ABSTRACT

Ventricular weight in spontaneously hypertensive rats (F26 generation, Okamoto-Aoki strain) was significantly higher ($P < 0.001$) than that in body weight-matched American Wistar and Kyoto-Wistar normotensive rats, not only among older groups of rats but also among younger groups that had not developed significant hypertension. Deoxyribonucleic acid (DNA) concentration in ventricular muscle was not different from normal in the youngest group ($P < 0.4$) but was significantly reduced in the older spontaneously hypertensive rats ($P < 0.01$). Plasma renin activity was significantly increased in younger spontaneously hypertensive rats before the development of established hypertension; moreover, ventricular weight and plasma renin activity were significantly correlated in younger rats ($r = 0.788, P < 0.005$ for all rats, $r = 0.644, P < 0.01$ for spontaneously hypertensive rats). Antihypertensive therapy with either $\alpha$-methyldopa or hydralazine reduced blood pressure, especially in hypertensive rats; however, ventricular weight was reduced by methyldopa ($P < 0.01$) but not by hydralazine. Plasma renin activity was reduced by methyldopa but increased by hydralazine ($P < 0.01$). DNA concentration was reversed toward normal by methyldopa but not by hydralazine. Similar results were obtained when methyldopa and hydralazine were given to younger rats to prevent hypertension. The changes in ventricular weight with the onset of hypertension and with its reversal or its prevention suggest that blood pressure might not be the sole factor contributing to cardiac hypertrophy in the spontaneously hypertensive rat and that the renin-angiotensin system might play a permissive role enhancing myocardial hypertrophy.

KEY WORDS deoxyribonucleic acid blood pressure myocardium renin ventricle $\alpha$-methyldopa hydralazine

Cardiac hypertrophy in hypertension has usually been regarded as a secondary response to the increased pressure load; indeed, in some studies, the assumption of a close parallelism between the degree of hypertrophy and the level of hypertension has been considered "basic to the conclusions drawn" (1). However, as Grant pointed out in 1953 (2), there are many exceptions to this rule; at autopsy, many patients with marked hypertension show little or no hypertrophy even though the duration and the severity of the disease have not differed substantially from those seen in patients who do show left ventricular hypertrophy (3-5).

The possibility that other factors in addition to arterial blood pressure are involved in the cardiac hypertrophy associated with hypertension was examined in spontaneously hypertensive rats. The rat's heart is known to vary rapidly in weight in response to various experimental maneuvers (1, 6, 7), and spontaneously hypertensive rats provide a reliable model of a naturally developing pressure load akin to essential hypertension (8). In addition, the effects of antihypertensive therapy at different stages in the evolution of the hypertension were studied. Since our initial observations suggested a possible dissociation between the blood pressure level and the degree of cardiac hypertrophy under some circumstances, variations in plasma renin activity were also measured in view of recently reported effects of angiotensin II on myocardial protein synthesis (9, 10).

Methods

Materials.—The spontaneously hypertensive rats utilized were either bred at the Cleveland Clinic Foundation or obtained from Purina Laboratories. They belonged to the F26 generation derived from the spontaneously hypertensive Kyoto-Wistar strain developed by Okamoto and Aoki (8, 11). Two sets of normal control rats, one from the American Wistar strain and the other from the Kyoto-Wistar strain, were used; both sets were obtained from the same laboratory and handled in the same way as the spontaneously hypertensive rats. Six groups of rats from each of the three strains were studied to differentiate the course of hypertension and cardiac alterations in the spontaneously hypertensive rats from that in the normal rats. Each group was made up of eight
rats of equal body weight and the same approximate age. The groups were planned to cover the span of normal growth (50, 100, 150, 200, 250, and 300 g body weight) up to that point when the body weights of normal and spontaneously hypertensive rats diverge excessively (8). Both normal and spontaneously hypertensive rats were kept under the same conditions, properly housed and fed (regular rat chow), and handled by the same person at the same preset regular intervals.

In addition, four groups of spontaneously hypertensive rats were followed under the same conditions except that either methyldopa or hydralazine was added to their drinking water. Methyldopa was given in a concentration of 5 g/liter and hydralazine at 80 mg/liter. This route of administration was chosen because it has been shown to lower blood pressure effectively and probably more persistently than do intermittent intramuscular injections (12). In two groups, treatment with either drug was begun very early (body weight < 100 g) before the rats were hypertensive. The two other groups were given methyldopa or hydralazine only after they had attained a body weight of 200 g and were definitely hypertensive.

