Introduction

As described by Sander and Lawler [1], the borderline hypertensive rat (BHR) is the F1 of a cross between a hypertensive spontaneously hypertensive rat (SHR) and a normotensive Wistar–Kyoto (WKY) rat. 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 remain normotensive (BHR-1%) [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.

In examining the influence of a high dietary NaCl intake on aspects of the regulation of RSNA and the neural control of renal function in BHR and in rats of the parental strains, WKY rats and SHR, phenotypic features were identified [3–18] (Table 1) that occur both in the hypertensive SHR parent and in the hypertensive BHR-8% but not in the normotensive WKY rat parent and the normotensive BHR-1%. It was considered that these phenotypic features dealing with renal sympathetic neural mechanisms constitute a complex quantitative trait that could represent an intermediate phenotype.

Methods

A backcross population, developed by mating borderline hypertensive rats with Wistar–Kyoto rats, was fed 8% NaCl food for 12 weeks from age 4 to 16 weeks. Responses to intravenous isotonic saline volume loading (10% body weight/30 min) in 81 backcross rats chronically instrumented for measurement of mean arterial pressure, renal sympathetic nerve activity, and urinary sodium excretion were determined.

Objective

To determine whether exaggerated natriuresis and exaggerated renal sympathoinhibition during volume loading constitute an intermediate phenotype in spontaneously hypertensive rats.

Design

The borderline hypertensive rat, the F1 of a cross between a spontaneously hypertensive rat and a normotensive Wistar–Kyoto rat, is a NaCl-sensitive model of genetic hypertension. In addition to hypertension, borderline hypertensive rats fed 8% NaCl food develop characteristic alterations in regulation of renal sympathetic nerve activity and neural regulation of renal function similar to those in the spontaneously hypertensive rat parent. Like the Wistar–Kyoto rat parent, borderline hypertensive rats fed 1% NaCl food remain normotensive and do not exhibit these alterations in renal sympathetic neural mechanisms. These renal sympathetic neural mechanisms constitute a complex quantitative trait that could represent an intermediate phenotype.

Results

Mean arterial pressure was 105–180 mmHg and was not correlated to the magnitude either of the decrease in renal sympathetic nerve activity or of the increase in urinary sodium excretion during volume loading.

Conclusions

These two aspects of the complex quantitative trait, exaggerated natriuresis and exaggerated renal sympathoinhibition during volume loading, are not part of an intermediate phenotype in spontaneously hypertensive rats. J Hypertens 16:85–90 © 1998 Rapid Science Ltd.

Keywords: renal nerves, hypertension, sodium

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Table 1 Phenotypic features common to spontaneously hypertensive rat and borderline hypertensive rat (BHR-8%) but absent in Wistar–Kyoto rat and BHR-1%

1. Hypertension
2. Increased RSNA responsiveness to air jet stress (excitatory)
3. Increased RSNA responsiveness to intracerebroventricular guanabenz (inhibitory)
4. Exaggerated natriuretic response to volume loading
5. Exaggerated RSNA inhibitory response to volume loading
6. Resetting of arterial baroreflex control of heart rate and RSNA
7. Enhanced cardiac baroreflex inhibition of RSNA
8. Decreased renal sensory receptor responsiveness

RSNA, renal sympathetic nerve activity.

Exaggerated inhibition of RSNA is found in SHR and BHR-8% but not in WKY rats and BHR-1% [13]. Because prior renal denervation attenuates the exaggerated natriuresis both in SHR and in BHR-8% but has no effect on WKY rats and BHR-1%, the inhibition of RSNA during volume loading is a significant contributor to the exaggerated natriuresis. Thus, exaggerated natriuresis is another manifestation of an alteration of renal sympathetic neural control of renal function.

The current experiments tested the hypothesis that these two additional aspects of the complex quantitative trait of altered renal sympathetic neural control of renal function, namely exaggerated natriuresis and exaggerated renal sympatho-inhibition during volume loading, cosegregate with hypertension in a backcross population (F1 × WKY rat) consuming an 8% NaCl diet.

