Cardiac Hypertrophy in Early Postnatal Stroke-Prone Spontaneously Hypertensive Rats

Wei-Wen Kuo[1], Kwo-Chang Ueng[2], Tsung-Po Tsai[2], Der-Jinn Wu[2], Chung-Jung Liu[1], Jung-Min Yu[2], Yih-Shou Hsieh[1], Jer-Yuh Liu[1], Hsien-Ping Huang[1], Chih-Yang Huang[1*]

This study, for the first time, investigates the growth of the heart during the early postnatal period (1-20 days) in pups born to stroke-prone spontaneously SP and SF, respectively. Growth rate of the heart was measured by weight as well as by concentrations of total DNA, RNA, and protein. Measurements of growth of the heart in pups born to normotensive WKY rats served as controls. At day 1 postnatal, the heart weights of SP and SF pups were larger by 35% and 15% respectively, than that of WKY rats. Between 3 and 10 days, heart weight increases about 3-fold in SF and only 2-fold in SP and WKY. However, by 20 days, the heart weight of SF is the same as that of WKY and 15% less than SP. Relative water content of the hearts of the three strains did not differ at any time during the 20 days postnatal period. The hearts of the three strains showed an increased DNA concentration for up to 5 days, though the concentrations in SF and SP were significantly higher than those in WKY rats. By 20 days, however, all three strains had the same the DNA concentrations. At day 1 postnatal, only the hearts of SP pups had increased concentrations of RNA and protein. At 20 days, the RNA and protein concentrations were the same in all three strains. In summary, growth patterns of the hearts in SF and SP pups differ from those of WKY rats especially during the early postnatal period. Furthermore, the course of growth of the heart in SP pups in the early period after birth can be modified environmentally, i.e., by excess salt intake by the parent animals.

Key words: cardiac hypertrophy, stroke-prone spontaneously hypertensive rats, salt feeding

1. Institute of Biochemistry, Chung Shan Medical University, Taichung, Taiwan (R.O.C.)
2. Department of Medicine and Division of Cardiology, Chung Shan Medical University, Taichung, Taiwan (R.O.C.)
* Reprints and Corresponding Author: Chih-Yang Huang
Address: Institute of Biochemistry, Chang-Shan Medical University No. 110, Section 1, Chien Kuo N. Rd., Taichung, 402, Taiwan, R.O.C. Tel: 886-4-24730022 ext 1679 E-mail: chuang1@csmu.edu.tw

Introduction

Animal models for the study of hypertension began in the late 1950's and early 1960's [1]. Among these models, the spontaneously hypertensive rat (SHR) developed by Okamoto and Aoki [2] has been widely studied [1]. A stroke-prone SHR (SP) also has also been developed [3]. The SP model shows, spontaneously, a high incidence of cerebrovascular lesion. In the SP, the predisposition to strokes correlates with a high degree of hypertension [4]. This result is reminiscent of the situation in the human in which high blood pressure has been known for many years to be one of the causative factors of stroke [5,6]. The development of hypertension [3,7] and the predisposition to stroke [4] in SP are both genetically controlled. Further, an abnormally high salt intake further elevates blood pressure in SP [4] as it does in humans [8] and experimental animals including SHR [9]. SP develops a more severe hypertension within a shorter period of time than does SHR when given drinking water containing 1% saline [9]. Also, excessive salt-fed SP (SF) has a higher incidence of strokes than do non salt-fed SP [4].

Genetic hypertensive models, such as SHR and SP, are valuable for studies on the pathogenesis and treatment of hypertensive diseases [1,10,11]. In SHR cardiac hypertrophy (cardiomegaly) occurs in the early postnatal period, within 21-30 days after birth [12,13,14,15] in the absence of a sustained hypertension [7,16,17]. This early cardiac hypertrophy is exacerbated later (at about 10 weeks of age) when sustained hypertension occurs in SHR [2]. SP shows an even more severe hypertension than does SHR at 10-15 weeks of age [7], and this severe hypertension is accompanied by cardiac hypertrophy. However, whether or not an early cardiac hypertrophy also develops in SP in the early postnatal period as it does in SHR has not been investigated. Herein, we report on such an investigation using hearts obtained from SP pups at 1 to 20 days postnatal. Results show that cardiac hypertrophy was evident in SP rats compared to normotensive WKY rats as early 1 day postnatal. Furthermore, the pattern of growth of the heart in SP during the 20 days postnatal period could be modified by continuous excess salt intake by the SP parents.

