Original Article

Sympathetic Regulation of the Renal Functions in Rats Reciprocally Congenic for Chromosome 1 Blood Pressure Quantitative Trait Locus

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The role of the chromosome 1 blood pressure quantitative trait locus (QTL) on the sympathorenal interaction was studied using congenic strains. The two reciprocal congenic strains, WKYpch1.0 and SHRSPwch1.0, were respectively constructed by introgressing the stroke-prone spontaneously hypertensive rat (SHRSP)–derived fragment for the QTL into a Wistar-Kyoto rat (WKY) and *vice versa*. The role of the sympathetic nervous system in the kidney was evaluated by comparing the renal functions between denervated and sham-operated kidneys under anesthesia. The denervation was performed by stripping the adventitia off and applying 10% phenol to the blood vessels at the left renal hilus. Polyfructosan was continuously injected intravenously to determine the renal plasma flow and the glomerular filtration rate. A reciprocal and significant alteration in the renal norepinephrine (NE) content was observed between WKY and WKYpch1.0 and between SHRSP and SHRSPwch1.0. Concomitantly, the renal vascular resistance differed significantly between the congenic and the background parental strains. By contrast, no significant difference was observed in the fractional excretion of sodium, an index of the tubular function. While the denervation elicited a significant decrease of the renal NE content in all of the four strains studied, the significant effects of the denervation on the renal functions were observed only in SHRSP and WKYpch1.0, both of which harbored the SHRSP-derived QTL fragment. These results indicated that the chromosome 1 blood pressure QTL modulated the renal functions through the sympathetic nerve activity in the kidney. (*Hypertens Res* 2008; 31: 561–568)

***Key Words*: sympathetic nervous activity, renal function, congenic rats, stroke-prone spontaneously hypertensive rat, blood pressure**

Introduction

The sympathetic nervous system (SNS) plays a pivotal role in the development as well as the maintenance of arterial hypertension both in humans (*1*) and in laboratory model animals (*2*). Among the functions controlled by the SNS, the regulation of the water balance by the kidney is the most important when the long term regulation of blood pressure (BP) is considered.

A number of studies have suggested the important role of the SNS in the spontaneously hypertensive rat (SHR) and the stroke-prone SHR (SHRSP); SHR have been shown to exhibit elevated efferent renal sympathetic nerve activity (RSNA) before hypertension is established (*3*). In addition, the renal norepinephrine (NE) content has been reported to be 2 times and 1.5 times greater in newborn (*4*) and adult SHR (*5*), respectively, when compared with age- and sex-matched Wistar-Kyoto rats (WKY). These observations suggest that

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the modulation of renal function by sympathetic hyperactivity plays a key role in the establishment of hypertension in this rat model. This hypothesis was further supported by the fact that the renal denervation in young SHR delayed the development of hypertension (*6*).

In previous studies, Nabika and colleagues showed that a WKY-based congenic strain that harbored the SHRSP-derived fragment of the BP quantitative trait locus (QTL) on chromosome 1 (chr-1) showed exaggerated reactivity of the SNS to a variety of environmental stresses (*7*–*9*). This observation implied that the SNS activity was influenced by a gene or genes located in this QTL region, which might contribute to the development of hypertension in SHRSP.

Based on this observation, in the present work, we extended our study to the role of the SNS in renal functions in the congenic strains. For this purpose, we denervated the renal nerves to evaluate the significance of the SNS in the regulation of renal functions. Further, we used the reciprocal congenic strains that harbored the mutually exchanged fragments of the chr-1 QTL, which made it possible to show the functional role of the target QTL independent of the genetic background.

We show here that the chr-1 QTL influenced the renal NE content as well as parameters for the renal functions in the reciprocal congenic rats. Further, the renal denervation elicited discernible effects on the tubular function as well as the renal vascular resistance (RVR) only in the strains that carried the SHRSP-derived fragment of the chr-1 QTL, suggesting the pivotal role of the QTL on the sympathetic regulation of the renal functions.

Methods

Animals

SHRSP and WKY rats of the Izumo colony were provided by the Disease Model Cooperative Research Association (Kyoto, Japan). The WKY-based congenic strain, WKY.SHRSP-(*D1Wox29–D1Arb21*)/Izm (abbreviated as WKYpch1.0 hereafter), was established by the transfer of the chromosomal segment between *D1Wox29* and *D1Arb21* from SHRSP onto the genetic background of WKY as previously described (*10*). The SHRSP-based congenic rat, SHRSP.WKY-(*D1Wox29–D1Arb21*)/Izm (abbreviated as SHRSPwch1.0), was designed to possess the genomic composition corresponding to that of the WKYpch1.0 (*11*). It was developed by reversing the donor and recipient strains in the construction of the WKYpch1.0 and the introgressed segment is smaller than that reported by Kato *et al*. (*12*). The congenic region was about 40 cM, which covered the 100:1 confidence interval for the BP QTL (*13*). To avoid double recombination inside the congenic region, three to four markers in the target region were monitored throughout the breeding process. Further information about the strains is available at the website of the National BioResource Project for the Rat (http://www.anim.med.kyoto-u.ac.jp/nbr) (*14*). All procedures in this study were reviewed and approved by the Ethics Committee for Animal Use and Care in Shimane University.

