Differential Development of Vascular and Cardiac Hypertrophy in Genetic Hypertension
Relation to Sympathetic Function

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We compared blood pressure, hindquarter vascular resistance properties, left ventricular weight, and norepinephrine kinetics, in spontaneously hypertensive rats (SHR) and weight-matched normotensive Wistar-Kyoto (WKY) rats at 4, 9, 14, 20, 30, and 50 weeks of age. At 4 weeks, systolic and mean blood pressure measurements were the same in both strains, but the vascular resistance of the fully dilated hindquarter bed was significantly higher in SHR than in WKY rats, with a much larger difference during maximum constriction. Plots of resistance at maximum dilatation and at maximum constriction against body weight suggest that a component of the increase in vascular muscle mass in SHR occurred in the neonatal period preceding hypertension followed by a later component related to the rise in blood pressure. By contrast, left ventricular hypertrophy was minimal at 4 weeks and most of its development paralleled the rise in blood pressure. Sympathetic activity, assessed by norepinephrine fractional rate constant, was higher in SHR than in WKY rats in the left ventricle and kidney through most of the period between 4 and 50 weeks, but was similar in both strains in the muscle bed. This pattern of sympathetic activity will accentuate hypertension once cardiac and vascular hypertrophy are fully established. In all regions, norepinephrine tissue concentration was higher in young SHR and could potentiate the trophic effects of growth factors in early vascular hypertrophy. We suggest that the initial (primary) component of vascular hypertrophy precedes the rise in blood pressure and may be critical in the pathogenesis of hypertension. Possible reasons for the short delay in the rise in blood pressure in young SHR, once the vascular “amplifier” has been established, include high vascularity, immaturity of smooth muscle, and delay in the development of left ventricular hypertrophy. (Hypertension 1989;14:191-202)

Folksow1,2 and colleagues were the first to demonstrate the importance of structural changes associated with hypertrophy of the cardiovascular musculature in hypertension. In the spontaneously hypertensive rat (SHR), they showed that, as a result of the increase in wall thickness and narrowing of the lumen, changes in vascular resistance were “amplified” when compared with the responses of Wistar-Kyoto (WKY) normotensive rats.3 The concentrically hypertrophied left ventricle also has amplifier properties, which help in the maintenance of cardiac output against a higher pressure load.4,5 In chronic secondary hypertension due to renal artery stenosis, the cardiovascular amplifiers contribute about 70% to the maintenance of the elevated blood pressure (BP) and this is probably similar in primary hypertension.6

In secondary hypertension, the hypertrophy is clearly a consequence of the elevated BP, and it has generally been assumed that this is also the case in primary hypertension. There has been speculation whether, in primary hypertension, the early development of cardiovascular hypertrophy could initiate the rise in BP.7-9 This hypothesis is not readily testable in humans, because of the difficulty of performing an adequate longitudinal study. Such a study is feasible in SHR, where the time course of the BP changes has been documented in great detail,10 but the range of ages in previous longitudinal studies on the development of cardiac and vascular hypertrophy has been too small for an adequate sequential analysis (see References 10-14). For example, we still do not know whether in SHR the vascular and cardiac hypertrophy develop over the same age span.

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Present evidence suggests that sympathetic nervous system is important in the development of hypertension in SHR.\textsuperscript{2,15-19} Folkow et al\textsuperscript{15} showed that immunosympathectomy in very young rats attenuated the development of hypertension. Recently Lee et al,\textsuperscript{20} using an improved ablation technique, found that hypertension could be prevented completely. One way in which increased sympathetic neural activity in SHR could elevate BP is through a net increase in vasoconstrictor tone associated with a low threshold of the “defense” reaction,\textsuperscript{1,2,16,17} so that the hypertrophy will occur as a direct consequence of the rise in BP. An alternative hypothesis is that the sympathetic nervous system exerts a local trophic role in the development of cardiovascular hypertrophy.\textsuperscript{7,18,19}

Such a role presupposes elevation of regional norepinephrine tissue concentration [NE] and high turnover at the time of development of cardiovascular hypertrophy. However, there has been only one detailed longitudinal study by Patel et al,\textsuperscript{21} who determined the fractional rate constant of norepinephrine, but did not relate it to the development of cardiovascular hypertrophy.

In the present experiments, we examined the time course of development of cardiac and vascular hypertrophy and how this related 1) to the development of hypertension and 2) to the various measures of regional sympathetic function. Accordingly, we compared the vascular properties of the hindquarters in SHR and WKY rats and measured the left ventricular and right ventricular weights at six time points over an age span of 4–50 weeks of age. Indexes of regional sympathetic function were obtained in a parallel study in skeletal muscle vessels, heart, and kidney.