Materials and Methods

Animals

Normotensive Wistar-Kyoto (WKY) and stroke-prone spontaneously hypertensive (SP) rats were originally obtained from the National Institutes of Health. Separate colonies for the WKY, salt-fed SP, and non-salt-fed SP rats were established and maintained in a University of IIIinois animal care facility. Male and female breeders were housed together at 60 days of age. In the case of the salt-fed SP, the breeders, upon pairing and thereafter, were given drinking water containing 1% salt ad libitum. The term SF animals refer to pups born to rats fed the drinking water containing salt. WKY and SP were given water without the added salt ad libitum. All animals were fed standard rat chow ad libitum. WKY, SP, and SF pups were used at 1, 3, 5,10, and 20 days after birth. Breeders and offspring were exposed to 14h light/10h dark cycle. All of the following procedures performed on the animals were in accordance with university and federal regulations, which govern the care and usage of animals involved in research. All rat pups were collected from the parents between 1 and 4 PM. Body weights were recorded and animals were sacrificed by decapitation. Hearts were removed by cutting the blood vessels at the atria, drained of blood, blotted, weighed, flash-frozen in liquid nitrogen and stored at -70°C until used. When dry

weights were determined, hearts were placed in at 78℃ oven after the initial weighing and reweighing at 24-h intervals until a constant dry weight was attained.

DNA, RNA, and Protein Measurements

Frozen hearts were thawed at room temperature, rinsed in 5 ml ice-cold distilled water, and completely homogenized in 1 ml of fresh cold distilled water (Brinkmann Polytron model PCU 111, setting 11). For 1 and 3 day old rat pups, three hearts were homogenized together. Hearts of 5 to 20 day old pups were homogenized individually. The volume of each homogenate was recorded, and a 100 μl sample was removed and frozen at 0℃ for subsequent total protein analysis according to method previously described by Bradford [18].

The remaining homogenate was used for total DNA and total RNA analyses. Two ml of ice-cold 10% trichloroacetic acid (TCA) were added and the mixture was kept on ice for 20 min and then centrifuged for 2 min in a clinical centrifuge at 1600xg. The supernatant was removed. The pellet was resuspended in 2.5 ml 1 N KOH and incubated at 37℃ for 20h. Then, 0.4 ml 6 N HCl was added, followed by 2 ml 5% TCA solution, and the mix was centrifuged as described above. The supernatant was then collected and analyzed for total RNA using the orcinol method [19]. The pellet was resuspended in 2.6 ml 5% TCA and incubated at 90℃ for 15 min. After cooling to room temperature, the suspension was centrifuged as described above. The supernatant was analyzed for total DNA by the diphenylamine method [20].

Blood Pressure Measurements

Using 20-day-old WKY, SP, and SF pups, systolic blood pressures were measured using the tail-cuff method (IITC Model Mark 12 with R.C. Electronics' Scopedriver [1SC-67] software) between 10 AM and 12 PM. Five readings from each animal were averaged.

Results

Table 1 shows body weights, heart weights, and heart weight to body weight ratios for neonatal WKY, SP, and SF pups. The body weights of all three strains were significantly different from each other at 1 and 3 days postnatal. During this early postnatal period, SF pups weighed significantly less and SP pups weighed significantly more than WKY pups. Therefore, salt feeding of the SP parents resulted in pups (SF) with small body weights early in the postnatal period compared to those born of non-salt-fed SP. Thereafter and up to 20 days postnatal, body weights were about the same for the 3 rat strains.

At day 1 postnatal, the heart weights of SF and SP pups are larger by 15% and 35%, respectively, than that of the WKY pups (Table 1). The growth rate of the heart then slowed between 1 and 3 days for SP, and even more so for SF, compared to WKY. During this period, heart weights increased 70%, 26%, and 19% for WKY, SP, and SF, respectively. Between 3 and 10 days postnatal, heart weights continued to increase in all three strains as expected. However, heart weight increased the most in SF, i.e., nearly 3-fold compared to only 2-fold in both SP and WKY during the same period of time. In contrast, between 10 and 20 days, increased in heart weight is the smallest in the SF pups and greatest in the SP pups. The heart weight of the SF was about the same as that of the WKY at this time but 15% less than that of the SP. Therefore, salt-feeding of the SP parents resulted in pups that have a postnatal heart growth pattern that differs from pups born to both the non-salt-fed SP parents and the normotensive WKY parents. These different heart growth patterns among