Surgical Procedures

Experiments were carried out on 6- to 8-week-old male rats. At this age, hypertension is not yet established in SHRSP (*15*, *16*). Anesthesia was induced by intraperitoneal injection of thiopental sodium (110 mg/kg body weight; Ravonal, Tanabe, Osaka, Japan), which was supplemented *via* the rectum when needed during the experiment. The animals were placed on a servo-regulated heating table to maintain their body temperature at 37°C. A tracheotomy was performed to facilitate spontaneous breathing. The right external jugular vein was cannulated with a PE-50 polyethylene catheter (Clay Adams, Parsippany, USA) and 10% polyfructosan (Inutest; Fresenius, Linz, Austria) in 0.9% saline was continuously infused at a rate of 4.5 ml/kg body weight/h. Another PE-50 catheter was placed in the right femoral artery for monitoring arterial pressure and drawing blood samples.

The left kidney was exposed through a flank incision, dissected free from the adhering tissue, and placed horizontally in a Lucite cup. The kidney was loosely surrounded with saline-soaked cotton, immobilized in agar and bathed in warmed (37.5°C) mineral oil to prevent it from drying. The pelvis was cannulated with a PE-10 catheter (Clay Adams) to collect urine samples. A 25-gauge needle connected to a PE-50 catheter was inserted into the left renal vein to obtain the venous blood samples.

The left kidney was then denervated by cutting all visible nerves entering the renal hilus, removing the adventitia from the renal artery and vein, and applying 10% phenol in ethanol to the vessels for 30 min. Intense care was taken to protect the kidney and the adjacent tissue from the detrimental effects of phenol. In the control animals, the sham-operation was accomplished by leaving the left kidney innervated, and saline instead of phenol was placed around the renal vessels. The clearance study was started 1 h after surgery when the physiological status of the rats became stable.

Determination of the Renal Functions

BP was monitored with a pressure transducer (Gould, Cleveland, USA). Urine was collected into preweighed tubes during three 40-min experimental periods and the urine volume (UV) was determined gravimetrically. Arterial and venous blood samples were taken at the midpoint of each period. Polyfructosan and electrolyte concentrations in the plasma and the urine were measured by the anthrone method (*17*) and by a flame photometer (Model 775; Hitachi, Tokyo, Japan), respectively. Each measurement was made in duplicate. The glomerular filtration rate (GFR) was derived from the polyfructosan clearance and other renal parameters were calculated as previously described in detail (*18*).

Table 1. Physiological Characteristics of the Rats Used in the Experiment

	SHRSP		SHRSPwch1.0		WKYpch1.0		WKY	
	Sham	DN	Sham	DN	Sham	DN	Sham	DN
Age (weeks)	6.8±0.4	6.8±0.3	6.9±0.3	7.0±0.3	7.1±0.3	7.1±0.4	7.2±0.2	7.3±0.2
Hct (%)								
Before-exp	46±0.9	44±1.3	46±0.5	47±0.6	43±0.8	43±0.6	44±0.9	44±0.8
During-exp	46±0.3	46±1.3	47±0.2	48±0.5	43±1.2	44±0.9	45±1.2	45±1.1
BW (g)	152±15*	157±14	152±13*	153±7	179±12	171±14	197±11	198±5
KW (mg)	670±50*	720±70	640±40*	660±20	750±40	740±50	830±40	820±30
KW/BW (mg/g)	4.4±0.2	4.6±0.1	4.2±0.1	4.3±0.1	4.2±0.1	4.4±0.1	4.2±0.1	4.2±0.1
MBP (mmHg)								
Before-exp	146±7*,†	141±7*,†	143±4*,†	144±3*,†	119±3	118±4	118±2	119±2
During-exp	143±6*,†	138±8*,†	138±3*,†	137±3*,†	115±2	112±4	117±2	115±2
n	6	6	7	7	7	7	6	6

Values are mean±SEM. Sham, the sham-operated rats; DN, the denervated rats; Hct, hematocrit; before-exp, the value measured just before the experiment started; during-exp, the average during the experiment period; BW and KW, body and kidney weight, respectively; MBP, mean blood pressure. *$p<0.05$ *vs*. WKY sham group. †$p<0.05$ *vs*. WKYpch1.0 sham group.