Materials and Methods

Animals and Blood Pressure Measurements

Male SHR and WKY rats (Animal Resources Centre, Perth, Western Australia) were housed in groups of three rats to a cage in a room with a 12-hour light/dark cycle and an ambient temperature of 22–24° C, with food and water provided ad libitum. We had intended to take special care to weight-match pairs of SHR and WKY rats at the different ages (see below). However, no special selection procedure turned out to be necessary, and in all experiments the difference in body weight was less than 5%. The rats came originally from the National Institutes of Health in the United States and the strain of WKY rats that we used was that in which the age–body weight relation was similar to that of SHR.\textsuperscript{22}

Systolic BP and heart rate were measured in conscious rats with an automated multichannel system (IITC Life Science Instruments, Woodland Hills, California, 24 channel) that used tail cuffs and photoelectric sensors to detect the tail pulses. A test chamber maintained at 27–28° C was used to place rats in holders appropriate for their body weight. Over the last 2 weeks of each time period, there was a minimum of four BP recording sessions: the first two were regarded as training sessions and the data was discarded; the systolic BP was taken as the mean of the last two sessions (4–6 sets of measurements) recorded over the last 2–3 days. In addition, mean arterial pressure was measured directly in a separate series of 4-week-old SHR and WKY rats which were anesthetized with methohexitane anesthesia (Brietal, Eli Lilly, Indianapolis, Indiana, 30 mg/kg i.p.) and were cannulated with aortic catheters 4–7 days before recording, as described by Head and McCarty.\textsuperscript{23} In these rats pressure was recorded for a period of 3–4 hours on the day of the study.

Hindquarter Preparation

Hindquarter perfusions were carried out in pairs of age- and weight-matched SHR and WKY rats by using a slight modification of the method of Folkow et al.\textsuperscript{24} The system included a common reservoir for both rats, an injection port and bubble trap/mixing chamber in line with a peristaltic pump (Minipuls 2, Gilson Medical Elec., Inc., Middleton, Wisconsin). The perfuse consisted of 1.5% dextran T-40 (Pharmacia, Upsala, Sweden) in Tyrode’s solution, which was aerated with 95% O\textsubscript{2} and 5% CO\textsubscript{2}. The composition (mM) of the Tyrode’s solution was: KCl 20, CaCl\textsubscript{2} 2H\textsubscript{2}O 32.3, MgCl\textsubscript{2} 6H\textsubscript{2}O 5.1, Na\textsubscript{2}H\textsubscript{2}PO\textsubscript{4} 2H\textsubscript{2}O 6.2, NaHCO\textsubscript{3} 100, glucose 100, and NaCl 800 mg/100 ml fluid. The perfuse in the reservoir was warmed, and we used a heating pad to maintain rectal temperature at 36–38° C.

The rats were anesthetized with sodium pentobarbital (60 mg/kg i.p.) and the lower abdominal aorta was exposed at the iliac bifurcation through a midline abdominal incision. After heparinization (1,000 IU/kg), the aorta was cannulated with a 19- or 23-gauge needle, with the tip immediately proximal to the iliac bifurcation. The middle caudal and the caudal mesenteric arteries, which are close to the bifurcation, were ligated and not perfused. Flow of perfusate was started immediately after transection of the spinal cord and vena cava adjacent to the cannula entrance. The heart and other tissues were rapidly removed for catecholamine assays (see below). About 5 minutes after the start of perfusion, when the vasculature had been flushed clear of blood, papaverine HCl (12 mg/ml) was given as a bolus (1.5 mg/100 g body wt) and the perfusion pressure at maximum vasodilatation (P\textsubscript{Pmax} di) remained constant over a 20-minute washout period, indicating that edema was minimal. Subsequently, we obtained cumulative concentration–vascular resistance (≡PP) response curves to the \(\alpha\)-adrenergic receptor agonist methoxamine (0.4–300 \(\mu\)g/ml, Burroughs Wellcome, Research Triangle Park, North Carolina) by stepwise increases in the infusion rate (0.66–66 \(\mu\)l/min i.v.) from a syringe pump (Perfusor IV, Braun, Munich, FRG) contain-
The rats were perfused at a constant flow of 4 ml/min/100 g body wt, which in adult rats corresponds to a flow of 10 ml/min/100 g hindquarter wt.24 The relation between hindquarter weight and body weight was closely similar in SHR and WKY rats, but differed at different stages of growth (Table 1). Thus, in low body weight (young) rats of each strain, the hindquarter weight was a lower fraction of the total body weight than in adult rats. For example, in a 50-g rat, hindquarter weight was 26.5±0.4% body wt, whereas in a 450-g rat, hindquarter weight averaged 38.8±0.6% body wt, which was closer to the value of 40% that Folkow et al24 reported in adult rats.

The rats were anesthetized with sodium pentobarbital (60 mg/kg i.p.) and killed by exsanguination.25 The left ventricle plus septum, the kidney, and the forelimb muscle (caput longum of triceps brachii) were quickly dissected from the other tissues, weighed, rapidly frozen in liquid nitrogen, and then stored at −80°C until analysis.

Norepinephrine was extracted from the tissue by a slight modification of a procedure described previously.26 Briefly, the tissues were homogenized (Polytron PT 20) in ice-cold 0.4 M perchloric acid (4.0 ml,

