Heart Size in Inbred Strains of Rats
Part 2. Cardiovascular DNA and RNA Contents during the Development of Cardiac Enlargement in Rats

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SUMMARY
Enlargement and nucleic acid content of the cardiovascular system of several strains (SHRSP/N, SHR/N, OM/N, M520/N) of rats were compared with the WKY/N strain in an attempt to characterize cardiac enlargement. Cardiac enlargement in rats can be due to either hypertrophy (increase in myocyte size), hyperplasia (increase in cell number including supporting tissue), or a combination of both. The sum of the indices of the degree of hypertrophy and hyperplasia calculated from the difference of the heart and aorta deoxyribonucleic acid (DNA) concentration and total DNA content between each strain and the WKY/N was almost equal to the degree of heart and aorta enlargement. The SHRSP/N revealed a striking hypertrophy of myocardial cells from the prehypertensive stage, and hyperplasia appeared gradually with the elevation of blood pressure. In contrast, the SHR/N developed a marked hyperplasia with some hypertrophy at the prehypertensive stage. Cardiac enlargement of the OM/N was attributed to both hypertrophy and hyperplasia. A large heart weight of the M520/N was recognized at only a young age, and was due almost entirely to hyperplasia. Aortic enlargements were related to hyperplasia. An increased ribonucleic acid (RNA) concentration was observed in both ventricles of the SHRSP/N, SHR/N, and M520/N rats at 4 weeks of age, and in all of the four strains at 16 weeks of age. A significantly higher RNA concentration was indicated in the aorta of three hypertensive strains of SHRSP/N, SHR/N, and OM/N at established hypertensive stage. These changes might be related to manifestations of genetic or other factors such as the effect of elevated blood pressure. (Hypertension 4: 872-880, 1982)

KEY WORDS  •  hypertrophy  •  hyperplasia  •  heart  •  aorta  •  hypertension  •  SHR  •  SHRSP  •  DNA  •  RNA

CARDIAC enlargement caused by pressure and volume overload may be due to an increase of cell size (hypertrophy), an increment in cell number (hyperplasia), or both. Although in rats cardiac muscle cells show considerably high mitotic activity during the fetal period, they abruptly lose their mitotic activity and cell divisions are no longer seen after 3 weeks of age. Thereafter, the heart enlarges by muscle cell hypertrophy and nonmuscle cell proliferations. It is generally accepted that experimentally induced cardiac hypertrophy in adult animals is accompanied with hypertrophy of myocytes and hyperplasia of interstitial and endothelial cells. On the other hand, hy-
perplasia of myocytes is only present when cardiac hypertrophy is induced in the neonatal period. One method of distinguishing between hypertrophy and hyperplasia is by measurement of tissue deoxyribonucleic acid (DNA). In general, cardiac DNA content has been observed to increase in experimentally induced cardiac hypertrophy. It was confirmed that the increased cardiac DNA content results from hyperplasia of nonmuscle cells. In addition, one of the most well-documented changes in the enlarging myocardium is an increase in the amount of ribonucleic acid (RNA).

It is well known that the spontaneously hypertensive rat (SHR) develops left ventricular hypertrophy as well as hypertrophy of the myocytes. An increase of RNA content was also observed in the SHR heart. However, there are two conflicting reports concerning perplasia as standards.

Materials and Methods

Experimental Animals

The SHRSP/N, SHR/N, OM/N, M520/N, and WKYN strains of rats were obtained from the Small Animal Resources Section, Veterinary Resources Branch, Division of Research Services, National Institutes of Health. Only male rats were used in this study. In previous experiments described in Part 1 (pp 864–872), it was shown that the heart weight of rats is a highly heritable trait, and that the effect of genetic factors upon cardiac enlargement is larger than that of elevated blood pressure at a young age. In those Part I experiments, cardiac enlargement was demonstrated in the SHRSP/N, SHR/N, OM/N, and M520/N strains. Cardiac enlargement in these strains might develop through different causes since their gene constitutions are apparently different from each other. Therefore, the present paper deals with strain and age differences in the amount of cardiac and aortic nucleic acids in an attempt to characterize cardiac enlargement in these strains as compared with the WKYN/N. It should be noted that these measurements are only indices of cellular changes and do not give as much information as detailed morphometrical analysis.

Experimental Animals

The SHRSP/N, SHR/N, OM/N, M520/N, and WKYN strains of rats were obtained from the Small Animal Resources Section, Veterinary Resources Branch, Division of Research Services, National Institutes of Health. Only male rats were used in this study. After arrival in the laboratory they were maintained in a room with a temperature of 24°C ± 1°C and a humidity of 55% ± 5%, and fed commercial pellets (Ralston Purina Company, NIH Rat and Mouse Ration Form 1). Tap water and food were available ad libitum.