Renal Norepinephrine Content

At the end of the experiment and 3.5 h after the initiation of the denervation procedures, the operated left kidney was rapidly removed for the analysis of the NE content. The right kidney was concomitantly harvested and used as a control. Both kidneys were rapidly decapsuled, weighed and placed in an ice-cold solution containing 0.4 Eq/L perchloric acid–0.1% EDTA sodium metabisulfite. The kidney samples were then stored at −70°C until measurement. For the NE measurement, the samples were homogenized on ice and the protein was removed by centrifugation. The supernatants were then applied to a full-automatic catecholamine analyzer (HLC-725CA II; Tosoh Inc., Tamaguchi, Japan) (*19*). The results were represented as nmol/g kidney weight (g KW).

The NE turnover rate was calculated according to the method originally described by Brodie *et al*. (*20*) and used more recently by Caplea *et al*. (*5*).

Statistical Analysis

Values are expressed as the mean±SEM. Student's *t*-test was used for sham *vs*. denervation comparisons for each of the four strains. Analysis of variance, with Scheffe's post-hoc test applied when appropriate, was performed for the comparison of the four strains under the sham-treated conditions. Statistic analysis was conducted with StatView (SAS Institute Inc., Cary, USA). Values of $p<0.05$ were considered to be statistically significant.

Results

In both the sham-operated and the denervated groups, the ages and hematocrit levels were not significantly different among the four rat strains (Table 1). The hematocrit was stable throughout the experiment. The kidney weight and the body weight were comparable between the rats with a common genetic background, while both weights were significantly smaller in the two SHRSP-based strains than in WKY. The ratio of the kidney weight to the body weight, however, was not significantly different among the strains.

Mean blood pressure (MBP) was not affected by the unilateral renal denervation (Table 1). Further, no significant difference in MBP was observed between SHRSP and SHRSPwch1.0, nor between WKY and WKYpch1.0 at the ages used in this study. By contrast, the MBP was significantly higher in the two SHRSP-based strains than in the WKY-based strains even at this age (Table 1).

As illustrated in Fig. 1A, the NE content in the sham-operated kidney was greater in SHRSP than in WKY, which was consistent with previous reports (*21*). The renal NE contents in the two reciprocal congenic strains were between those in SHRSP and WKY. The difference was statistically significant both between SHRSPwch1.0 and SHRSP, and between WKYpch1.0 and WKY. This observation clearly indicated that the chr-1 QTL had a substantial effect on the renal NE content. The NE level in the sham-operated left kidneys was comparable with that in the right ones, indicating that this experimental manipulation *per se* did not affect the RSNA (data not shown). To estimate the effects of anesthesia, the renal NE contents were determined in the rats euthanized with carbon dioxide. The results from kidneys harvested immediately after euthanasia confirmed the inter-strain difference in the renal NE content (179.3±13.6, 108.5±5.1, 88.6±7.3 and 61.0±3.2 nmol/g KW in the SHRSP, SHRSPwch1.0, WKYpch1.0 and WKY, respectively; $n=5$).

Figure 1 summarizes the parameters for the renal function in the four strains of rats with or without denervation. Under the innervated condition (see open columns in Fig. 1), significant differences between WKY and SHRSP were observed