Table 1. Linear Regression Equations in Spontaneously Hypertensive Rats and Wistar-Kyoto Rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Regression equation</th>
<th>SE_β</th>
<th>r</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW-HQW relation</td>
<td>SHR+WKY 4-40: HQW=24.97+0.031 BW</td>
<td>0.0023</td>
<td>0.95</td>
</tr>
<tr>
<td>PP-flow relation</td>
<td>SHR 4: PP_max_eq=10.07+0.72 HQQ</td>
<td>0.088</td>
<td>0.85</td>
</tr>
<tr>
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<td>WKY 4: PP_max_eq=6.83+0.72 HQQ</td>
<td>0.047</td>
<td>0.94</td>
</tr>
<tr>
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<td>SHR 14: PP_max_eq=13.45+1.48 HQQ</td>
<td>0.181</td>
<td>0.91</td>
</tr>
<tr>
<td></td>
<td>WKY 14: PP_max_eq=9.84+1.48 HQQ</td>
<td>0.144</td>
<td>0.90</td>
</tr>
<tr>
<td></td>
<td>SHR 27: PP_max_eq=12.89+1.43 HQQ</td>
<td>0.387</td>
<td>0.95</td>
</tr>
<tr>
<td></td>
<td>WKY 27: PP_max_eq=8.70+1.43 HQQ</td>
<td>0.543</td>
<td>0.91</td>
</tr>
<tr>
<td>PP-BW relation</td>
<td>SHR 40-50: PP_max_eq=13.34+0.046 BW</td>
<td>0.0039</td>
<td>0.91</td>
</tr>
<tr>
<td></td>
<td>WKY 40-50: PP_max_eq=11.33+0.034 BW</td>
<td>0.0039</td>
<td>0.85</td>
</tr>
<tr>
<td></td>
<td>SHR 40-50: PP_max_eq=225+0.319 BW</td>
<td>0.32</td>
<td>0.89</td>
</tr>
<tr>
<td></td>
<td>WKY 40-50: PP_max_eq=159+0.274 BW</td>
<td>0.27</td>
<td>0.85</td>
</tr>
</tbody>
</table>

n, number of rats; SE_β, standard error of regression coefficient; r, correlation coefficient; BW, body weight (g); HQW, hindquarter weight (% of BW); SHR, spontaneously hypertensive rats; WKY, Wistar-Kyoto rats; PP, perfusion pressure; max dl, maximum dilatation; HQQ, hindquarter flow (ml/min/100 g HQW); max con, maximum constriction.

*For PP-flow curves, number of data points were, in order, spontaneously hypertensive rats, Wistar-Kyoto rats: 4 weeks, 32 and 28; 14 weeks, 17 and 24; 27 weeks, 12 and 12.
0.4 M) containing 3,4-dihydroxybenzylamine (DHBA). After the homogenate was centrifuged to pellet-precipitated proteins, the pH of the supernatant was adjusted to pH 8.6 with 1 M Tris buffer 8.6 containing ethylenediaminetetraacetic acid (EDTA, 10 mg%). Norepinephrine was then extracted from this solution onto 300 mg alumina (active/neutral form, Merck Sharp & Dohme, West Point, Pennsylvania), which was washed twice with water. Norepinephrine was eluted with 0.4 M perchloric acid.

Quantitation of norepinephrine was carried out by high-performance liquid chromatography (HPLC), using either fluorometric (Schoeffel, FS-970) or electrochemical (LC-4A controller, Bioanalytical Sys., Inc., West Lafayette, Indiana) detection.22 Fluorescence of catecholamines was measured through a Corning (Corning Glass Works, Corning, New York) 7-60 glass filter (band pass 250–400 nm) on excitation at 200 nm. For electrochemical estimation of the catecholamines, an electrochemical cell with a glassy carbon electrode (TL-5A, Bioanalytical Sys., Inc.) was used, with the potential set at 0.8 V against a Ag/AgCl reference electrode. The use of 0.8 V was validated by comparison of redox curves (detector response vs. oxidation potential) for norepinephrine and for alumina eluates containing norepinephrine extracted from heart, kidney, and muscle tissue. Signals from these detection systems were recorded on a Shimadzu data system (C-R3A Chromatopac, Shimadzu Sci. Instrs., Columbia, Maryland), and norepinephrine was quantified by using peak area ratios relative to the internal standard (DHBA). The liquid chromatographic system consisted of a Varian 5000 Pump equipped with a refrigerated autosampler (AS-48, Bio-Rad Labs., Richmond, California) and an Altex Ultrasphere-ODS column (3 μm; 46×7.5 cm). When fluorescence detection was used, the mobile phase consisted of 10 mM perchloric acid (pH 2). Since with electrochemical detection sensitivity is relatively poor at this pH, the mobile phase for this system consisted of dihydrogen phosphate (0.05 M), sodium citrate (0.05 M), EDTA (2 mM), and octyl sodium sulfate (100 mg%), dissolved in methanol/water (3%/10% vol/vol). The pH of the prepared solution was adjusted to 4.5. Quantitatively the two systems provided identical estimates of norepinephrine in the three tissues.

Norepinephrine Turnover

The rate of norepinephrine synthesis (turnover) can be expressed as $K \times [\text{NE}]$, where $K$ (the fractional rate constant) is the fraction of norepinephrine lost in unit time after suppression of norepinephrine biosynthesis by inhibition of the enzyme tyrosine hydroxylase by a-methyl-DL-p-tyrosine methyl ester HCl (AMPT) and [NE] is the steadystate tissue norepinephrine concentration. $K$ is closely related to sympathetic nerve impulse traffic21,29,30 and was calculated from the rate of decline of the tissue [NE] according to the equation

$$K = \frac{\ln [\text{NE}] - \ln [\text{NE}_{\text{t}}]}{t}$$

where $[\text{NE}_{\text{t}}]$ and $[\text{NE}]$ are the norepinephrine concentrations at zero time and after 4 or 8 hours of inhibition of synthesis with 300 mg/kg AMPT i.p. every 4 hours, in a solution of 60 mg/ml in 0.9% NaCl, which is known to inhibit tyrosine hydroxylase.29,30 For determination of $K$, we used all data points in three subgroups of rats killed at 0, 4, or 8 hours after administration of AMPT, using the slope of the ln [NE]–time regression equation. Norepinephrine turnover is the amount of norepinephrine released per gram of tissue per unit time, and is calculated from the product of $K$ times the endogenous steady-state tissue [NE]. An individual $K$ was estimated in each rat, based on the rate of decline from the animals' own zero time concentration to the average [NE] of the group, after 4 or 8 hours inhibition of synthesis. This method provides a reasonable estimate of the standard error of the mean of the group's norepinephrine turnover rate, and makes allowance for the errors associated with the determination of both $K$ and [NE].