Blood Pressure and Cardiac Weight.—In all groups, arterial blood pressure was measured by a tail-cuff method similar to that described by Friedman and Freed (13). The apparatus was obtained from Narco Biosystems Inc. The pressures were consistently recorded by the same person at approximately the same time of day. The rats were weighed twice weekly, and the last determination of weight and blood pressure was obtained immediately before they were killed. The blood pressures reported in this paper are the averages of the last three recordings.

Rats were killed by rapid decapitation and autopsied immediately for gross signs of cardiac failure, inflammatory lesions, or congenital defects. The heart was then removed and the ventricles carefully cleaned; both atria were removed and the aorta and the pulmonary artery were cut flush with the ventricular surface. The ventricles were washed repeatedly with saline, blotted dry, and weighted with a Mettler precision balance. They were then cut transversely into two parts; one part was used to determine deoxyribonucleic acid (DNA) content and the other was used to obtain the dry weight/wet weight ratio of the tissue. Dry weight was obtained by drying the lyophilized tissue in an oven at 100°F until its weight became constant.

Analytical Methods.—Plasma renin activity was determined the day before the rats were killed. Blood was drawn as previously described (14) under light ether anesthesia from the tail artery using a Vacutainer with tripotassium ethylenediaminetetraacetate (EDTA) as the anticoagulant. Renin activity was measured in 0.1-ml samples of plasma by the micromethod of Boucher et al. (15) using substrate prepared from 48-hour bilaterally nephrectomized rats. The plasma was incubated in the presence of Dowex 50 × 2 (NH₄⁺) for 15 hours with an excess of substrate, and the angiotensin generated was measured by bioassay (16). Results were expressed as ng/0.1 ml 15 hours⁻¹. The DNA content of ventricular tissue was determined by the method of Ceriotti (17). The intensity of the yellow color produced in the presence of indole and HCl was read with a Beckman spectrophotometer at 490 nm against an appropriate blank. Results were expressed in mg/g ventricular tissue.

Standard methods (18) were used to calculate statistical significance, t-tests, and correlation coefficients (r). Unless otherwise indicated, all values are reported as averages ± 1 SE.

Results

GROWTH CURVE AND EVOLUTION OF HYPERTENSION

In the early stages, there were no differences between normal and hypertensive rats with regard to body weight or growth, but the spontaneously hypertensive rats lagged behind in growth after they attained a body weight of 200 g, as has been previously described (8). Comparisons between the different groups were therefore limited at the 300-g mark. The spontaneously hypertensive rats did not develop significant hypertension before they reached about 140 g in weight (Fig. 1). During the 100–120-g period, blood pressure was not significantly different in the three strains of rats studied (P < 0.4). In accordance with Okamoto’s original description, the elevation of arterial blood pressure in the spontaneously hypertensive rats could be divided into three stages: a prehypertensive stage when pressure was not different from that in normal controls, a stage of labile hypertension (140–200 g), and finally the stage of established steady hypertension which began when the rats had matured to a weight of 200 g or more (usually when they were 8 weeks old). In contrast, arterial blood pressure in normal control (American Wistar or Kyoto-Wistar) rats was more or less the same throughout their growth.

VENTRICULAR WEIGHT

Significant cardiac hypertrophy was found in spontaneously hypertensive rats from the youngest
SPONTANEOUS HYPERTENSION AND CARDIAC HYPERTROPHY

5.0 -
4.5 -
4.0 -
3.5 -
3.0 -
2.5 -
2.0 -

BJPOnmHg)
125^20 I50J20 185-120 190-120 200-120

FIGURE 2
Ventricular weight (H. WT.) in normal rats (NR) and spontaneously hypertensive rats (SHR). The results are expressed as mg/g body weight ± SE. The horizontal axis gives both blood pressure and body weight. Blood pressures in rats of the 50-g weight group were too small to record with the present setup.

age investigated (group with a body weight of 50 g corresponding to 3–4 weeks of age) (Fig. 2). This finding held regardless of the index of comparison (absolute ventricular weight or ventricular weight relative to body weight) or the normotensive strain (American or Kyoto) used as the control (Table 1). The dry weight–wet weight ratios of the ventricles of normal and spontaneously hypertensive rats (whether treated or untreated) were not significantly different.