Table 1 Body weights, heart weights, and body weight : heart weight ratios of postnatal WKY, SP and SF rat pups

	Days Postnatal	WKY	SP	SF
BW(g)	1	5.78±0.10(47)	6.76±0.15(43)*	5.32±0.11(40)≠,*
	3	7.44±0.17(48)	8.11±0.21(23)§	6.64±0.19(36)**,*
	5	9.25±0.29(33)	10.19±0.46(12)o	10.87±0.37(19)*,o
	10	18.23±0.52(27)	18.28±0.80(17)o	19.44±0.64(24)o,o
	20	35.38±1.52(29)	36.25±1.03(29)o	33.88±1.40(19)o,o
HW(mg)	1	24.76±0.55(46)	33.46±1.21(43)*	28.56±0.89(39)*,**
	3	42.02±1.22(46)	42.04±1.16(22)o	34.06±0.83(34)*,*
	5	50.53±1.79(32)	59.17±1.67(12)*	53.26±3.31(19)o,o
	10	88.30±4.65(27)	86.94±4.09(17)o	98.33±3.39(24)≠,//
	20	172.80±7.95(26)	195.90±4.27(29)§	165.61±12.91(18)o,//
HW:BW(mg/g)	1	4.29±0.08(46)	4.94±0.14(43)*	5.41±0.15(39)* ≠
	3	5.71±0.16(46)	5.20±0.11(22)≠	5.29±0.16(34)o,o
	5	5.47±0.11(32)	5.96±0.35(12)≠	4.88±0.20(19)§,≠
	10	4.83±0.20(27)	4.76±0.11(17)o	5.07±0.09(24)o,//
	20	4.83±0.08(26)	5.51±0.21(29)+	4.81±0.16(18)o,//

Data are mean ±SEM. Number of animals given in parentheses. BW=body weight; HW=heart weight. Symbols given for SP and SF (first symbol) represent levels of significance compared to the corresponding WKY animals as determined by the sampling for one variable (comparison of means) test (Bailey, 1995, p.42). For SF, the second symbol represents the level of significance compared to the corresponding SP animals. *p<.001, **<.002,t <.OO5, ≠ < 01,§ <.02, // <.05, o=n.s.

the three strains were not due to differences in relative water content. Hearts were removed from SF, SP, and WKY pups at 1, 3, 5, 10, and 20 days postnatal and dried to a constant weight. Percent dry weight to wet weight ratios, when heart weights from all postnatal days were pooled, were 19.8 ±6% (mean ±SEM), 19.1 ± 0.6%, and 19.7 ±0.5% for the SF, SP, and WKY pups, respectively.

The heart weight to body weight ratios (Table 1) of the SF and SP pups at day 1 postnatal were higher than those of the WKY pups, with SF showing the highest ratio. This ratio further emphasized the finding that although the body weight of the SF was less than that of WKY at this time, the heart weight of SF was higher than that of WKY. Also, the ratios showed that, though the body weight of the SP pups at day 1 was larger than that of WKY, their heart weight showed an even greater increase. By 20 days postnatal, the heart weight to body weight ratio of SF had become similar to that of WKY. In contrast, the heart weight to body weight ratio of SP was higher than that of WKY.

Figure 1 shows the concentration of total DNA per mg of heart tissue at 1 to 20 day postnatal ages for the three different rat strains. All three strains showed an increased DNA concentration up to 5 days. DNA concentrations had changed little between 5 and 10 days, but they came to a common value by 20 days in all three strains. At day 1, both the SF and the SP pups had significantly higher DNA concentrations than

the WKY pups. Though the SF at day 1 postnatal showed, on an average, a higher DNA concentration than does the SP, the values for the two strains were not significantly different from each other. The DNA concentrations in the SF and the SP remained higher than those in the WKY for up to 6-10 days postnatal at least. However, the SF and SP DNA concentrations were not found to be significantly different from each other at any time measured. Therefore, salt feeding of the SP parents did not result in a significantly changed concentration of DNA in hearts of SP pups through 20 days postnatal. Similar results occurred when the total DNA content of the whole heart was considered (Fig. 2). Total DNA content of the SF and SP hearts was consistently higher than that of the WKY up to 6-10 days postnatal. Once again, the values for SF and SP were not significantly different from one another during this period. The DNA content of the three heart types all came to a common level by 20 days. DNA content increased essentially linearly in the WKY hearts throughout the 20 days. In contrast, the DNA contents of the SF and SP hearts increased almost linearly for only up to 10 days and then increased more slowly to 20 days.