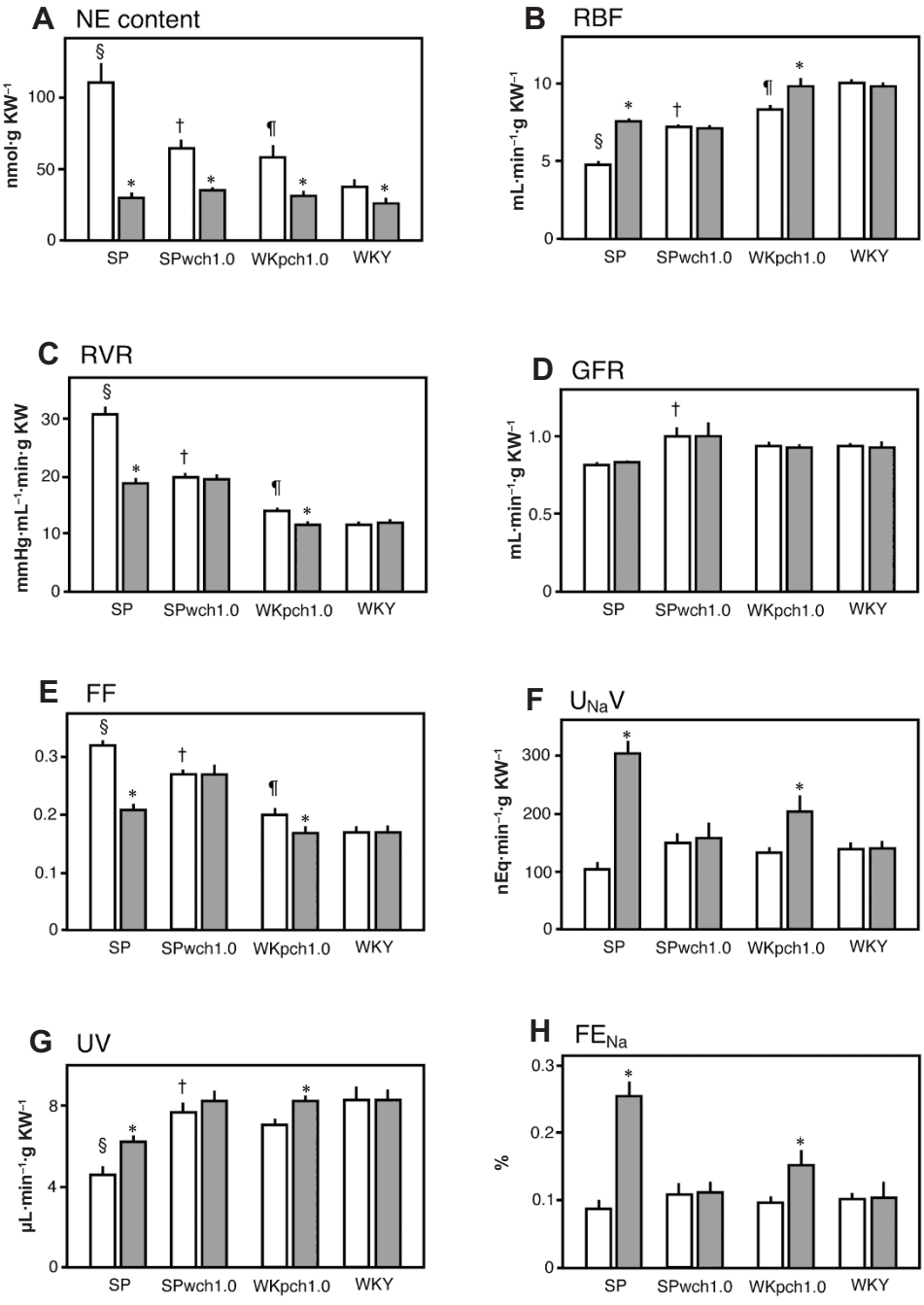

Fig. 1. *The renal NE content and renal functional parameters in the reciprocal congenic and the parental strains with and without renal denervation. The columns and the vertical bars represent the mean and the standard error of the mean. The open and shaded columns represent the sham-operated and denervated kidney, respectively. RBF, renal blood flow; RVR, renal vascular resistance; GFR, glomerular filtration rate; FF, filtration fraction; $U_{Na}V$, urinary sodium excretion; UV, urine volume; FE_{Na}, fractional excretion of sodium; SP, SHRSP; SPwch1.0, SHRSPwch1.0. See text for the abbreviations of the strains. *$p < 0.05$, significantly different from the corresponding sham-operated control. §$p < 0.05$, sham-operated SHRSP vs. sham-operated WKY. †$p < 0.05$, sham-operated SHRSPwch1.0 vs. sham-operated SHRSP. ¶$p < 0.05$, sham-operated WKYpch1.0 vs. sham-operated WKY.*

in renal blood flow (RBF), RVR, filtration fraction (FF) and UV (§ in Fig. 1), which was consistent with previous reports (*22*). No difference in urinary sodium excretion ($U_{Na}V$) or fractional excretion of sodium (FE_{Na}) was observed between

Table 2. Effects of the Denervation on the Renal Vascular and Tubular Functions

	SHRSP	SHRSPwch1.0	WKYpch1.0	WKY
Chr-1 QTL	*SHRSP*	*WKY*	*SHRSP*	*WKY*
Genetic background	*SHRSP*	*SHRSP*	*WKY*	*WKY*
MBP	no change	no change	no change	no change
RVR	↓	no change	↓	no change
GFR	no change	no change	no change	no change
FE_{Na}	↑	no change	↑	no change

Increase (↑), decrease (↓) and no change in each parameter due to the denervation are indicated. SHRSP, stroke-prone spontaneously hypertensive rat; WKY, Wistar-Kyoto rat; chr-1, chromosome 1; QTL, quantitative trait locus; MBP, mean blood pressure; RVR, renal vascular resisitance; GFR, glomerular filtration rate; FE_{Na}, urinary sodium excretion.

the two parental strains, indicating that the sodium handling in SHRSP was comparable with that in WKY under the innervated condition.