Methods

Animals

Female SHR and male WKY rats were purchased from Taconic Farms (Germantown, New York, USA). Female SHR were mated with male WKY rats to produce BHR. BHR of both sexes were mated with WKY rats of the other sex to produce the backcross population. Backcross population rats of both sexes were weaned aged 4 weeks. They were fed 8% NaCl food and had free access to tap water drinking solution until they were aged 16 weeks, 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 methohexital (Brevital, 20 mg/kg intraperitoneally supplemented with 10 mg/kg intravenously if necessary; Eli Lilly, Indianapolis, Indiana, USA).

Procedures

Catheterization

The rats were instrumented with polyethylene catheters in the right jugular vein and right carotid artery for infusion of isotonic saline at 0.05 ml/min during the surgical preparation and the measurement of mean arterial pressure (MAP) and heart rate. The catheters were tunneled to the dorsum of the neck where they were exteriorized. A bladder catheter was inserted through a midline suprapubic incision [13].

Renal sympathetic nerve activity recording electrode

The left kidney was exposed through a left-flank incision via a retroperitoneal approach. With the use of a dissecting microscope (×25), a renal nerve branch from the aorticorenal ganglion was isolated and carefully dissected free. The renal nerve branch was then placed on a bipolar platinum wire (Cooper Wire Company, Chatsworth, California, USA) electrode. RSNA was amplified (×50 000) and filtered (low, 30 Hz; high, 3000 Hz) with a high-impedance probe and preamplifier (Grass P511, respectively; Grass Instrument Co., Quincy, Massachusetts, USA). The output of the amplifier was led to a Tektronix 5113 oscilloscope (Tektronix, Inc., Beaverton, Oregon, USA) for visual evaluation and to an audio amplifier/loudspeaker (Grass Model AM 8 audio monitor) for auditory evaluation. The quality of the RSNA signal was assessed in terms of its pulse synchronous rhythmicity and by examining the magnitude of decreases in recorded RSNA during sinoaortic baroreceptor loading with an intravenous bolus injection of 3 mg norepinephrine. The RSNA remaining after maximum inhibition after administration of norepinephrine was within 5% of the background noise observed approximately 30 min post mortem or after intravenous administration of 30 mg/kg hexamethonium (ICN Pharmaceuticals, Cleveland, Ohio, USA) [20]; this background noise value was subtracted from all experimental values of RSNA. When an optimal RSNA signal (pulse synchronous rhythmicity, abolition by norepinephrine-induced increase in arterial pressure) was observed, the recording electrode was fixed to the renal nerve branch with a silicone cement (Wacker Sil-Gel 604; Wacker-Chemie, Munich, Germany). The electrode cable was then secured in position by suturing it to the abdominal trunk muscles and further tunneled to the dorsum of the neck where it was exteriorized. The flank incision was closed in layers, the vascular catheters were plugged, and the rat was returned to its home cage and allowed free access to 8% NaCl food and tap water drinking solution.

Experimental protocol

All rats were studied while they were conscious on the day after the above-described chronic instrumentation. For continuous complete collection of urine, the rat was placed in a device that permitted forward and backward movement but did not permit the rat to turn around. Isotonic saline was infused into the jugular venous catheter at 0.05 ml/min, the carotid arterial catheter was connected to the electronic pressure transducer, the RSNA electrode was connected to the high-impedance probe, and the bladder catheter was led to a collection...
A 1 h period of continuous recording of basal MAP was used prior to beginning the experimental protocol. With the onset of the experimental protocol, continuous recording of MAP, heart rate, and RSNA was begun and urine was collected in consecutive 10 min periods. The first three periods (C1–C3), during which isotonic saline was infused at 0.05 ml/min, constituted the control phase. The next three periods (E1–E3), during which a volume of isotonic saline equivalent to 10% body weight was infused, constituted the experimental phase. The next six periods (R1–R6), during which there was no infusion, constituted the recovery phase.

At the end of the experimental protocol, the rat was killed with an overdose of methohexital injected intravenously and post-mortem RSNA was recorded for 30 min; this value was subtracted from all experimental protocol RSNA data. Both kidneys were removed, drained, blotted, and weighed.

**Analytical**

The amplified and filtered renal neurogram was full-wave rectified, integrated (Grass 7P3 resistance–capacitance integrator, 20 ms time constant), averaged and stored as RSNA on videotape (Vetter 4000A PCM; Vetter, Stroudsburg, Pennsylvania, USA) along with MAP (Statham 23Db pressure transducer; Statham-Gould, Hato Rey, Puerto Rico, USA) and heart rate (Grass 7P4 tachograph) for later offline analysis.