Blood Pressure Measurements

At 4, 8, 12, and 16 weeks of age, their body weight, blood pressure, and heart rate were measured. Ten rats were used in each strain and age group. Blood pressure was measured from the caudal artery of unanesthetized animals using a modification of the tail-cuff method.

Heart and Aorta Weights

After the measurement of blood pressure, animals were sacrificed by bleeding from the carotid artery under light ether anesthesia. The whole heart was removed immediately, and dissected into the atria and ventricles. Ventricles were excised into the right ventricular free wall and left ventricle including the septum. All tissues were washed with saline, blotted to dryness, and weighed on a precision balance. The whole aorta from the ascending aorta down to the turning point of femoral artery was also weighed after removing fat and other tissues carefully.

Analytical Methods

In rats 4, 8, and 16 weeks of age, DNA and RNA in both ventricles and aorta were extracted by a modification of the method of Cutilletta et al. The ages were defined as pre-, early, and established hypertensive stages in the SHRSP/N and SHR/N, respectively. All tissues were homogenized in 4 ml of 95% alcohol in a Duall ground glass homogenizer. The centrifuged sample was washed twice with 4 ml of 0.2 N perchloric acid (PCA). The precipitate was extracted twice with 4 ml of 1N PCA for 30 minutes at 80°C. The extract was assayed for DNA and RNA according to method of Ceriotti and Dische, respectively. Calf thymus DNA and yeast RNA (Sigma Biochemicals) were used as standards.

Results

Blood Pressure and Heart Rate

The changes of average blood pressure and heart rate in each strain with age are shown in figure 1. Body weight is presented for comparative purposes. The blood pressure in the SHRSP/N and SHR/N rats showed rapidly at 4 to 12 weeks of age and more slowly thereafter. The blood pressure of the OM/N rats increased moderately and exceeded 150 mm Hg after 12 weeks of age. It was clearly demonstrated that the OM/N is a strain with mild hypertension, as shown in Part 1 (pp 864–872). In these three hypertensive strains, 4, 8, and 16 weeks of age corresponded with pre-, early, and established hypertensive stages, respectively. In contrast, the blood pressure in the WKYN/N rats showed only a slight increase at these ages.

The heart rate in the SHRSP/N, OM/N, and WKYN/N rats decreased slightly with age, and did not differ from each other. The heart rate of the WKYN/N rats was consistently low at all stages.

Heart and Aorta Weights

Average left and right ventricular and total heart weights as a function of age are shown in figure 2. At 4 weeks of age, the heart size and left ventricular weight in the M520/N, OM/N, and SHR/N rats were already significantly larger than those of the WKYN/N. A simi-
A highly significant increase in body weight, blood pressure, and heart rate was observed in the SHRSP/N strain at 8 weeks of age. Although at this age the heart size in the SHRSP/N was not significantly different from that of the WKY/N, left ventricular weight in this strain was significantly larger than that of the WKY/N. A similar tendency was observed at 12 weeks of age, and at 16 weeks of age the SHRSP/N exhibited a significantly larger heart size as well as a greater left ventricular weight in comparison with the WKY/N. On the other hand, the increase of heart size and left ventricular weight in the M520/N progressed slowly after 12 weeks of age, and both were significantly smaller than those of the WKY/N at 16 weeks of age. A significantly larger right ventricle than that of the WKY/N was observed in the OM/N at 8 weeks of age and in the SHR/N after 8 weeks of age.

Changes in aorta weight with age are presented in figure 3. The OM/N and SHR/N strains had a significantly greater aorta weight in comparison with the WKY/N at all stages. A significantly large aorta weight was also seen in the SHRSP/N only at 16 weeks of age. On the other hand, aorta weight in the M520/N was almost similar to that of the WKY/N at all stages. Since in the previous study the heart and aorta weights were expressed as the ratio with regard to body weight, the data were transformed in this manner (fig. 4). Results showed that the heart weight in the SHRSP/N, SHR/N, and M520/N was significantly larger than that of the WKY/N at any stage. Especially notable was the relative heart weight of the SHRSP/N, which...
Changes in DNA

Changes in left ventricular DNA concentration and total content with age are presented in figure 5. On the whole, DNA concentration was reduced, as expected, with the growth of myocardial cells. In contrast, the total content was increased with enlargement of the heart. Left ventricular DNA concentration of the SHRSP/N was decreased significantly in comparison with the WKY/N at all stages, especially at the prehypertensive stage. A slight but distinct decrease of DNA concentration by the SHR/N was apparent at pre- and early hypertensive stages as compared with the WKY/N, and this decrease was more pronounced at the established hypertensive stage. In the OM/N strain, DNA concentration was not significantly different from the WKY/N at the prehypertensive stage. Thereafter, its concentration decreased rapidly in parallel with the increase of blood pressure, becoming similar to the SHRSP/N. The DNA concentration in the left ventricle of the M520/N was not significantly different from that in the WKY/N at any age.