Data Analysis

We found that a logistic function curve described the relation between methoxamine concentration and change in perfusion pressure; a computer program was used to fit the data points using the algorithm of Marquardt.31 Each curve was characterized by the following parameters: 1) $PP_{\text{max}}$; 2) $EC_{50}$, the concentration of methoxamine to reach 50% of the pressure range of the curve between minimum and maximum pressures with methoxamine; 3) the maximum slope of the concentration-response curve with methoxamine, and 4) the range between $PP_{\text{max}}$ and $PP_{\text{min}}$ with methoxamine or Ang II. The slope of the logistic curve depends in part on the range between the two plateaus (i.e., $PP_{\text{max}} - PP_{\text{min}}$) and we therefore also calculated the range-independent (normalized) slope of the logistic function, where the range is regarded as 100% under all conditions.32 The various age and strain differences were compared by one-way analysis of variance and covariance.33 Individual comparisons between strains at particular ages were done using an unpaired Student's $t$ test and, where appropriate, the Mann-Whitney test. A $p<0.05$ was taken to indicate statistical significance.

Results

Age Relation to Body Weight, Blood Pressure, and Heart Rate

The relation between age and body weight was closely similar in SHR and WKY rats (Figure 2). The rate of body weight gain was about 25 g/wk over the period 4–14 weeks, compared with the average increase of about 4 g/wk over the subsequent 36 weeks. The similar body weights in the paired perfusion experiments ensured that, at any
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40
30
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Q 20
-X
10
Q/100g BVV,
ml/mln/100g BW
2 4 6 8
wk
10 15 20 0 5 10
FLOW- ml/min/100a HQW 15 20
FIGURE 1. Left panel, relation of perfusion pressure at maximum dilatation (PPmax_dil) to flow (Q) in isolated hindquarters of 4-week-old spontaneously hypertensive rats (SHR) (n=8), plotted as ml/min/100 g body wt (BW) (left line) and ml/min/100 g hindquarter wt (HQW). In rats of 75.5 g, PPmax corresponds to flows of 4 ml/min/100 g BW and to 14.6 ml/min/100 g HQW. PPmax_dil at a flow of 10 ml/min/100 g HQW lies at the tip of the arrowhead. Right panel, relation of PPmax_dil to flow (ml/min/100 g HQW) in 4-week-old SHR (lower solid line and symbols; n=8) and Wistar-Kyoto (WKY) rats (lower interrupted line and symbols; n=7) and in corresponding pooled data in 14- and 27-week-old SHR (n=10) and WKY rats (n=12). Points are expected means from the regression lines±SEM.

given age, strain differences in vascular resistance properties did not depend on differences in body size.
The difference in systolic BP between the two strains increased progressively during the period of rapid growth between 4 and 14 weeks (Figure 2). The difference was minimal and not significant at 4 weeks (n=5 in each strain). We also performed direct measurements of mean arterial pressure in 4-week-old SHR and WKY rats, which was 113±5 and 110±6 mm Hg, respectively (n=7 and 6; Δ N.S.), in agreement with the systolic BP findings when using tail-cuff measurements. At all times after and including 9 weeks, the systolic BP was higher in SHR than in WKY rats (p<0.0001). In WKY rats the “adult” BP value was reached by about 9 weeks of age, whereas the corresponding value in SHR was reached by about 14 weeks (Figure 2). There was a progressive increase in the difference in systolic BP throughout the period of rapid growth, to a stable difference in the adult of about 75 mm Hg.

In both SHR and WKY rats, heart rate declined with increasing age at a rate of 1–2 beats/min/wk. The rate of decline was similar in both strains, but at all ages the heart rate was higher in SHR than in WKY rats. The average heart rate over all time intervals was 371±38.9 beats/min in SHR and 349±40.3 beats/min in WKY rats (Δ=22; F(1,264)=23.2, p<0.001).

Heart Weight
The left ventricle plus septum weight/body weight ratio and the ratio of right ventricular weight/body weight declined in both SHR and WKY rats from 4 weeks to reach stable values at about 14–20 weeks (Figure 2). At 4 weeks the left ventricle plus septum weight/body weight ratio was only 8% higher in SHR than in WKY rats (p=0.05), rising to 25% at 14 weeks and to a final value of 40%
above the corresponding values in WKY rats after 20 weeks (p<0.001; Figure 2). At no time period was there a significant difference in right ventricular weight/body weight ratio between SHR and WKY rats (Figure 2).