An analog-to-digital converter (Data Translation 2801; Data Translation, Marlboro, Massachusetts, USA) and standard data-acquisition software (LabTech Notebook 7.3; LabTech, Wilmington, Massachusetts, USA) were used. For the 1 h recording of basal MAP which preceded the experimental protocol, data were sampled at 1 Hz and averaged over 10 min periods. Because the averages for the six 10 min periods differed by less than 5%, they were averaged to give a single basal MAP for each rat. For the experimental protocol, RSNA, MAP, and heart rate were sampled at 1 Hz and averaged for each of the 10 min periods during the control (C1–C3), experimental (E1–E3), and recovery periods (E1–E6).

Urine volume was determined gravimetrically and urine sodium concentration was measured with flame photometry (IL 943; Instrumentation Laboratories, Lexington, Massachusetts, USA). Urinary sodium excretion (U_{NaV}), was expressed per g kidney weight (gKW).

**Data analysis**

The values of RSNA for C1–C3 were averaged and the average value set to 0%; the values of RSNA for E1–E3 and R1–R6 were normalized with respect to this and expressed as percentage changes from control. The values of U_{NaV} for C1–C3 were averaged to give a single control phase value. After the volume load, the decreases in RSNA and the increases in U_{NaV} that occurred during E1–E3 and R1–R6 were analyzed as areas over and under the curve, respectively [21].

One-way analysis of variance with repeated measures and Scheffé’s test were used for comparison of responses of MAP, heart rate, RSNA, and U_{NaV} to volume loading. The correlation coefficient values (r) for correlation between basal MAP and the responses of RSNA and U_{NaV} responses to volume loading were calculated. P < 0.05 was considered statistically significant. Data in text, tables, and figures are means ± SEM.

**Results**

The 1 h period of continuous recording of basal MAP before beginning the experimental protocol gave values of MAP that were not significantly different than those for the control phase (C1–C3) of each individual rat
experiment protocol. Of the 81 backcross population rats studied, there were 41 male rats (MAP 137 ± 3 mmHg) and 40 female rats (MAP 136 ± 2 mmHg).

Figure 1 shows mean data on heart rate, MAP, UNaV, and RSNA for all rats during each period. The values for heart rate, MAP, UNaV, and RSNA were stable during the control phase (C1–C3) and the values have been averaged to give a single value. During the volume loading (E1–E3) and recovery (R1–R6) phases, heart rate, MAP, and RSNA progressively decreased. UNaV increased sharply to a peak in E3 and returned to levels slightly above control by R6.

Calculation of areas under and over the curve permitted more complete measurement both of the magnitude and of the duration of the effects of volume loading on UNaV and RSNA, respectively. For each rat, the MAP from the preprotocol 1 h continuous monitoring period was plotted against the respective values for the decrease in RSNA (Fig. 2), expressed as area (–%min) over the curve, and the increase in UNaV (Fig. 3), expressed as area (mmol/min per gKW per min) under the curve. Neither variable exhibited significant correlation to MAP when analyzed for the entire group (Figs 2, 3) or by sex (data not shown).

Discussion
The BHR (F1) inherits genetic information from a hypertensive SHR parent and a normotensive WKY rat 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. 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 rat parent. 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 renal sympathetic neural mechanisms as a complex quantitative trait for suitability as an intermediate phenotype. 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 albino versus pigmented.

With regard to the criteria put forth by Rapp [22] for complex quantitative traits as intermediate phenotypes, renal sympathetic neural mechanisms through their multiple actions on various aspects of renal function [23] have a plausible pathophysiologic role in the pathogenesis of hypertension (criterion 1). An increase in RSNA is known to increase renal vascular resistance, which, in view of the fraction of cardiac output delivered to the kidneys, could represent a substantial contribution to total peripheral vascular resistance. RSNA at levels below the threshold for effects on renal blood flow and glomerular filtration rate directly increase renal tubular reabsorption.
of sodium and water throughout the nephron. In this way, an increase in RSNA opposes pressure diuresis and natriuresis, resulting in the need for a higher level of MAP to achieve the same level of excretion of water and sodium (i.e. a rightward shift of the renal function curve along the MAP axis) [24]. Graded increases in RSNA produce graded increases in release of renin, beginning at levels of RSNA that are subthreshold for effects on renal blood flow, glomerular filtration rate, and renal tubular reabsorption of sodium and water.