Though both the heart weights (Table 1) and the DNA concentrations (Fig. 1) in the hearts of SF and SP pups were higher than the WKY pups at day 1, only the SP pups showed higher concentrations of RNA and protein at this time (Figs. 3,4). Therefore, salt-feeding of the SP parents resulted in pups whose hearts had total RNA and protein concentrations more like pups from WKY parents than pups from non-salt-fed SP parents. The lowered total RNA concentration in the hearts of the SF pups was further reflected in the total RNA to total DNA ratio of the hearts at day 1 postnatal (Fig. 5). This ratio was only about 45% of that in WKY pups whereas that of SP pups was almost the same as WKY. However, the total protein to total RNA ratio of the hearts of SF pups, though on the average diminished from that of WKY and SP pups, was not significantly different from the latter two strains at day 1 postnatal (Fig. 6).

At 20 days postnatal, when the DNA concentrations of the hearts of SF, SP, and WKY pups had come to a common level (Fig. 1), the concentrations of total RNA and total protein were also about the same in the hearts of all 3 strains (Figs. 3-6).

In SP rats, sustained hypertension is known to occur by about 30-40 days of age [7] and severe hypertension develops by 70-105 days [1,11]. Furthermore, salt feeding of these animals results in an even earlier onset of severe hypertension [4]. The question was asked here whether or not increased blood pressure would be found in pups born to salt-fed SP parents. The answer was that it was not, at least not at 20 days postnatal. The blood pressure of the SF pups at this time was not significantly different from that of the same aged SP and WKY pups (Table 2).

Discussion

This study, for the first time, investigates the growth of the heart in SP and SF pups during the early postnatal period. Growth of the heart was measured by weight as well as by concentrations of total DNA, RNA, and protein. Growth patterns of the hearts of pups born to both salt-fed SP and non-salt fed SP parents were different than that of pups born of normotensive WKY rats. Furthermore, salt feeding of the SP parents resulted in pups with hearts that had growth pattern altered from that of hearts in pups of non-salt fed SP parents.

Cardiomyocyte (CM) growth in the heart during the first 3 weeks postnatal has been divided into 3 phases, i.e., an initial hyperplastic phase lasting about 4 days followed by a transi-

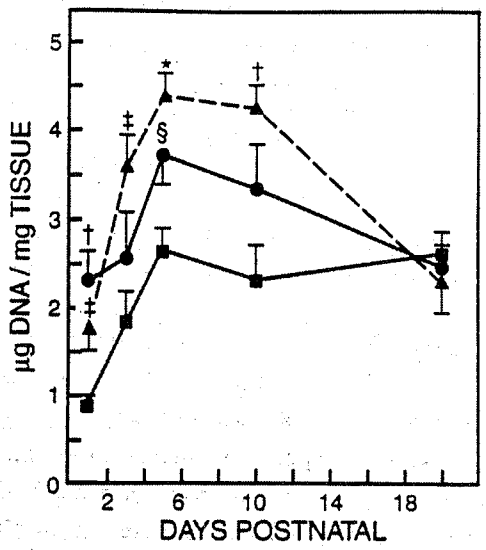

Fig. 1 The concentration of total DNA per mg wet heart tissue in SF (circle), SP (triangle), and WKY (square) pups as a function of postnatal age. Each data point represents the mean ± SEM for 6-14 animals. Symbols given for SF and SP represent levels of significance compared to the correspondingly aged WKY animals. * p<.001, + <.005, ≠ <01, § <02.

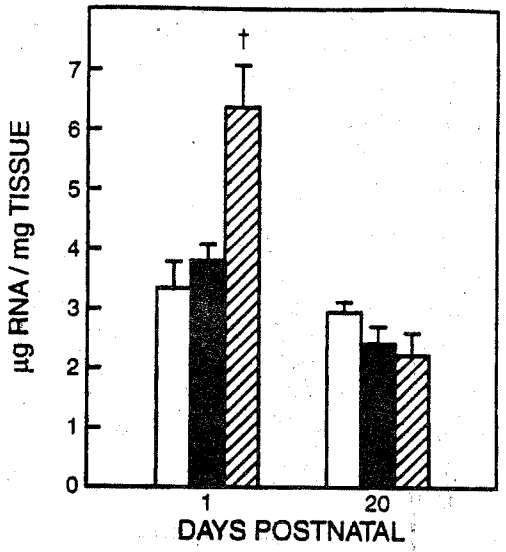

Fig. 3 The concentration of total RNA per mg wet heart tissue in SF (solid bar), SP (hatched bar), and WKY (open bar) pups as a function of postnatal age. Each data point represents the mean ± SEM for 3-12 animals. Symbol given for SF and SP represents level of significance compared to the WKY animals the same age. + p<.005.