The exchange of the chr-1 QTL region between the parental strains elicited significant reciprocal changes in RBF, RVR and FF († and ¶ in Fig. 1). By contrast, GFR and UV were significantly different only between SHRSP and SHRSPwch1.0. Since no significant difference in GFR was observed between the two parental strains, the physiological importance of the greater GFR in SHRSPwch1.0 is not clear at the moment.

Under the denervation (shaded columns in Fig. 1), the renal NE content was significantly reduced in all four strains (Fig. 1A). As such, the calculated NE turnover rates were 42.5±6.1, 14.6±2.8, 17.5±3.3 and 6.9±0.8 nmol/g KW/h for SHRSP, SHRSPwch1.0, WKYpch1.0 and WKY, respectively. Between the innervated and the denervated conditions, however, significant changes of RBF, RVR, FF, UV, $U_{Na}V$ and FE_{Na} were observed only in SHRSP and WKYpch1.0 (✻ in Fig. 1). The denervation did not cause significant changes in GFR in any of the four rat strains. The patterns of response to the sympathetic denervation in the studied strains are summarized in Table 2.

Discussion

In this communication, we conducted a comprehensive study to evaluate the effects of the chr-1 QTL region on renal functions in the reciprocal congenic strains between SHRSP and WKY. This extended our previous observation on adult WKY and WKYpch1.0 substantially (*22*) by indicating that, even in the early developing stage of hypertension, the alteration of the renal response to the sympathetic regulation was largely dependent on the target QTL region and not on the genetic background of the rats.

To avoid secondary BP effects, including the renal sclerosis found in adult SHRSP (*23*, *24*), we employed relatively young rats in the experiment. However, due to the technical difficulties posed by the small body sizes of very young rats, we used rats of 6 to 8 weeks of age. At this age, SHRSP rapidly develop hypertension (*15*, *16*). Accordingly, a significant difference in BP was observed between the SHRSP-based and the WKY-based strains, while BP was not different between the two SHRSP-based strains nor between the two WKY-based strains. Consequently, the effects of different BP could at least be excluded when making comparisons between SHRSP and SHRSPwch1.0 and between WKY and WKYpch1.0 under our experimental conditions (Table 1). In this study, we therefore focused on the reciprocal changes in phenotypes observed between the two pairs of strains, WKY *vs*. WKYpch1.0 and SHRSP *vs*. SHRSPwch1.0.

The major findings of the study were: 1) the congenic transfer of the QTL region influenced the renal NE content, RBF, RVR, FF and UV; and 2) the effects of the denervation on the renal parameters were observed only in SHRSP and WKYpch1.0, both of which bear the SHRSP-fragment of the chr-1 QTL region.

The renal NE content was greater in SHRSP than WKY, which was consistent with the previous findings (*21*). Our study clearly illustrated a major effect of the chr-1 QTL on this phenotype, which was independent of the genetic background. This contrasted well with the case of BP *per se*; in the adult congenic strains, BP was only marginally different between WKYpch1.0 and WKY (*7*, *22*), but was significantly lower in SHRSPwch1.0 compared to SHRSP (*11*, *12*). This means that BP is a more "remote" phenotype under more complex control by a number of genetic and environmental factors. This in turn suggests that the renal NE content may be a good intermediate phenotype for the chr-1 QTL (*10*).

The renal NE content represented the density and/or the activity of the sympathetic nerves in the kidney. In this study, we obtained the kidney samples for NE measurement approximately 3.5 h after the denervation (*i.e.*, at the end of the physiological experiment under the denervated condition). Under this condition, the denervation elicited significant decreases of the NE levels in all four of the strains compared to the level of the sham-operated controls. Caplea *et al*. showed in their recent communication that the chemical denervation caused a time-dependent log-linear reduction of the renal NE content up to 6 h, which could be used to calculate the NE turnover rate (*5*). Based on their work, the NE turnover rates were provisionally calculated in the present study. Although we did not confirm the linearity of the reduction in our experiments,

the estimates for SHRSP and WKY were in good agreement with those reported previously (*16*, *21*). These results suggested that RSNA was different among the four strains (*25*). The idea that RSNA differed among the strains was further supported by the findings of Iigaya *et al*., who observed an elevated neuronal activity in the rostral ventrolateral medulla, the sympathetic center, of WKYpch1.0 when compared with WKY (*26*). Further, Yamazato *et al*. actually indicated that RSNA was greater in WKYpch1.0 than WKY (*9*). These observations together with our results suggest the genetic influence of the chr-1 QTL on RSNA.

Recent reports have indicated that the augmented superoxide production in the brain stem might be responsible for the activation of the SNS and contribute to the elevation of BP in SHRSP (*27–29*). It would be of interest to measure the oxidative stress and the NADPH oxidase activity in the brain stem of the reciprocal congenic strains studied in the present report.

