontaneously Hypertensive Rats

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Abstract—This study tested the hypothesis that decreased responsiveness of renal mechanosensitive neurons constitutes an intermediate phenotype in spontaneously hypertensive rats (SHR). Decreased responsiveness of these sensory neurons would contribute to increased renal sympathetic nerve activity and sodium retention, characteristic findings in hypertension. A backcross population, developed by mating borderline hypertensive rats with Wistar-Kyoto rats (WKY) (the F1 of a cross between an SHR and a normotensive WKY), was fed 8% NaCl food for 12 weeks from age 4 to 16 weeks. Responses to increases in ureteral pressure to 20 and 40 mm Hg in 80 backcross rats instrumented for measurement of mean arterial pressure and afferent renal nerve activity were determined. Mean arterial pressure ranged from 110 to 212 mm Hg and was inversely correlated with the magnitude of the increase in afferent renal nerve activity during increased ureteral pressure. Thus, decreased responsiveness of renal mechanosensitive neurons cosegregated with hypertension in this backcross population. This aspect of the complex quantitative trait of altered renal sympathetic neural control of renal function, ie, decreased renal mechanoreceptor responsiveness, is part of an intermediate phenotype in SHR. (Hypertension. 1999;33[part II]:472-475.)

Key Words: afferent renal nerve activity ■ rats, inbred SHR ■ mechanoreceptors

As described by Sanders and Lawler,1 the borderline hypertensive rat (BHR) is the F1 of a cross between a spontaneously hypertensive rat (SHR) and a normotensive Wistar-Kyoto rat (WKY). Exposure of BHR either to environmental stress or to a high dietary NaCl intake (8% NaCl) produces sustained hypertension (BHR-8%); in the absence of these interventions, the BHR remains normotensive (BHR-1%). BHR subjected to prior renal denervation exhibit an attenuated increase in arterial pressure compared with BHR with intact renal innervation.2 This finding suggests that renal sympathetic nerve activity (RSNA) plays an important role in the development of hypertension in BHR.

Phenotypic features of BHR fed both 1% and 8% NaCl have been compared with those in the parental WKY and SHR strains. Several aspects of the regulation of RSNA and the neural control of renal function were seen to occur in the hypertensive SHR parent and in the hypertensive BHR-8% but not in the normotensive WKY parent or the normotensive BHR-1%3–7 (Table). It was considered that these phenotypic features constitute a complex quantitative trait of altered renal sympathetic neural control of renal function and may serve as an intermediate phenotype for hypertension in SHR.8,9

Thus far, the evaluation of aspects of this complex quantitative trait as part of an intermediate phenotype has dealt with alterations in efferent RSNA and its influence on renal function. However, one aspect of the complex quantitative trait, decreased responsiveness of renal mechanosensitive neurons, deals with afferent renal nerve activity (ARNA).

The present experiments tested the hypothesis that this aspect of the complex quantitative trait of altered renal sympathetic neural control of renal function, decreased responsiveness of renal mechanosensitive neurons, cosegregates with hypertension in a backcross population (F1 x WKY) consuming an 8% NaCl diet.

Methods

Animals
Female SHR and male WKY were purchased from Taconic Farms (Germantown, New York). Female SHR were mated with male WKY to produce BHR. BHR of both sexes were mated with WKY of the other sex to produce the backcross population. Backcross population rats of both sexes were weaned at age 4 weeks. They were fed 8% NaCl food and had free access to tap water drinking solution until they were 16 weeks old, which is when they were studied. All animal procedures were in accordance with the guidelines of the University of Iowa Animal Care and Use Committee.

Anesthesia
The rats were anesthetized with 20 mg/kg IP methohexital supplemented with 10 mg/kg IV as required (short-duration procedures) and 30 mg/kg IV sodium pentobarbital supplemented with 10 to 20 mg/kg IV as required (long-duration procedures).

Procedures
Under methohexital anesthesia, the rats were instrumented with polyethylene catheters in a femoral vein and a femoral artery. The
catheters were filled with heparinized isotonic saline and plugged, and the rats were returned to their home cages. The next day the rats were placed in a device that permitted forward and backward movement but did not allow the rats to turn around. The femoral vein catheter was connected to an infusion pump that delivered isotonic saline at a rate of 50 μL/min, and the femoral artery catheter was connected to an electronic pressure transducer for the measurement of mean arterial pressure (MAP). After a 30-minute equilibration period, basal MAP in the conscious state was recorded over a 1-hour period.

After the recording of basal MAP in the conscious state, the rats were anesthetized with sodium pentobarbital introduced at 25 mg/kg IV and maintained with an IV infusion of sodium pentobarbital at 10 mg/kg per hour IV in isotonic saline at 50 μL/min using the femoral vein catheter. The left kidney was approached through a flank incision, and a PE-10 catheter was placed in the right ureter for collection of urine. A PE-60 catheter was placed in the left renal pelvis via the ureter. To administer test agents into the left renal pelvis at the conclusion of the experiment, a nonobstructing PE-10 catheter was inserted into the PE-60 catheter and advanced into the renal pelvis, so that its tip extended 1 to 2 mm beyond the tip of the PE-60 catheter.11,12 Left ureteral pressure (UP) was recorded with a P23Db Statham transducer connected to the left ureteral catheter by a T-tube connector.