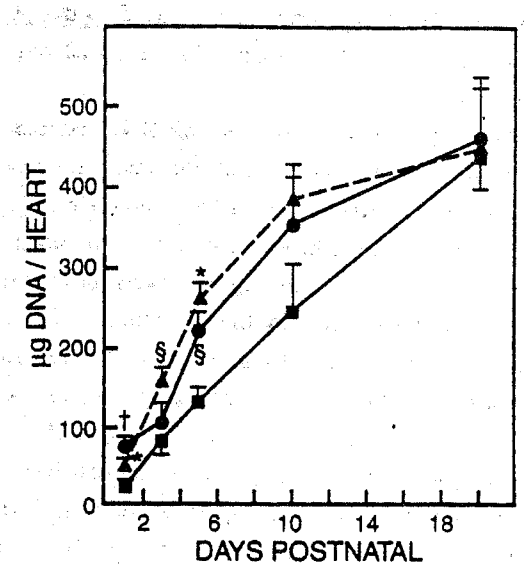

Fig. 2 The amount of total DNA per heart in SF (circle), SP (triangle), and WKY (square) pups as a function of postnatal age. Each data point represents the mean ± SEM for 6-14 animals. Symbols given for SF and SP represent levels' of significance compared to the WKY animals the same age. * p<.001, + <.005. § <.02.

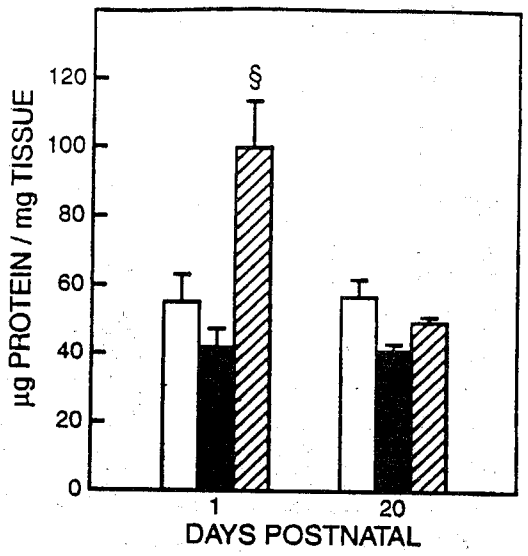

Fig. 4 The concentration of total protein per mg wet heart tissue in SF (solid bar), SP (hatched bar), and WKY (open bar) pups as a function of postnatal age. Each data point represents the mean ± SEM for 3-12 animals. Symbol given for SF and SP represents level of significance compared to the WKY animals the same age. § p<.02.