Total left ventricular DNA content of the SHRSP/N was significantly lower than that of the WKY/N at prehypertensive stage. However, a significant difference was not seen after the early hypertensive stage. The SHR/N and OM/N exhibited a significantly greater total DNA content in this tissue after the early hypertensive stage in comparison with the WKY/N. Only at 8 weeks of age was the total DNA content of the M520/N significantly increased.
If one assumes that each cell contained a constant amount of DNA, then a decrease of DNA concentration might reflect hypertrophy of myocardial cells. On the other hand, an increase of total content might be attributable to hyperplasia. We therefore calculated an index of the degree of hypertrophy from the difference in left ventricular DNA concentration between the WKY/N and each strain as follows:

\[ \frac{\text{WKY/N DNA concentration} - \text{each strain DNA concentration}}{\text{WKY/N DNA concentration}} \times 100. \]

Similarly, an index of the degree of hyperplasia (the term "hyperplasia" is not limited to myocytes but reflects all cell types of the myocardium) was calculated as follows:

\[ \frac{\text{Each strain DNA content} - \text{WKY/N DNA content}}{\text{WKY/N DNA content}} \times 100. \]

In addition, the degree of left ventricular enlargement was calculated as follows:

\[ \frac{\text{LV weight in each strain} - \text{LV weight in WKY/N}}{\text{LV weight in WKY/N}} \times 100. \]

Results (table 1) show that the sum of the indices for the degrees of hypertrophy and hyperplasia corresponds with the degree of left ventricular enlargement in any strain and age. Left ventricular enlargement of the SHRSP/N was almost entirely due to hypertrophy of myocardial cells. A negative degree of hyperplasia at the prehypertensive stage indicated a significantly severe hypertrophy. Thereafter, myocardial hyperplasia appeared gradually with the elevation of blood pressure. In contrast, left ventricular enlargement of the SHR/N resulted from hyperplasia of the myocardium and in part from hypertrophy. In the OM/N strain, both hypertrophy and hyperplasia were observed. The left ventricle of the M520/N exhibited hypertrophy and hyperplasia relative to the WKY/N at 4 weeks of age, but the hypertrophy was not seen at 8 weeks. Negative degrees of hypertrophy and hyperplasia in this strain at 16 weeks of age reflected a smaller left ventricle than that of the WKY/N.

Changes in right ventricular DNA concentration and total content are shown in figure 6. A significant decrease of DNA concentration was observed in the SHRSP/N at the prehypertensive stage and in the OM/N at early hypertensive stage as compared with the WKY/N. On the other hand, a significant increase was indicated in the M520/N at 16 weeks of age. Total DNA content of the SHRSP/N at prehypertensive stage decreased significantly in comparison with the WKY/N. In contrast, a significant increase was seen in the SHR/N and OM/N strains after an early hypertensive stage. No other significant difference was observed.

Degrees of right ventricular hypertrophy and hyperplasia as well as the degree of enlargement were calculated in the same manner as described for the left ventricle (table 2). The sum of degrees of hypertrophy and hyperplasia again coincided with the degree of right ventricular enlargement as observed in the left ventricle. Hypertrophy of the right ventricular myocardium was observed in the SHRSP/N at the prehypertensive stage in the absence of right ventricular enlargement. Right ventricular enlargement of the SHR/N at the prehypertensive stage was due to hyperplasia of the myocardial cells. This hyperplasia was maintained up to the established hypertensive stage, although a significant ventricular enlargement was not shown at this stage. Both hyperplasia and hypertrophy were observed in the OM/N strain at the early hypertensive stage, but hypertrophy was no longer seen at the established hypertensive stage. A slight increase in the degree of hyperplasia was seen in the M520/N at 4 weeks of age. The hyperplasia continued up to 16 weeks of age, although right ventricular enlargement was not seen at this age.

Changes in aortic DNA concentration and total content are given in figure 7. At 4 weeks of age only the SHR/N had a reduction in aortic DNA concentration;
no significant difference was noted at 8 weeks, but by 16 weeks all strains had significantly higher DNA concentrations than the WKY/N.