The difference in RVR probably reflected the difference of the SNS activity among the strains; NE is a potent vasoconstrictor and has been shown to be involved in the increased RVR in SHR (*30*). Further, the aggravated renal vasoconstriction is suggested to be a key pathomechanism for the development of hypertension in SHR (*31*, *32*) and SHRSP (*33*). Consistent with these reports, the RVR was greater in SHRSP and SHRSPwch1.0, both of which were phenotypically hypertensive, than in WKYpch1.0 and WKY. Although the BP was similar between the two pairs of strains at the age used in the study, the RVR was 36±3% lower in SHRSPwch1.0 than in SHRSP, while it was 17±2% higher in WKYpch1.0 than in WKY. This difference in RVR at this age might contribute to the BP difference observed in the older rats (*7*, *11*, *22*). These observations again suggested the complex makeup of the phenotype of BP in these strains.

The kidney participates in the genesis of hypertension through the excessive retention of water and sodium due to the enhanced tubular reabsorption (*34*). In this study, however, no inter-strain difference was identified in either $U_{Na}V$ or FE_{Na} under the innervated condition. Instead, the denervation elicited significant increases in $U_{Na}V$ and FE_{Na} only in SHRSP and WKYpch1.0, suggesting that the sympathetic drive of the sodium reabsorption in the renal tubules was greater in the strains carrying the SHRSP-derived fragment of the QTL. The effect of the renal denervation in SHRSP was consistent with the previous observation in SHR (*35*), and the lack of natriuresis after the acute denervation in WKY also echoed previous studies (*22*, *36*, *37*). Such a difference in the tubular responsiveness to the denervation could not be explained fully by the greater RSNA in SHRSP and the congenic strain, because WKYpch1.0 did not necessarily show a greater RSNA than SHRSPwch1.0. The fact that multiple systems coordinately regulate the tubular function (*38*) implies that the chr-1 QTL modulated the relative importance of the SNS in the regulatory systems of the tubular function. Alternatively, a currently unknown system in the WKY may compensate for the acute withdrawal of neural control and account for the unaltered tubular function in the denervated WKY and SHRSPwch1.0. Exactly the same "lack of response" to the denervation was observed in RVR (Fig. 1C), and at present we are limited to interpreting these identical phenomena in the same way.

If the levels of renal sodium absorption were identical under the innervated condition, how did renal sodium contribute to the difference in BP among the four strains studied? There are two possible interpretations. 1) Renal sodium did not make any *bona fide* contribution. In the QTL region, the genes responsible for hypertension and for the tubular responsiveness are separately located. 2) The anesthesia may have affected the neural influence on the renal hemodynamics, as the renal NE contents were higher in the unanesthetized rats than in the anesthetized ones. Conscious rats may have greater sympathetic tone due to a variety of environmental stresses, which may induce larger sodium reabsorption and later result in hypertension in the adult rats. Although the latter hypothesis is an attractive explanation for the difference in BP between SHRSP and SHRSPwch1.0, it can not provide a satisfactory explanation for the marginal BP difference between WKY and WKYpch1.0. Additional genetic components in the background genome may be essential to obtain an unequivocal increase of BP in WKYpch1.0. Regarding the complexity of the BP regulation *in vivo*, it will be necessary to carefully evaluate the intermediate phenotypes before reaching a conclusion.

As the congenic region is still quite large and expected to contain hundreds of genes, it is extremely difficult to select the "right" candidate genes. The present findings should provide an important clue to selecting proper candidate genes in the region. In this respect, a group of genes potentially affecting the structure and/or the activity of the SNS, such as *Calca*, *Arix*, *Homer-2* and *Arrb1*, would be interesting candidates. Nevertheless, it is important that the present study proposed a target organ through the physiological study of congenic rats. When comprehensive transcriptome analyses are planned, it is important to select proper target organs and proper development stages to study, which is not always an easy task in hypertension (*39*). This ambiguity of the primary target organs may hamper proper interpretations of comprehensive transcriptome analysis (*40*). In this regard, the present study provides useful information by suggesting that the organs involved in the SNS are good targets for transcriptome analysis.

A combination of two strategies is essential to track down the genes responsible for hypertension: 1) the use of a series of congenic substrains that carry a smaller segment of the QTL region, and 2) the exploration of the intermediate phenotypes in the congenic strains. Applying this methodology, a region of 12.8 cM on chr-1 that is responsible for impaired autoregulation of RBF in a hypertensive rat was recently located (*41*). Future studies using the chr-1 congenic substrains and focusing on the sympathorenal interaction may

thus be of help in the search for causative genes of hypertension.