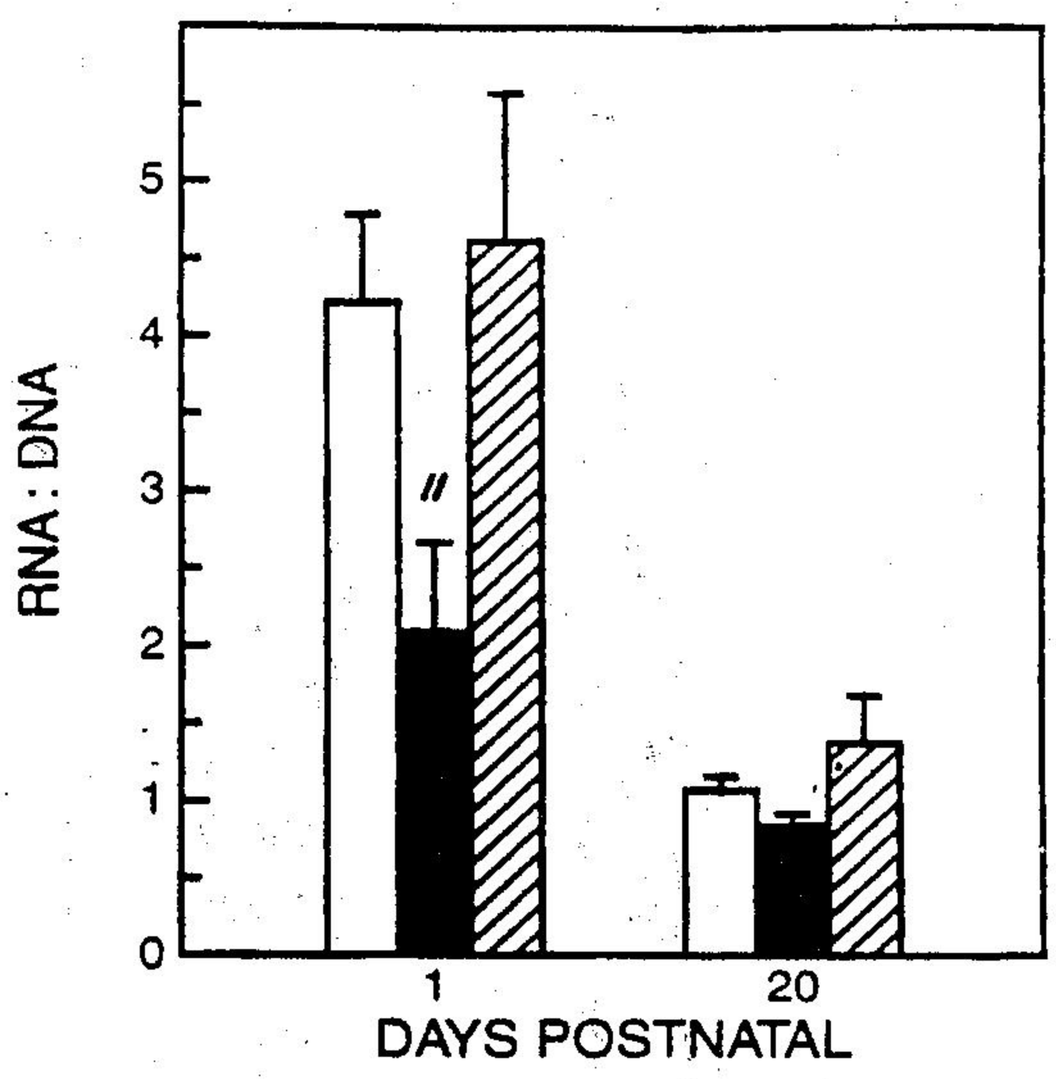

Fig. 5 The total RNA/total DNA ratio in SF (solid bar), SP (hatched bar), and WKY (open bar) pups as a function of postnatal age. Each data point represents the mean±SEM for 3-12 animals. Symbol given for SF and SP represents level of significance compared to the WKY animals the same age. $p < 0.05$.