**Hindquarter Vascular Resistance Properties**

**Perfusion pressure–flow relation.** We obtained perfusion pressure–flow curves in the fully dilated vascular bed of 4-, 14-, and 27-week-old SHR and WKY rats (see Table 1 for numbers). In 4-week-old rats of both strains, the relation between PP_{max,di} and flow was linear up to flow rates of about 25 ml/min/100 g hindquarter wt (Figure 1 and Table 1). From analysis of covariance of the regression lines in SHR (n=8) and in WKY rats (n=7), there was no significant strain difference in regression coefficients, but there was a significant difference in intercepts (Table 1, p<0.001); PP_{max,di} was on average 3.2 mm Hg higher in SHR than in WKY rats. The pressure–flow curves in 14- and 27-week-old rats were linear up to flow rates of about 15 ml/min/100 g hindquarter wt. Over this range, the relation was virtually the same at the two time periods, so that we pooled the results from both groups (Figure 1). Again, there were no significant differences in regression coefficients but the intercepts differed, with PP_{max,di} on average 3.7 mm Hg higher in SHR than in WKY rats (Table 1, p<0.001). The slopes of the 14- and 27-week-old SHR and WKY rats were about twice as steep as those of the 4-week-old rats, indicating a greater resistance to flow in the older animals (Table 1 and Figure 1). At a flow of 10 ml/min/100 g hindquarter wt, PP_{max,di} in the young and older SHR averaged 17.3 and 27.8 mm Hg, respectively, with the corresponding figures in WKY rats 13.6 and 24.1 mm Hg (both p<0.01). Thus, there was a similar age-related increase in vascular resistance in both strains.

In 4-week-old SHR, increasing flow beyond the linear part of the pressure–flow curve resulted in a plateau value of PP_{max,di} of ≈37 mm Hg at high flows greater than 35–40 ml/min/100 g hindquarter wt. In WKY rats, the slope of the perfusion pressure–flow curve at these high flows was significantly less than in the more physiological range, but there was no definite plateau at flows as high as 50 ml/min/100 g hindquarter wt. The difference between SHR and WKY rats suggests that the hindquarter bed was less distensible in young SHR. In the older SHR, we did not study flows greater than 20–25 ml/min/100 g hindquarter wt and the perfusion pressure–flow curves were of similar appearance in both strains.

**Responses to methoxamine.** In other SHR and WKY rats, we obtained dose–response curves to methoxamine at different ages adjusted to constant flow perfusion of 10 ml/100 g hindquarter wt (see Materials and Methods) (Figure 3). When the hindquarters were maximally dilated with papaverine, PP_{max,di} was higher at all ages in SHR than in WKY rats (p<0.01 except at 4 weeks, Figure 4). The relation between age and PP_{max,di} was curvilinear in each strain (Figure 4). The (PP_{max,di} in SHR)/ (PP_{max,di} in WKY rats) ratio was similar at all ages and averaged 1.26.

Similarly, at every age PP_{max,con} was higher in SHR than in WKY rats (Figures 3 and 4). One finding common to both strains was that in 4- and 9-week-old rats, the maximum methoxamine-induced constrictor response was below the maximum constriction induced by Ang II or BaCl2 (Table 2). But in rats at or older than 14 weeks, α1-adrenergic receptor stimulation elicited the same maximum constrictor responses as supramaximal doses of Ang II or BaCl2. The (PP_{max,con} in SHR)/ (PP_{max,con} in WKY rats) ratio (produced by either methoxamine or Ang II) was similar at all time periods and averaged 1.35.
In both SHR and WKY rats, we determined the relation between body weight on the one hand and PP\textsuperscript{max dill} and PP\textsuperscript{max con} on the other (Figure 5 and Table 1). Analysis of covariance showed that, with PP\textsuperscript{max dill}, the slopes of the regression lines diverged significantly (p<0.05), and the slope in SHR was about 35% steeper than in WKY rats. Thus, in a 60-g rat the PP\textsuperscript{max dill} averaged 16.1±0.9 in SHR and 13.4±0.9 mm Hg in WKY rats (Δ=2.7 mm Hg; p=0.05); at the end of the rapid growth phase (400-g rat), PP\textsuperscript{max dill} in SHR was 31.8±0.7 mm Hg compared with 25.1±0.6 mm Hg in WKY rats (Δ=6.7 mm Hg; p<0.001). By contrast, the slopes of the lines relating body weight to PP\textsuperscript{max con} were only 16% steeper in SHR than in WKY rats (N.S., Table 1 and Figure 5), with the perfusion pressure in 60-g rats 69 mm Hg higher in SHR than in WKY rats, as compared with an only slightly greater difference in 400-g rats of 85 mm Hg (p<0.001 for both sets).

In both SHR and WKY rats, EC\textsubscript{50} decreased progressively after the age of 9 weeks, with the values in the two strains virtually identical after about 14 weeks (Figure 4). Between 4 and 14 weeks, EC\textsubscript{50} was significantly less in SHR than in WKY rats, so that only about 50% of the concentration of methoxamine was required to elicit the same constrictor response (p=0.02).