References

1. Mancia G, Grassi G, Giannattasio C, Seravalle G: Sympathetic activation in the pathogenesis of hypertension and progression of organ damage. *Hypertension* 1999; **34**: 724–728.
2. Lee RM, Borkowski KR, Leenen FH, Tsoporis J, Coughlin M: Combined effect of neonatal sympathectomy and adrenal demedullation on blood pressure and vascular changes in spontaneously hypertensive rats. *Circ Res* 1991; **69**: 714–721.
3. Judy WV, Farrell SK: Arterial baroreceptor reflex control of sympathetic nerve activity in the spontaneously hypertensive rat. *Hypertension* 1979; **1**: 605–614.
4. Gattone VH 2nd, Evan AP, Overhage JM, Severs WB: Developing renal innervation in the spontaneously hypertensive rat: evidence for a role of the sympathetic nervous system in renal damage. *J Hypertens* 1990; **8**: 423–428.
5. Caplea A, Seachrist D, Daneshvar H, Dunphy G, Ely D: Noradrenergic content and turnover rate in kidney and heart shows gender and strain differences. *J Appl Physiol* 2002; **92**: 567–571.
6. Winternitz SR, Katholi RE, Oparil S: Role of the renal sympathetic nerves in the development and maintenance of hypertension in the spontaneously hypertensive rat. *J Clin Invest* 1980; **66**: 971–978.
7. Cui ZH, Ikeda K, Kawakami K, Gonda T, Nabika T, Masuda J: Exaggerated response to restraint stress in rats congenic for the chromosome 1 blood pressure quantitative trait locus. *Clin Exp Pharmacol Physiol* 2003; **30**: 464–469.
8. Cui ZH, Ikeda K, Kawakami K, Gonda T, Masuda J, Nabika T: Exaggerated response to cold stress in a congenic strain for the quantitative trait locus for blood pressure. *J Hypertens* 2004; **11**: 2103–2109.
9. Yamazato M, Ohya Y, Nakamoto M, *et al*: Sympathetic hyperreactivity to air-jet stress in the chromosome 1 blood pressure quantitative trait locus congenic rats. *Am J Physiol Regul Integr Comp Physiol* 2006; **290**: R709–R714.
10. Nabika T, Kobayashi Y, Yamori Y: Congenic rats for hypertension: how useful are they for the hunting of hypertension genes? *Clin Exp Pharmacol Physiol* 2000; **27**: 251–256.
11. Yao H, Cui ZH, Masuda J, Nabika T: Congenic removal of a QTL for blood pressure attenuates infarct size produced by middle cerebral artery occlusion in hypertensive rats. *Physiol Genomics* 2007; **30**: 69–73.
12. Kato N, Nabika T, Liang YQ, *et al*: Isolation of a chromosome 1 region affecting blood pressure and vascular disease traits in the stroke-prone rat model. *Hypertension* 2003; **42**: 1191–1197.
13. Mashimo T, Nabika T, Matsumoto C, *et al*: Aging and salt-loading modulate blood pressure QTLs in rats. *Am J Hypertens* 1999; **12**: 1098–1104.
14. Serikawa T: Colourful history of Japan's rat resources. *Nature* 2004; **429**: 15 (Letter).
15. Ikeda K, Nara Y, Nabika T, *et al*: Genetic factors regulate the rise in blood pressure in F2 generation crossed between stroke-prone spontaneously hypertensive rats and Wistar-Kyoto rats. *Clin Exp Pharmacol Physiol* 1991; **18**: 593–597.
16. Patel KP, Kline RL, Mercer PF: Noradrenergic mechanisms in the brain and peripheral organs of normotensive and spontaneously hypertensive rats at various ages. *Hypertension* 1981; **3**: 682–690.
17. Fuhr J, Kaczmarczyk J, Kruttgen CD: A simple colorimetric method of inulin determination in renal clearance studies on metabolically normal subjects and diabetics. *Klin Wochenschr* 1955; **33**: 729–730.
18. Wang T, Takabatake T: Effects of vasopeptidase inhibition on renal function and tubuloglomerular feedback in spontaneously hypertensive rats. *Hypertens Res* 2005; **28**: 611–618.
19. Kai T, Shimada S, Sugimura K, *et al*: Tissue-localized angiotensin II enhances cardiac and renal disorders in Tsukuba hypertensive mice. *J Hypertens* 1998; **16**: 2045–2049.
20. Brodie BB, Costa E, Dlabac A, Neff NH, Smookler HH: Application of steady state kinetics to the estimation of synthesis rate and turnover time of tissue catecholamines. *J Pharmacol Exp Ther* 1966; **154**: 493–498.
21. Fujita T, Sato Y: Role of hypothalamic-renal noradrenergic systems in hypotensive action of potassium. *Hypertension* 1992; **20**: 466–472.
22. Wang T, Kobayashi Y, Nabika T, Takabatake T: Enhanced sympathetic control of renal function in rats congenic for the hypertension-related region on chromosome 1. *Clin Exp Pharmacol Physiol* 2005; **32**: 1055–1060.
23. Nakaya H, Sasamura H, Kitamura Y, *et al*: Effects of angiotensin inhibitors on renal injury and angiotensin receptor expression in early hypertensive nephrosclerosis. *Hypertens Res* 1999; **22**: 303–312.
24. Nakaya H, Sasamura H, Hayashi M, Saruta T: Temporary treatment of prepubescent rats with angiotensin inhibitors suppresses the development of hypertensive nephrosclerosis. *J Am Soc Nephrol* 2001; **12**: 659–666.
25. Esler M, Jennings G, Korner P, *et al*: Assessment of human sympathetic nervous system activity from measurements of norepinephrine turnover. *Hypertension* 1988; **11**: 3–20.
26. Iigaya K, Kumagai T, Nabika T, *et al*: Chromosome 1 quantitative trait locus in congenic strains induces different response of rostral ventrolateral medulla neurons to angiotensin II. Proceedings of the 12th International Symposium on SHR. Kyoto, 2006, p 27.
27. Hirooka Y, Kimura Y, Nozoe M, Sagara Y, Ito K, Sunagawa K: Amlodipine-induced reduction of oxidative stress in the brain is associated with sympatho-inhibitory effects in stroke-prone spontaneously hypertensive rats. *Hypertens Res* 2006; **29**: 49–56.
28. Kishi T, Hirooka Y, Kimura Y, Ito K, Shimokawa H, Takeshita A: Increased reactive oxygen species in rostral ventrolateral medulla contribute to neural mechanisms of hypertension in stroke-prone spontaneously hypertensive rats. *Circulation* 2004; **109**: 2357–2362.
29. Ishiguro K, Sasamura H, Sakamaki Y, Itoh H, Saruta T: Developmental activity of the renin-angiotensin system during the "critical period" modulates later L-NAME–induced