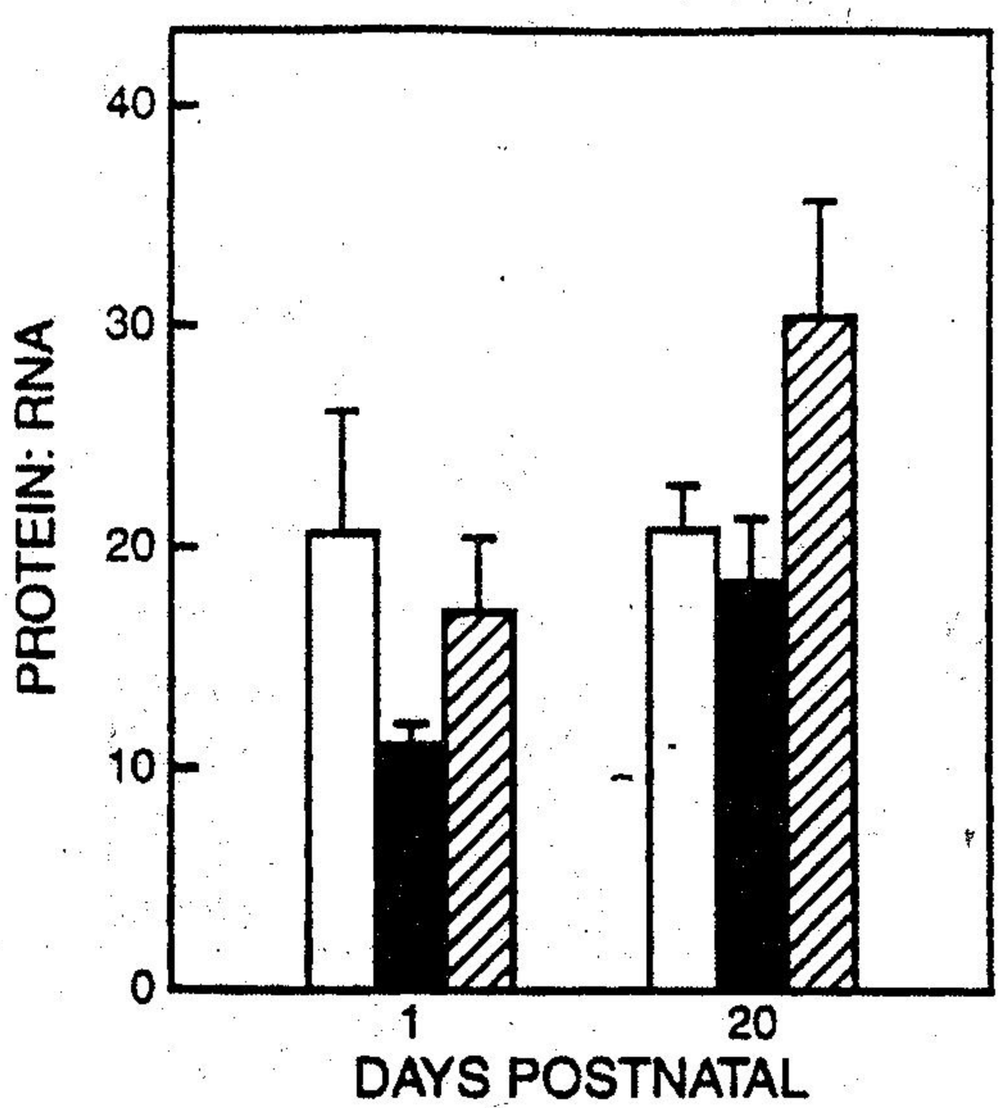

Fig. 6 The total protein/total RNA ratio in SF (solid bar), SP (hatched bar), and WKY (open bar) pups as a function of postnatal age. Each data point represents the mean±SEM for 3-9 animals.

Table 2 Systolic blood pressures of 20 day-old WKY, SP, and SF rat pups.

Animal	Blood Pressure (mm Hg)
WKY	106±7(3)
SP	113±1(4)
SF	113±7(4)

Data are mean±s.d. Numbers of animals given in parentheses.

tion phase from hyperplasia to hypertrophy lasting from about day 6 to day 14 and finally a predominantly hypertrophic phase which lasts to day 21[21,15]. CM's continue to proliferate to the age of weaning (21 days), but the major increase in CM number occurs within the first few days after birth [22,23,21,24]. The overall pattern of DNA concentrations in the hearts of the SF, SP, and WKY pups reflects this previously established CM growth pattern for the first 2O days postnatal. In each of the 3 strains, DNA concentrations increased most rapidly during the first 5 postnatal days, displayed no changes from day 5 to day 10 and finally came to a common value by 20 days postnatal.

The present measurements of DNA concentration and DNA content of the heart do not distinguish between CM's and non-CM's. The normotensive heart, however, is known to contain predominantly CM-s during the early postnatal period. For example, in the ventricles of the normotensive heart, CM's have been reported to account for 86% of the tissue volume at day 1, 81% at day 5, and 75% at day 11 postnatal [23]. Also, the number of diploid mononucleated CM's remains above 95% up to at least 4 days postnatal in the ventricles [23,24,21,17]. Therefore, in the present study, the rapid increase of DNA concentration in the heart up to 5 days postnatal indicated CM hyperplasia in the WKY pups (Fig. 1). The same conclusion holds for the SP and the SF pups, assuming there was no change in the relative distribution of CM's and

non-CM's or in the percentage of mononucleated CM's in these animals compared to the case in the WKY in the first 3 to 5 days postnatal.

In the hearts of SF, SP, or WKY pups, there was little difference in DNA concentration at 10 days compared to 5 days postnatal (Fig. 1), indicating a decreased level of hyperplasia in the hearts of all 3 strains during this time. This decrease corresponds with the expected transition phase from hyperplasia to hypertrophy which is known to occur in normotensive rats at this time [21,15]. The third phase, i.e. hypertrophy of CM's, was especially evident in the SF and SP pups which showed a decreased DNA concentration per unit heart weight at 20 days compared to 10 days postnatal.