Of particular interest was the observation that the degree by which the ventricular weight of the spontaneously hypertensive rats (SHR) exceeded normal ventricular weight was as high in the youngest group (50 g) as it was in the 250-g group. The degree of hypertrophy was calculated as follows:

\[
\text{SHR ventricular weight} - \text{control ventricular weight} \\
\text{control ventricular weight} \times 100
\]

where all ventricular weights were expressed as mg/g body weight. The ventricular weight of spontaneously hypertensive rats was 16.4% greater than that in controls for the 50-g group, 18.75% greater for the 100-g group, and 15% greater for the 250-g group.

TABLE 1
Ventricular Weight and Blood Pressure in Two Normotensive Wistar Strains

<table>
<thead>
<tr>
<th>Weight group (g)</th>
<th>Ventricular weight (mg/g body wt)</th>
<th>Blood pressure (mm Hg)</th>
<th>Ventricular weight (mg/g body wt)</th>
<th>Blood pressure (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>125</td>
<td>3.2 ± 0.02</td>
<td>120 ± 5</td>
<td>3.2 ± 0.1</td>
<td>115 ± 10</td>
</tr>
<tr>
<td>200</td>
<td>2.6 ± 0.06</td>
<td>120 ± 10</td>
<td>2.5 ± 0.05</td>
<td>120 ± 10</td>
</tr>
</tbody>
</table>

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CARDIAC HYPERTROPHY AND ANTIHYPERTENSIVE THERAPY

Both methyldopa and hydralazine controlled arterial blood pressure in the spontaneously hypertensive rats. Blood pressure averaged 188 ± 10 (SE) mm Hg in untreated rats but only 149 ± 8 mm Hg with methyldopa treatment and 123 ± 10 mm Hg with hydralazine. Neither drug interfered with growth, and the body weights of treated and untreated rats were not significantly different. In contrast, ventricular weights were significantly different between the treated groups. In rats treated with hydralazine, ventricular weight was 3.4 ± 0.09 mg/g body weight (not significantly different from the value for untreated spontaneously hypertensive rats). In methyldopa-treated rats, ventricular weight (2.8 ± 0.7 mg/g body weight) was significantly lower than it was in untreated hypertensive rats (P < 0.01) or in hydralazine-treated rats (P < 0.01) (Fig. 3).

The effects of preventive therapy on arterial blood pressure and ventricular weights were similar to those of treatment begun at an older age (Fig. 4). Hypertension did not develop in either the methyldopa- or the hydralazine-treated groups. Arterial blood pressure after 6 weeks of therapy averaged only 135 ± 10 mm Hg in rats given methyldopa and 115 ± 15 mm Hg in those given hydralazine compared with 200 ± 8 mm Hg in the 200-g untreated spontaneously hypertensive rats. The ventricular weight–body weight ratios were significantly different in the two treated groups, averaging 3.25 ± 0.06 mg/g in hydralazine-treated rats vs. 3.25 ± 0.06 mg/g in methyldopa-treated rats (P <
VENTRICULAR WEIGHT AND PREVENTIVE TREATMENT

Results are expressed as mg/g body weight ± SE. BP = blood pressure.

0.01). When it was given to normotensive Wistar rats, methyldopa reduced ventricular weight slightly (2.30 ± 0.11 mg/g), whereas hydralazine had no significant effect (2.5 ± 0.06 mg/g). Neither drug altered blood pressure appreciably in normotensive rats.

VENTRICULAR DNA CONTENT

Ventricular DNA concentration was stable in normotensive rats during the period of growth investigated (50-250 g body weight). In contrast, DNA concentration changed as the spontaneously hypertensive rats developed; in general DNA content decreased with age, and it was reduced in the oldest group relative to controls (P < 0.01) (Table 2). Spontaneously hypertensive rats treated with methyldopa after they became hypertensive had a ventricular DNA content equal to the expected normal for their body weight (2.2 ± 0.05 mg/g for 220-g rats). However, those treated with hydralazine had the same ventricular DNA concentration (1.6 ± 0.01 mg/g) as untreated spontaneously hypertensive rats (1.6 ± 0.10 mg/g) (Table 3).