Total DNA content of the aorta in the three hypertensive strains increased progressively up to the established hypertensive stage; a significant elevation was observed after the early hypertensive stage, especially in the SHRSP/N and SHR/N rats. On the other hand, total content of the M520/N increased more slowly with age, and a significant increase was observed at only 16 weeks of age.

Degree of hypertrophy and hyperplasia as well as the degree of aortic enlargement were calculated in the same manner (table 3); the sum of the degrees of hypertrophy and hyperplasia again corresponded with the degree of aortic enlargement. The enlargement of the aorta in the three hypertensive strains was mainly attributed to hyperplasia, although hypertrophy was observed in the aorta of the SHRSP/N at the prehypertensive stage. A large negative degree of hypertrophy might imply severe hyperplasia. The M520/N also exhibited hyperplasia at 16 weeks of age in spite of the absence of aortic enlargement.

### Changes in RNA

Left and right ventricular and aortic RNA concentrations (fig. 8) decreased with age, on the whole. A significant increase of left ventricular RNA concentration was shown in the SHRSP/N, SHR/N, and M520/N as compared with the WKY/N at 4 weeks of age. There was no significant difference at 8 weeks of age. At 16 weeks of age, these four strains exhibited a significantly higher RNA concentration than that of the WKY/N. Almosts the same result was obtained for right ventricular RNA concentration. RNA concentration of the aorta in each strain was slightly higher than that of the WKY/N up to 8 weeks of age. A significant increase was observed in the three hypertensive strains at the established hypertensive stage as compared with the WKY/N.

### Discussion

The present study indicated that the sum of the degrees of hypertrophy and hyperplasia calculated from the cardiac or aortic DNA concentration and total content is almost equal to the degree of cardiac or aortic enlargement. Therefore, from the viewpoint of cellular
hypertrophy and hyperplasia, it is possible to characterize the enlargement of the heart and aorta in each strain as compared with the WKY/N. Cardiac cells consist of muscle and nonmuscle cells. However, the proliferation of muscle cells is no longer seen after 3 weeks of age in rats, nor observed in experimentally induced cardiac hypertrophy, although mitotic activity of nonmuscle cells is increased considerably.

Therefore, a decrease of ventricular DNA concentration may be attributed to hypertrophy of the myocytes. Nonmuscle nuclei compose approximately 75% of the total nuclei; 80% of the total volume is myocytes. Therefore, the contribution of nonmuscle cell hypertrophy to decreased DNA concentration would be small, even if it existed. On the other hand, an increase of total DNA content reflects proliferation of nonmuscle nuclei.
cle elements, as shown in experimentally induced cardiac hypertrophy. However, further studies are needed with regard to the increase of proliferation of myocytes during the neonatal period, as indicated in the SHR by Cutilletta et al. The effect of polyploidization of myocytes on total DNA content was also suggested, but there was no difference in the frequency of polyploid among the SHR and normotensive strains. Furthermore, the increase of mitochondrial DNA during the development of cardiac enlargement can contribute very little to the increase of total DNA content. Therefore, in view of the above-mentioned points, it can be said that the left ventricular enlargement of the SHRSP/N was due to a striking hypertrophy of myocytes and hyperplasia of nonmuscle cells developing gradually with the elevation of blood pressure. In contrast, the SHR/N revealed mainly hyperplasia of nonmuscle cells and in part hypertrophy of myocytes. The OM/N showed both hypertrophy and hyperplasia. On the other hand, the M520/N exhibited a different basis for enlargement of the left ventricle, that is, a large ventricle was recognized at only a young age and could be attributed to hyperplasia of nonmuscle cells.

The degree of hypertrophy in the SHR/N seemed to be small. However, morphometrical studies clearly indicated that myocytes of the SHR were significantly larger than those of normotensive strains. Therefore, it is suggested that hypertrophy of myocytes in the SHR/N is “diluted” by severe hyperplasia of nonmuscle cells. On the other hand, the degree of hyperplasia in the SHRSP/N seemed to be very small, although hyperplasia appeared with the elevation of blood pressure. The enlargement in this strain appears to be related to severe hypertrophy of myocytes.

The SHR/N revealed a remarkable increase of left ventricular total DNA content in contrast to a slight decrease of DNA concentration. Cutilletta et al. reported the increased left ventricular DNA concentration and total content in the SHR. In contrast, Sen et al. indicated a significant decrease of DNA concentration in the SHR although an increased total content was shown in the young SHR. An explanation for the discrepancy is the difference in the normotensive control rats used in each experiment. Cutilletta et al. used the outbred WKY. However, the WKY/N in our present study is an inbred strain. Therefore, their gene constitutions are different from each other. Since a remarkable difference was not observed in total DNA content among the SHR strains used in these three experiments, it can be said that the nonmuscle cells of the SHR/N are hyperplastic in comparison with the WKY/N.