One renal nerve branch was isolated at the angle between the aorta and the left renal artery and placed on a bipolar silver wire electrode for recording of multifiber renal nerve activity. The signals were led by a high-impedance probe (Grass H5P11) to a bandpass amplifier (Grass P511) with a high-frequency cutoff at 3000 Hz, a low-frequency cutoff at 30 Hz, and × 20 000 amplification. The output of the bandpass amplifier was fed into an oscilloscope (Tektronix 5113) and to a full-wave rectifying capacitance voltage integrator with a 20-ms time constant (Grass 7P3). Assessment of renal nerve activity was done by its pulse synchronous rhythmicity. After identification and verification of the electrode for recording ARNA,7,10–12 The electrode was fixed to the afferent renal nerve with silicone cement (Wacker Sil-Gel 604, Wacker-Chemie).

Experimental Protocol

After a 1-hour equilibration period, control period measurements of MAP, UP, and ARNA were made over a 10-minute period. The left ureteral catheter then was elevated above the level of the rat to increase UP by 40 mm Hg, and a 3-minute experimental UP-40 period was established. The left ureteral catheter then was lowered to the baseline position, and a 10-minute recovery period was established. After a 10-minute collection period, a second 10-minute control period was established. Then, the left ureteral catheter was elevated above the level of the rat enough to increase UP by 20 mm Hg, and a 3-minute experimental UP-20 period was made. Then, the left ureteral catheter was lowered to the baseline position and a 10-minute recovery period was established. At the conclusion of each experiment, the general responsiveness of renal sensory receptors was tested by assessing the ARNA response to an injection of 50 μL of a capsaicin solution (2.5 μg/mL) into the inner PE-10 catheter in the left ureter; this was followed by a flush of 50 μL isotonic saline.12 All afferent renal nerve preparations in this study responded to this maneuver with an increase in ARNA. To determine background renal nerve activity, the decentralized renal nerve bundle was cut peripheral to the recording electrode, and the recorded value was subtracted from all values of ARNA. Rats were killed with an overdose of sodium pentobarbital.

Analytical Techniques

An analog-to-digital converter and standard data acquisition software were used. For the 1-hour continuous recording of basal MAP, data were sampled at 1 Hz and averaged over 10-minute periods. Because the average for the six 10-minute periods differed by <5%, they were averaged to give a single basal MAP for each rat. In the experimental protocol, MAP and UP were sampled at 0.1 Hz and ARNA was sampled at 4 Hz, and the data were averaged to give a single value for each variable for each period.

Data Analysis

The level of ARNA during the control period was set to 100%, and the ARNA values during increased UP and recovery periods were normalized to it. The ARNA response to increased UP was taken as the change from the average of the control and recovery period values bracketing each of the UP-20 or UP-40 periods.

The linear regression lines with 95% confidence intervals and correlation coefficient values for the relationships between basal MAP and the responses of ARNA to increases in UP to 20 and 40 mm Hg were calculated.13 P < 0.05 was considered statistically significant. Data in text and figures are expressed as mean±SE.

Results

The 1-hour period of continuous recording of basal MAP in the conscious state gave values of MAP that ranged from 110 to 212 mm Hg. Of the 80 backcross population rats studied, MAP averaged $144 \pm 3$ mm Hg in 50 male rats and $140 \pm 4$ mm Hg in 30 female rats. The distributions were unimodal (5-mm Hg bins), with the mode being 140 mm Hg in both male and female rats.

For each rat, the MAP from the basal 1-hour continuous recording period in the conscious state was plotted against the respective percent change in ARNA during an increase in UP to 20 and to 40 mm Hg. As seen in the Figure, the magnitude of the increase in ARNA produced by increased UP was inversely related to the level of the basal MAP. Thus, the increases in ARNA in hypertensive rats were less than those in normotensive rats. When UP was increased to 20 mm Hg, the correlation coefficient ($r$) was 0.21 and was of borderline statistical significance ($P<0.07$). When UP was increased to 40 mm Hg, the correlation coefficient ($r$) of 0.27 was statistically significant ($P<0.02$). The slope of the linear regression line when UP was increased to 40 mm Hg, $-0.29$, was approximately twice that when UP was increased to 20 mm Hg, $-0.16$. Thus, a doubling of the stimulus (from UP-20 mm Hg to UP-40 mm Hg) resulted in an approximate doubling of the ARNA response.

Discussion

The BHR inherits genetic information from a hypertensive SHR parent and a normotensive WKY parent. When BHR ingest an 8% NaCl diet, they develop hypertension and exhibit aspects of regulation of RSNA and the neural control of renal function that are similar to those of the hypertensive SHR parent phenotype. When BHR ingest a 1% NaCl diet,
they remain normotensive and exhibit aspects of regulation of RSNA and the neural control of renal function that are similar to those of the normotensive WKY parent phenotype (see the Table). These results suggest that a high dietary NaCl intake is able to induce or unmask the capabilities for these responses, which are genetically conveyed to the BHR by the hypertensive SHR parent in latent forms.