The maximum slope of the sigmoid curve was significantly greater in SHR than in WKY rats, and in both strains there was a linear increase with age, with the two lines approximately parallel (Figure 4). The slope parameter is related to the perfusion pressure range between PP\textsuperscript{max dill} and PP\textsuperscript{max con} (see Data Analysis under Materials and Methods), so that overall the greater maximum slope in SHR compared with WKY rats at a given age was partly due to the greater perfusion pressure range in SHR at a given age. However, there was also a "true" increase in responsiveness in SHR between 4 and 20 weeks, since their range-independent slope parameter was significantly greater than in WKY rats (Table 3). At 30 and 50 weeks, however, the range-independent slope was similar in both strains (Table 3), so that during this period the absolute value of...
TABLE 2. Differences Between Perfusion Pressure at Maximum Constriction Produced by Methoxamine and by Angiotensin II

<table>
<thead>
<tr>
<th>Age (wk)</th>
<th>Methoxamine</th>
<th>Angiotensin II</th>
<th>Methoxamine</th>
<th>Angiotensin II</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>176.5±4.19</td>
<td>231.5±4.82*</td>
<td>130.2±15.09</td>
<td>161.8±19.39*</td>
</tr>
<tr>
<td>9</td>
<td>287.3±5.53</td>
<td>314.8±7.15*</td>
<td>210.0±9.51</td>
<td>231.2±10.36*</td>
</tr>
<tr>
<td>14-50†</td>
<td>347.0±5.00</td>
<td>347.0±5.00</td>
<td>264.3±3.90</td>
<td>264.3±3.90</td>
</tr>
</tbody>
</table>

Values for differences in perfusion pressure (mm Hg) are mean±SEM. SHR, spontaneously hypertensive rats; WKY, Wistar-Kyoto rats.

* p for difference <0.05.
†Values for methoxamine and angiotensin II responses between 14 and 50 weeks were similar at all times.

the slope of the SHR was higher only because of elevation of the perfusion pressure range.

Regional Sympathetic Function

In the left ventricle plus septum there is a substantial sympathetic innervation of the myocardium. However, in skeletal muscle and kidney most of the innervation goes to blood vessels.34-35

In the left ventricle plus septum, [NE] was nearly twice as great in SHR as in WKY rats from 4 to 14 weeks of age (Figure 6, p<0.01). However, at 20 weeks and older the [NE] was closely similar in both strains. The left ventricle plus septum fractional rate constant K tended to be higher in SHR than in WKY rats at most times between 4 and 50 weeks, but the variability was large, so that, at the individual time intervals, only the strain differences at 9 and 50 weeks were statistically significant. The average norepinephrine turnover (i.e., K×[NE]) was also higher at most ages in SHR than in WKY rats, but again because of the variability the difference was statistically significant only at 4 and 9 weeks.

Forelimb muscle [NE] was about 40% higher in SHR than in WKY rats during the rapid growth period from 4 to 14 weeks (p<0.01) (Figure 6), but after that period the difference in tissue concentration was much smaller and not significant. In contrast to the findings in heart and kidney, K was virtually identical in SHR and WKY rats between 4 and 30 weeks, but at 50 weeks K in SHR was about 40% below the value observed in WKY rats. Norepinephrine turnover tended to be higher at 4 and 9 weeks in SHR than in WKY rats, but the difference was significant only at 4 weeks (p<0.01).

In the kidney, [NE], K, and norepinephrine turnover were all higher in SHR than in WKY rats at most time periods (Figure 6). The only exception was at 20 weeks when K and norepinephrine turnover were the same as in WKY rats. On average, K and norepinephrine turnover in SHR were, respectively, 1.66 and 1.92 times the corresponding values in WKY rats.

Discussion

The present study provides the most detailed analysis to date of the time course of cardiovascular hypertrophy and sympathetic function in SHR during the first year of life. We found that: 1) a higher vascular resistance in SHR compared with WKY rats was already established at 4 weeks, suggesting that vascular hypertrophy was present at a time when there was no difference in BP between the two strains; 2) left ventricular hypertrophy lagged behind the development of vascular hypertrophy and occurred in parallel with the rise in BP; 3) in both strains α1-adrenergic receptor stimulation with methoxamine did not elicit maximum hindquarter bed vasoconstriction until after 14 weeks of age; between 4–14 weeks the resistance vessels of SHR were relatively hyperresponsive compared with those of WKY rats, as assessed by the differences in EC50 and in range-independent slope; and 4) [NE] and turnover was increased in the heart, kidney, and skeletal muscle vessels of young SHR; in the skeletal muscle bed these changes were indepen-

TABLE 3. Comparison of Range-Independent and Maximum Slopes in Spontaneously Hypertensive Rats and Wistar-Kyoto Rats of Methoxamine/Concentration-Response Curves

<table>
<thead>
<tr>
<th>Range-independent slope (normalized units)</th>
<th>Maximum slope (pressure/unit dose)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (wk)</td>
<td>SHR</td>
</tr>
<tr>
<td>4</td>
<td>119±16.9</td>
</tr>
<tr>
<td>9</td>
<td>90±4.6</td>
</tr>
<tr>
<td>14</td>
<td>122±11.8</td>
</tr>
<tr>
<td>20</td>
<td>127±11.8</td>
</tr>
<tr>
<td>30</td>
<td>130±3.6</td>
</tr>
<tr>
<td>50</td>
<td>181±12.8</td>
</tr>
</tbody>
</table>

*p for difference <0.05.
Values are mean±SEM. SHR, spontaneously hypertensive rats; WKY, Wistar-Kyoto rats.
dent of increased neural activity, as assessed from the fractional rate constant.