As noted above, the various renal sympathetic neural mechanisms operating in SHR and WKY rats differ substantially (criterion 2). Furthermore, the influence of dietary NaCl intake on the BHR 

\( F_{1} \)

is such that an 8% NaCl intake results in the BHR resembling the hypertensive SHR parent in becoming hypertensive and exhibiting these features whereas a 1% dietary NaCl intake results in the BHR resembling the normotensive parent and not exhibiting these features.

Exaggerated natriuresis dependent on an accompanying exaggerated inhibition of RSNA during volume loading is not a ubiquitous phenomenon, secondary to hypertension from any cause (criterion 3). For example, exaggerated natriuresis is not observed in hypertensive Dahl S rats [6]. This is likely due to their attenuated (lumbar) sympathoinhibitory response to volume loading [25].

Using a backcross population 

\( F_{1} \times WKY \)

fed an 8% NaCl diet, neither the exaggerated natriuresis nor the exaggerated renal sympathoinhibition with volume loading cosegregated with the hypertension (criterion 4). Both the range of MAP (105–180 mmHg) and the size (\( n = 81 \)) of this 8% NaCl-fed backcross population were similar to those observed in our previous study [19] wherein two other aspects of this complex quantitative trait were clearly identified as an intermediate phenotype. In that study, responses of RSNA to AJS (\( n = 81 \), MAP range 105–180 mmHg) and intracerebroventricular administration of the \( \alpha_{2} \)-adrenocceptor agonist guanabenz (\( n = 82 \), MAP range 100–170 mmHg) cosegregated with MAP. Higher levels of MAP were correlated to greater renal sympathoexcitatory responses to AJS and greater renal sympathoinhibitory responses to intracerebroventricular administration of guanabenz.

By using the phenotypic differences for the renal sympathoinhibitory and natriuretic responses to volume loading for SHR and WKY rats from our previous studies [3,6] and the variances of these two responses from the current 8% NaCl-fed backcross population, the phenotypic effects of quantitative trait loci (QTL) and their fractional contributions to explaining the backcross variances may be calculated [26]. For a single QTL, the phenotypic difference between SHR and WKY rats for the renal sympathoinhibitory response would explain 31% of the backcross variance whereas that for the natriuretic response would explain 71% of the backcross variance. For two QTL, these values decrease to 8 and 18%, respectively. Computerized simulations indicated that effects of this magnitude would be readily detected both by using the traditional method in which all progeny are genotyped and single markers are analyzed [26,27] and by using the interval and extremes method in which only progeny with the most extreme phenotypes are genotyped and interval mapping is used to analyze the data [26,28].

Thus, neither the size nor the MAP range of the backcross population would appear to be likely explanations for the failure of the exaggerated natriuresis and the exaggerated renal sympathoinhibition with volume loading to cosegregate with MAP in rats of the current backcross population. Therefore, these two aspects of this complex quantitative trait are not part of an intermediate phenotype in SHR.

Because these common phenotypic features deal with renal sympathetic neural mechanisms, it was initially thought that they constitute a complex quantitative trait and may serve as an intermediate phenotype. Although it is clear that two of these phenotypic features, the responses of RSNA to AJS and to intracerebroventricular administration of guanabenz, do serve as an intermediate phenotype, it is evident that two others of these phenotypic features, the responses of exaggerated natriuresis and exaggerated renal sympathoinhibition to volume loading, do not. Thus, even though all these four phenotypic features relate to alterations in responses of RSNA or to alterations in responses of renal function that are influenced by RSNA, there is specificity concerning their ability to serve as an intermediate phenotype. Those features that represent an integrated response of the central nervous system to the intervention, hypothalamic–limbic processing of the cortically perceived environmental stimulus of AJS and directly intracerebroventricularly administered guanabenz, cosegregate with hypertension. However, volume loading involves multiple alterations in several peripheral neurohumoral reflex mechanisms, albeit with obligatory engagement of the central nervous system, in the generation of renal sympathoinhibition and natriuresis. This suggests that central sites or mechanisms of regulation of RSNA, rather than peripheral reflex inputs, are more critically located on the pathogenetic pathway between the responsible gene(s) and the expression of hypertension in SHR.

References