This study evaluated decreased responsiveness of renal mechanosensitive neurons (ARNa) as one aspect of the complex quantitative trait of altered renal sympathetic neural control of renal function for suitability as an intermediate phenotype for hypertension. Complex traits refer to phenotypes or intermediate phenotypes that do not exhibit classic Mendelian inheritance attributable to a single gene locus. Variations in these traits may result from variations in multiple genes and environmental influences. Quantitative traits refer to continuous variables such as MAP, in contrast to discrete traits measured by a specific outcome, such as albinos versus pigmented.

Rapp set forth 4 criteria for a complex quantitative trait as an intermediate phenotype: (1) the trait should have a plausible pathophysiological role in hypertension; (2) there should be evidence for a difference in the trait in progenitor hypertensive and normotensive strains that would implicate the trait in the pathogenesis of hypertension; (3) the difference in the trait should not be secondary to the hypertension; and (4) there should be evidence for a difference in the trait in progenitor hypertensive and normotensive strains that would implicate the trait in the pathogenesis of hypertension.

Hypertension cosegregated both with the magnitude of the increase in RSNA produced by air-jet stress and with the magnitude of the decrease in RSNA produced by the intracerebroventricular administration of guanabenz, an α₂-adrenoceptor agonist.6 These results supported the hypothesis that these 2 phenotypic aspects of this complex quantitative trait are part of an intermediate phenotype.

Because renal denervation attenuates the exaggerated natriuresis both in SHR and in BHR-8% but has no effect in WKY and BHR-1%,3,9 the exaggerated inhibition of RSNA that occurs during volume loading in both SHR and BHR-8% (but not in WKY and BHR-1%)3,15 is a significant contributor to the exaggerated natriuresis. Therefore, exaggerated natriuresis is another manifestation of an alteration in renal sympathetic neural control of renal function. However, hypertension did not cosegregate with the magnitude of either the decrease in RSNA or the increase in urinary sodium excretion during volume loading.16 Thus, these 2 phenotypic aspects of the complex quantitative trait, exaggerated natriuresis and exaggerated renal sympathoinhibition during volume loading, are not part of an intermediate phenotype in SHR.

The present study focused on another phenotypic aspect of the complex quantitative trait of altered renal sympathetic neural control of renal function: decreased responsiveness of renal mechanosensitive neurons. In normal physiological circumstances, stimulation of renal mechanosensitive neurons by increasing UP results in a contralateral inhibitory renorenal reflex composed of an afferent limb of increased ipsilateral ARNa and an efferent limb of decreased contralateral efferent RSNA, resulting in a contralateral diuresis and natriuresis.10 This inhibitory renorenal reflex contributes importantly to the compensatory contralateral renal excretory responses following ipsilateral increases in UP, eg, during partial ureteral occlusion. Compared with normotensive WKY, SHR exhibit decreased responsiveness of renal mechanosensitive neurons5,17,18 (criterion 2 of Rapp). During increased UP, the increase in ipsilateral ARNa in SHR is markedly suppressed compared with WKY. In SHR, this is associated with an impaired contralateral inhibitory renorenal reflex response with sustained elevations in contralateral
efferent RSNA and absence of the contralateral diuretic and natriuretic response. The resultant excess sodium and water retention can contribute to the hypertension in SHR (criterion 1 of Rapp).

The present data indicate that hypertension cosegregates with decreased responsiveness of renal mechanosensitive neurons in a backcross population (criterion 4 of Rapp). Therefore, decreased responsiveness of renal mechanosensitive neurons as another phenotypic aspect of the complex quantitative trait of altered renal sympathetic neural control of renal function might serve as an intermediate phenotype for hypertension in SHR. However, an alternative explanation may relate to a possible desensitization of renal mechanosensitive neurons via chronic exposure to the increased arterial pressure and associated increase in intrarenal pressure. Support for this view comes from the finding that SHR treated from weaning with captopril to prevent the development of hypertension had normal responsiveness of renal mechanosensitive neurons.19 However, it is also possible that chronic captopril treatment might have influenced central nervous system mechanisms involved in the integrative control of the responsiveness of renal mechanosensitive neurons. It is known that the level of efferent RSNA influences the responsiveness of renal mechanosensitive neurons.19 As recordings of afferent renal nerve activity have not been accomplished in conscious rats, there is always the possibility that the responsiveness of renal mechanosensitive neurons is influenced by anesthesia, eg, pentobarbital in the present experiments. However, recordings from renal mechanosensitive neurons have been made in rats anesthetized with a variety of agents with generally uniform agreement as to the overall qualitative nature of the results obtained.17,18

In the hypertensive rats of the backcross population, the decreased responsiveness of renal mechanosensitive neurons would result in impaired renorenal reflex regulation of urinary sodium excretion. The initial step is a lesser activation of ARNA in response to increased UP, which in turn results in attenuated inhibition of contralateral RSNA and a diminished diuretic and natriuretic response. The resultant sodium and water retention could contribute to the development and maintenance of the hypertension observed in the backcross population.

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References
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