**Vascular and Cardiac Changes**

In our colony there were no significant differences at 4 weeks between SHR and WKY rats, in either systolic or mean arterial BP. However, by 6 weeks in the same colony, there was a definite rise in mean arterial pressure, which increased in subsequent weeks in a manner similar to our systolic pressure findings. Gray's review of blood pressure changes in young SHR suggests that BP may already be raised in SHR during the neonatal period, but, in our experiments, there was no evidence of this at 4 weeks. Thus, our findings indicate that in SHR there already was considerable vascular hypertrophy by 4 weeks, preceding the rise in BP. Later, there was a further increase in vascular hypertrophy, suggesting that there also was a component associated with the rise in BP. But at 4 weeks, left ventricle plus septum weight in SHR was only 8% above that in WKY rats. Thus, before the rise in BP, left ventricular hypertrophy was slight, and over 90% of the rise in left ventricular weight followed the rise in BP.

We have assumed that the greater vascular resistance at full dilatation and during maximum constriction in SHR is due to the greater muscle mass in the media of their resistance vessels. Medial hypertension in SHR is the main determinant of the increased wall/lumen ratio of individual resistance vessels, which, by causing narrowing of the vessel compared with the lumen of WKY rats, is responsible for the greater values of the vascular resistances at full dilatation and at maximum constriction and for the greater slope of the stimulus–response curve. There is controversy concerning to what extent anatomic “rarefaction” (i.e., reduction in vascular density of resistance vessels per unit tissue volume) contributes to the strain differences in resistance properties. Experimentally, microsphere-induced anatomic rarefaction in the hindquarter bed causes elevation of $P_{\text{max}}$ but leaves unaltered $P_{\text{max}}$ and slope. By contrast, an increased wall/lumen ratio and associated vascular narrowing increases all three parameters of the dose–response curve, both in individual small arteries and in the whole vascular bed.

From the perfusion pressure–flow curves, the differences in $P_{\text{max}}$ between SHR and WKY rats were similar at 4, 14, and 27 weeks (Figure 1). However, the main study, which encompasses a wider age and body weight range, suggests that the difference between the strains widened progressively and that at 4 weeks, the difference was about half that observed in adult rats (Figure 5). The two series included a substantial number of rats, thus providing strong evidence that, by 4 weeks, a considerable amount of vascular hypertrophy had developed. However, the strain difference in $P_{\text{max}}$ at 4 weeks was only $\approx 20\%$ below the value in adults (Figure 5). One reason why $P_{\text{max}}$ in SHR was closer to the final adult value than $P_{\text{max}}$ is that the amplification is accentuated during constriction, since resistance increases in proportion to the fourth power of the radius. In addition, the stiffer vessels in the young SHR compared with the WKY rats may accentuate the degree of luminal encroachment during maximum constriction.

We did not determine vascular resistance before the age of 4 weeks and extrapolation from the body weight–$P_{\text{max}}$ and body weight–$P_{\text{max}}$ relation in Figure 5 is somewhat hazardous. Had we extrapolated the body weight–$P_{\text{max}}$ relation to a lower body weight value, we would have concluded that the amount of vascular hypertrophy soon after birth was small or absent. However, the body weight–$P_{\text{max}}$ relation is consistent with some degree of hypertrophy at, or soon after, birth. The anatomic literature on this point is conflicting. To date, only the structure of large arteries has been examined in these very young animals, with evidence of hypertrophy in one study, but not in another.
Although the rise in $PP_{\text{max}}$ for a given increase in body weight was somewhat steeper in SHR than in WKY rats, in each strain there was a substantial increase in this parameter with increasing body weight and age (Figures 4 and 5). With increasing body weight, most of the narrowing in average cross-sectional area per unit tissue of the hindquarter bed was similar in SHR and WKY rats (e.g., Figure 1). We do not know the mechanisms involved in these body weight-related and age-related vascular resistance rises, but each of the following could play a role: 1) reduction in the rats' metabolic rate during the early postnatal period, which may be associated with a reduced density of resistance vessels and a rise in resistance as the rat gets older; 2) growth-related increases in the length of resistance vessels; 3) changes in the proportion of skeletal muscle to fat in the hindquarters at different stages of development; 4) changes in collagen and elastin composition of the fibrous matrix of the vessel, which affect wall distensibility; and 5) changes in proportion of different isoforms of vascular smooth muscle actin, reflecting alterations in the proportion of the various phenotypes of smooth muscle in the vasculature.

In the absence of autonomic support, the development of vascular hypertrophy ahead of left ventricular hypertrophy in SHR will result in some degree of mismatching of the intrinsic properties of the vascular amplifier and the cardiac pumping capacity. This occurs with normal left ventricular myocardial function per gram of myocardium, as indicated in studies performed under highly controlled conditions in renal hypertensive dogs and in SHR. The development of additional myocardial elements in left ventricular hypertrophy allows the heart to maintain a given stroke volume and cardiac output against a higher BP than is possible without hypertrophy. In the event, there is enhanced left ventricular neural activity at an early age (Figure 6, rate constant data), which could provide sufficient inotropic support to raise BP. Possibly, the reason why the pressure does not rise to higher values than in WKY rats is that renal sympathetic activity is also elevated at the time, so that there is still some degree of mismatch.