hypertension and renal injury. *Hypertens Res* 2007; **30**: 63–75.
30. DiBona GF, Kopp UC: Neural control of renal function. *Physiol Rev* 1997; **77**: 75–197.
31. Gebremedhin D, Fenoy FJ, Harder DR, Roman RJ: Enhanced vascular tone in the renal vasculature of spontaneously hypertensive rats. *Hypertension* 1990; **16**: 648–654.
32. Uyehara CF, Gellai M: Impairment of renal function precedes establishment of hypertension in spontaneously hypertensive rats. *Am J Physiol Regul Integr Comp Physiol* 1993; **265**: R943–R950.
33. Berecek KH, Schwertschlag U, Gross F: Alterations in renal vascular resistance and reactivity in spontaneous hypertension of rats. *Am J Physiol Heart Circ Physiol* 1980; **238**: H287–H293.
34. Guyton AC, Coleman TG, Cowley AV Jr, Scheel KW, Manning RD Jr, Norman RA Jr: Arterial pressure regulation. Overriding dominance of the kidneys in long-term regulation and in hypertension. *Am J Med* 1972; **52**: 584–594.
35. Beach RE: Renal nerve-mediated proximal tubule solute reabsorption contributes to hypertension in spontaneously hypertensive rats. *Clin Exp Hypertens A* 1992; **14**: 685–697.
36. Rudd MA, Grippo RS, Arendshorst WJ: Acute renal denervation produces a diuresis and natriuresis in young SHR but not WKY rats. *Am J Physiol Renal Physiol* 1986; **251**: F655–F661.
37. Takabatake T, Ushiogi Y, Ohta K, Hattori N: Attenuation of enhanced tubuloglomerular feedback activity in SHR by renal denervation. *Am J Physiol Renal Physiol* 1990; **258**: F980–F985.
38. Cowley AW Jr, Roman RJ, Krieger JE: Pathways linking renal excretion and arterial pressure with vascular structure and function. *Clin Exp Pharmacol Physiol* 1991; **18**: 21–27.
39. Hubner N, Yagil C, Yagil Y: Novel integrative approaches to the identification of candidate genes in hypertension. *Hypertension* 2006; **47**: 1–5.
40. Jaluria P, Konstantopoulos K, Betenbaugh M, Shiloach J: A perspective on microarrays: current applications, pitfalls, and potential uses. *Microb Cell Fact* 2007; **6**: 4.
41. Lopez B, Ryan RP, Moreno C, *et al*: Identification of a QTL on chromosome 1 for impaired autoregulation of RBF in fawn-hooded hypertensive rats. *Am J Physiol Renal Physiol* 2006; **290**: F1213–F1221.