PLASMA RENIN ACTIVITY

Confirming previous reports (19), plasma renin activity was higher in young spontaneously hypertensive rats than it was in normal controls (P < 0.01); it then progressively declined as the rats grew in size. In the younger control and hypertensive groups, 125 and 150 g, a significant correlation was found between ventricular weight and plasma renin activity (r = 0.788, P < 0.005); the correlation was significant in spontaneously hypertensive rats considered as a separate group (r = 0.644, P < 0.01) but not in normotensive controls (r = 0.383) (Fig. 5).

Plasma renin activity was depressed by methyldopa treatment in both normal rats (22.9 ± 1.8 ng/0.1 ml 15 hours⁻¹) and hypertensive rats (11.5 ± 1.0 ng/0.1 ml 15 hours⁻¹) but increased by hydralazine in both groups (33.11 ± 2.5 ng/0.1 ml 15 hours⁻¹ and 45.75 ± 2.2 ng/0.1 ml 15 hours⁻¹, respectively). The differences between the effects of the two types of therapy were highly significant (P < 0.001 for both normal and spontaneously hypertensive rats).

Discussion

Two unexpected results were obtained in this study: a significant increase in ventricular weight was found in very young spontaneously hypertensive rats, and a marked difference with regard to reversal or prevention of hypertrophy was seen between two equipotent antihypertensive drugs. The alterations in ventricular weight during the development or the reversal of cardiac hypertrophy were not due to changes in water content since the dry weight–wet weight ratios were basically the same in all groups investigated.

The early increase in ventricular weight was noticed before "hypertensive" pressure levels were recorded in the spontaneously hypertensive rats.

TABLE 2

<table>
<thead>
<tr>
<th>Weight group (g)</th>
<th>SHR</th>
<th>Normal</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>3.0 ± 0.04</td>
<td>2.8 ± 0.13</td>
<td>NS</td>
</tr>
<tr>
<td>125</td>
<td>2.05 ± 0.07</td>
<td>2.6 ± 0.07</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>150</td>
<td>2.2 ± 0.09</td>
<td>2.2 ± 0.11</td>
<td>NS</td>
</tr>
<tr>
<td>200</td>
<td>1.3 ± 0.06</td>
<td>2.1 ± 0.07</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>250</td>
<td>1.6 ± 0.10</td>
<td>2.2 ± 0.12</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>
Relationship between ventricular weight and plasma renin activity (PRA) in young normal (NR) (open circles) and spontaneously hypertensive (SHR) (solid circles) rats.

This difference from the expected parallelism between the rise in pressure and the increase in cardiac output (1, 8, 20) could be related to many factors including the arbitrary definition of the hypertensive level. The increase in weight could be related to an increase in pressure relative to that in normal control rats rather than to the passing of a certain conventional mark (140 or 150 mm Hg). Although there was no statistically significant difference in average arterial blood pressure between our youngest spontaneously hypertensive rats and either the American Wistar or the Kyoto-Wistar control groups investigated, differences in pressure cannot be ruled out with absolute certainty, partly because of the difficulties in measuring blood pressure in very young conscious rats and partly because of the marked lability in blood pressure that precedes the established phase of hypertension in spontaneously hypertensive rats (8, 11). These differences might be important biologically even though they are difficult to establish numerically. The increased cardiac weight in young spontaneously hypertensive rats would then be viewed as secondary to the increased pressure load.

Were this the whole explanation, it would be surprising to find that the degree of hypertrophy did not increase as the difference in blood pressure between normal rats and spontaneously hypertensive rats became more pronounced. However, other hemodynamic factors besides the increased pressure might be involved in young rats. Pfeffer et al. (21, 22) have demonstrated the occurrence of an early phase of increased cardiac rate and output in young spontaneously hypertensive rats and related it to an increased sympathetic drive to the heart (23). Folkow et al. (24-26) have postulated that repeated or exaggerated "arousal responses" could lead to early vascular hypertrophy thus setting the stage for increased vascular resistance in the later stages of hypertension. Repeated sympathetic stimulation to the heart and a fluctuating arterial blood pressure might therefore be visualized as possibly leading to early cardiac hypertrophy when hypertension is still borderline.