**Sympathetic Innervation**

At most periods there was elevation in heart rate and in regional sympathetic activity (assessed from the fractional norepinephrine rate constants) in heart and kidney, but sympathetic regional activity in the muscle was normal (Figure 6). Such a pattern resembles the defense reaction. The elevation of cardiac sympathetic activity in young SHR will help to compensate for any inadequacy in intrinsic pumping capacity (see above) and later on, will provide enhanced cardiac pumping once left ventricular hypertrophy has fully developed. In the hypertrophied vessels of the hindlimb bed, the amplifier properties will ensure that resting vascular resistance is elevated, even at normal levels of sympathetic activity. Assuming the early development of the renal vasculature is similar to that of the hindlimb, the rise of resting renal vascular resistance in SHR will tend to be relatively greater than in the hindlimb. We cannot say from the available data how the defense pattern of sympathetic activity will affect the mismatch between vascular amplifier and cardiac pump before left ventricular hypertrophy has fully developed. However, at the latter time, the elevated neural activity (fractional rate constant) will accentuate the elevation of BP.

Tissue [NE] was raised in all three regions up to 14 weeks of age, and in the kidney for most of the 50 weeks. Chronic increases in sympathetic activity have been shown to increase steady-state concentrations of the enzymes tyrosine hydroxylase and dopamine β-hydroxylase, thereby increasing tissue [NE]. In SHR, higher levels of both enzymes have been reported in the mesenteric vessels, compared with those in WKY rats. The effect of modulation of transmitter release on [NE] by, for example, increased reuptake are difficult to predict. However, another mechanism capable of increasing tissue [NE] independently of neural activity (as in the case in the muscle bed in our experiments) may be an increased innervation density in the vasculature of young SHR, as recently demonstrated by Head and colleagues. Thus, both a chronic increase in neural activity and an increased innervation could alter postjunctional properties and contribute to the lower $EC_{50}$ (threshold) and to the higher range-independent slope observed in 4–14-week-old SHR during methoxamine stimulation (Figure 4, Table 3). Neuromuscular coupling between vascular $\alpha_1$-adrenergic receptors and the contractile machinery of the smooth muscle cell develops slowly in both SHR and WKY rats, as judged by the inability to develop maximum constriction with methoxamine until they are above 14 weeks of age, as has also been observed in rabbits. Below this age both strains have a lower response for a given level of $\alpha_1$-adrenergic receptor stimulation than the corresponding group of older rats (Figures 3 and 4), but the lower threshold and increased slope of young SHR will tend to enhance the resistance vessel responses, compared with those of young WKY rats.

Recent studies by Lee et al have shown that hypertension and hypertrophy are completely prevented by sympathetic ablation (see above), but we do not know how this comes about. The observed pattern of sympathetic activity will elevate BP through enhanced constrictor and inotropic responses once cardiovascular hypertrophy has developed, but it may have an additional trophic growth-promoting role at an earlier stage, which is independent of the level of neural activity. In normal rabbits in vivo, trophic effects of the sympathetic innervation have been found to contribute to the development of the smooth muscle of the ear.
artery\textsuperscript{18} and to the development of medial hypertrophy of cerebral vessels in SHR.\textsuperscript{53} In tissue culture, catecholamines stimulate growth through adrenergic receptor-mediated mechanisms,\textsuperscript{19,54} but the process is complex and appears to involve interactions with numerous growth factors.\textsuperscript{7,55}

In the hindlimb bed, in contrast to the other regions, elevation of [NE] and turnover occurs at normal levels of sympathetic activity.\textsuperscript{47} From the time course of $PP^\text{max}$,\textsubscript{obs} hypertrophy develops in the immediate postnatal period and any trophic role of the sympathetic activity should be apparent by the age of 4–6 weeks. In the light of tissue culture studies,\textsuperscript{19,53} our findings of high [NE] at 4 weeks in the skeletal muscle bed provide a pointer of a possible trophic role of the sympathetic in early vascular hypertrophy. We may hypothesize that the transiently increased density of sympathetic vascular innervation\textsuperscript{49-50} elevate the amount of norepinephrine released in the tissue, where it can interact with local growth factors released by immature vascular smooth muscle.\textsuperscript{54,55} A more definitive answer must await further experiments.

\textbf{Is There Primary Cardiovascular Hypertrophy?}

Our findings suggest that a significant component of vascular hypertrophy precedes the elevation of hypertension, while the rest occurs pari passu with the rise in BP. The first can be regarded as the primary component that is critical to the development of hypertension, while the additional later hypertrophy occurs as the conventional adaptive response associated with the pressure rise. In contrast, there is little, if any, primary left ventricular hypertrophy and this process appears to be predominantly adaptive.

A priori we would expect that the simultaneous establishment of cardiovascular hypertrophy will produce an almost immediate elevation of BP, due to the properties of the vascular amplifier. However, though the latter was well established by 4 weeks, we observed no rise in BP at this time, though hypertension developed soon afterwards (e.g., Reference 37). We do not know either the reason for the slow delay, despite the defense pattern of sympathetic activity, or why it took 14–20 weeks before the adult BP level was reached in SHR. Factors discussed earlier, such as the metabolically related high vascularity in the young rat, slow development of adrenergic receptor coupling to the contractile apparatus of the smooth muscle cell, and mismatch between left ventricular pumping capacity and vascular impedance may all play a role in delaying the manifestation of the vascular amplifier on the BP of SHR.

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