Hyperplasia of nonmuscle cells is also related to increased fibrous tissue. An increase of fibrous tissue content was reported in renal hypertensive rats and in the SHR. However, left ventricular fibrosis in the SHR remained essentially unchanged up to 25 weeks of age. Accordingly, hyperplasia of the SHR/N in the present study could not be related to the amount of fibrosis.

It is well established that the heart weight of the SHR is significantly greater even at the prehypertensive stage than that of normotensive rats. An increased heart weight was also observed in the SHRSP at the prehypertensive stage. We observed similar results in our present study, although the absolute heart weight of the SHRSP/N was not significantly greater than that of the WKY/N. In addition, hypertrophy of myocardial cells in the SHRSP/N was already initiated at the prehypertensive stage. These results clearly support the hypothesis that blood pressure is not the sole factor responsible for cardiac hypertrophy, although it must be remembered that the SHRSP/N and SHR/N have a higher blood pressure than that of the WKY/N. One possible cause of the cardiac hypertrophy in the SHRSP/N and SHR/N could be genetic predisposition, which might be dependent or independent of genetic factors related to the hypertension. The existence of genetic predisposition to myocardial hypertrophy does not rule out other possible causes of cardiac hypertrophy such as hemodynamic, neurohumoral, and pathological factors. However, these factors might also be partly under genetic control. In Part 1 of this study we showed that heart weight, not cardiac hypertrophy, is determined by genetic factors to a considerable degree; we use the term ‘‘genetic factors’’ to mean gene constitution or genetic background in a broad sense. Accordingly, the genetic predisposition that we postulate here composes a part of such genetic factors. The OM/N and M520/N strains also exhibited a large heart weight at a young age. However, little is known about cardiac enlargement in these strains.

In the present study, it was indicated that cardiac enlargement is determined as additive effect of the hypertrophy of myocytes and hyperplasia of nonmuscle cells, and that cardiac enlargement of each strain is characterized by the extent of hypertrophy and hyperplasia. Therefore, the effect of genetic predisposition or genetic factors upon cardiac enlargement might be manifested through such a difference in the response of myocardium. The character could be modified by blood pressure or other factors.

On the whole, right ventricular DNA concentration was higher than that of the left ventricle. This might be related to myocytes being smaller in the right than left ventricle, due to a dramatic change of right and left ventricular pressure after birth. Furthermore, the number of myocytes in the right ventricle is smaller than that of left ventricle since right ventricular myocytes lose mitotic activity more rapidly than those of left ventricle. However, the SHR/N exhibited right ventricular enlargement, although only at the early hypertensive stage, in agreement with the report of Cutilletta et al. Right ventricular enlargement was also indicated in the OM/N after the early hypertensive stage, although there was no difference in relative weight. The meaning of the right ventricular enlargement is still obscure, at present.

Comparative studies of hypertensive and normotensive vessels in human and other animal species indicat-
ed that the vessels of hypertensives are generally thicker and stiffer than those of normotensives. Furthermore, the media was apparently responsible for most of the increased thickness of these vessels since the intima was only slightly thickened in association with hypertension. It was shown that the tunica media in the SHR and SHRSN exhibits both hypertrophy and hyperplasia as compared with normotensive rats. Furthermore, experimental hypertension in rabbits caused an increased arterial wall thickness consisting of hyperplasia and hypertrophy. However, enlargement of the aorta in the present experiment was almost entirely due to hyperplasia except for the aorta of the SHRSP/N at the prehypertensive stage. Therefore, it is suggested that the existence of cellular hypertrophy is concealed by severe hyperplasia, as discussed above for the heart of the SHR/N.

It was shown in Part I that aorta weight is also a highly heritable trait, but that it is not as strongly influenced by genetic factors as is heart weight. It is likely that aorta weight is influenced by blood pressure or other factors, as suggested in heart weight, which seem to be manifested as hyperplasia. However, the M520/N revealed a significant increase of DNA concentration and total content at 16 weeks of age in spite of being normotensive and having a low aorta weight. This may reflect the trait of small aortic cells in the M520/N. The differences in RNA content were relatively small, and although statistically significant, it is difficult to evaluate their biological significance.

Further comparative studies concerning cardiovascular enlargement of these strains are needed for the development of more useful models for the study of cardiac hypertrophy that eventually results in heart failure and myocardial infarction.

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