The surprising correlation between plasma renin activity and ventricular weight in young rats (Fig. 5) opens a new avenue for thought. A correlation naturally does not imply a causal association, but other observations from different fields suggest the possibility of a biologically important relationship. The elevated plasma renin activity in young spontaneously hypertensive rats has previously been reported by Sen et al. (19). It is significant that a similar observation of increased plasma renin activity has also been described in some human borderline hypertension (27). Dustan et al. (28) have reported a significant correlation between plasma renin activity and cardiac output but none between plasma renin activity and arterial blood pressure levels in essential hypertension. Finally, the correlation found in this study between plasma renin activity and ventricular weight among spontaneously hypertensive rats is in agreement with the work of Gross (29) in two-kidney renal hypertensive rats. Following renal arterial clipping, renin content of the ischemic kidney as well as plasma renin activity correlate with heart weight better than with arterial blood pressure. These correlations might be related to the fact that both renin release and cardiac work are markedly influenced by sympathetic activity (30), a suggestion already advanced by Dustan et al. (28). However, recent observations also suggest that metabolic mechanisms might be involved in addition to the more obvious hemodynamic factors. Khairallah et al. (9, 10) have found that angiotensin II stimulates myocardial protein synthesis; therefore, it is not inconceivable that renin might play a permissive or helping role in promoting cardiac hypertrophy under certain conditions. Whether the initial increase in ventricular weight in spontaneously hypertensive rats was related to hypertrophy or hyperplasia cannot be decided with certainty from our data. The reduction in myocardial DNA concentration in the older hypertensive rats indicates only ventricular hypertrophy at the more advanced stages (31). In the younger groups, DNA concentra-
tion was equal to that in control rats, but further studies are required before initial hypertrophy can be affirmed.

The effect of antihypertensive therapy on arterial blood pressure, ventricular weight, and plasma renin activity confirmed the tentative conclusions drawn from the first part of the study. Methyldopa and hydralazine were equally successful in controlling the hypertension in the older rats or in preventing its development in the younger spontaneously hypertensive rats. In fact, hydralazine was more potent under both conditions, leading consistently to better blood pressure control (123 ± 10 mm Hg) than methyldopa (149 ± 10 mm Hg). Yet ventricular weight was reduced by methyldopa (2.8 ± 0.7 mg/g body weight vs. 3.4 ± 0.1 mg/g in untreated rats) but not by hydralazine (3.4 ± 0.09 mg/g). Plasma renin activity was lowered by methyldopa but not by hydralazine. The dissociation between the arterial blood pressure response to hydralazine and the persistence of cardiac hypertrophy has also been noted by Masson et al. (12) in experimental renovascular hypertension and tentatively ascribed to its cardiac-stimulating effect. The reversal of hypertrophy with blood pressure control by methyldopa is consistent with the reduction in heart weight obtained by removing the cause of experimental hypertension (aortic constriction [6], deoxycorticosterone (DOC) pellet [32], or renal arterial clip [1]). The difference between the two drugs could be due to their different hemodynamic effects, although it has been argued by some that chronic hydralazine treatment in man may not be associated with more rapid heart rate than treatment with methyldopa (33).

Obviously, further studies are needed to characterize biochemically the reversal of hypertension and its hemodynamic correlates. The association of increased plasma renin activity with the persistence of cardiac hypertrophy during hydralazine therapy would again suggest that the link between the two might be more than coincidental. The importance of hemodynamic factors in hypertensive heart disease is so obvious that a more subtle or permissive role of associated hormonal or metabolic factors has been overshadowed. A possible link between renin and cardiac hypertrophy relates to protein synthesis. Not only is injected angiotensin II rapidly localized in cellular structures regulating protein synthesis (34) but also cardiac ribonucleic acid (RNA) content is rapidly increased by an angiotensin infusion (9). However, another possible mechanism is the modulating effect of angiotensin on sympathetic nerve activity and its inhibition of norepinephrine uptake (35). The occurrence of cardiac hypertrophy in "low-renin" types of hypertension such as essential hypertension (36), primary aldosteronism (37), or experimental DOC hypertension (24) obviously indicates that excess renin or angiotensin is not essential for the development of hypertension. However, the persistence of hypertension after blood pressure control by hydralazine may point to the possible importance of the renin system under certain conditions.

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