A number of studies have determined the percentage of heart cells that incorporate 3H-tnymidine in early postnatal rats. The percentage of 3H-thymidine labeled CM's in both ventricles, both atria, and the intraventricular septum [25,21,17], as well as the percentage of labeled non-CM's in the ventricles and the intraventricular septum [21], is highest the first few days after birth, is decreased at 6-10 days, and is further decreased by 21 days. In addition, this labeling index pattern holds for both neonatal WKY and spontaneously hypertensive rats (SHR) [17]. The pattern of DNA concentration in the hearts of SF, SP, and WKY pups over the 21 day postnatal period in the present study (Fig. 1) closely correlates with this previously established 3H-thymidine-labeling index pattern.

By day 1 postnatal, the DNA concentrations in the hearts of SF and SP pups were 2.6-fold and 2-fold higher, respectively, than those in the hearts of WKY pups. This indicates an increased level of cell division in developing SP and SF fetuses before birth compared to the case with WKY fetuses. A similar conclusion was drawn by Oparil et al [15], who presented evidence suggesting that myocardial cells divide more often in utero in the SHR strain (from which the SP strain was derived) than in the normotensive WKY strain. The DNA content of a single diploid rat nucleus is 6.2 picograms [26]. Using this value, the data in figure 1, and the fact that the heart cells are mononucleated and diploid up to at least 4 days postnatal as mentioned above, the calculated number of cells per mg heart tissue at day 1 postnatal for the SF, SP. and WKY pups was 0.37 x 106, 0.28 x 106, and 0.14 x 106 cells, respectively. This result indicates that the average heart cell in both SP and SF pups is smaller than that in WKY pups. Further, the average cell in SF pups is smaller than those in either SP or WKY pups at day 1 postnatal. Even so, the increased weight of SF and SP hearts at this time (Table 1) was apparently due to the greatly increased number of cells per heart in these pups (0.37 x 106 and 0.28 x 106, respectively) compared to the number of cells in the WKY pups (0.14 x 106). Calculations similar to those above also showed that the hearts of SF, SP, and WKY pups contained about 0.41 x 106, 0.58 x 106, and 0.29 x 106 cells per mg of tissue, respectively, at 3 days postnatal. At this time, the heart weight of SP was the same as WKY and that of SF was smaller than WKY. At 5 days postnatal, the hearts of the SF. SP, and WKY pups contained about 0.60 x 106, 0.70 x 106, and 0.42 x 106 cells per mg tissue, respectively. However, at this time, the heart weight of the SP was only 17% higher than WKY and that of SF was not significantly different than WKY. Also, the percent water conten t did not differ in the hearts of the 3 strains throughout the postnatal period. Therefore, during the hyperplastic phase of heart growth (1 to 5 days postnatal), the average cell size in SF and SP remained smaller than in WKY.

After 5 days, similar calculations and analyses were difficult to do because the degree of hypertrophy of CM's in SF and SP vs. WKY

was not yet known. Differences among the strains might be expected because, for example, the cardiomegaly determined in SHR (the parent strain of SP), during the transitional and hypertrophic phases of heart growth is due to a greater increase in myocardial cell volume than in WKY [15]. Furthermore, the degree of hyperplasia occurring in SF and SP vs. WKY was not known, though at 21 days postnatal, the number of CM's in the heart is known to be the same in SHR as in WKY [16]. Whether or not the number of CM's in SF or SP is the same as in WKY at 21 days is not yet known. At this time, the heart weights of SF and WKY were about the same while that of SP was 13% to 18% larger (Table 1). In any case, it cannot be determined yet to what degree hypertrophy of CM's vs. hyperplasia of CM's and non-CM's contribute to these weight differences in the three strains. Interestingly though, the levels of RNA, protein, and DNA per mg heart tissue as well as the RNA:DNA and protein: RNA ratios are essentially the same in all three rat strains at 20 days (Figs. 1, 3-6).

At birth to one day, the SP pups share several characteristics with pups of the SHR strain from which they were derived. For example, compared to the normotensive WKY, SHR shows a cardiomegaly [13,27,15,28], an increased heart weight to body weight ratio [28], and increased DNA and protein concentrations in the heart [28]. The same results were observed with 1-day old SP in the present study (Table 1, Figs. 1, 3, 4). Furthermore, between 1-3 days postnatal, the increase in heart weight in both SHR [15] and SP (Table 1) is less than that in WKY. The altered myocardial development in the early neonatal period in SHR compared to WKY could be genetically based and/or the result of differences in the in utero environment [15,28]. In any case, since the early postnatal cardiomegaly that occurs in SP parallels that of SHR, it is likely that similar genetic and/or environmental influences are at work in both strains.

Further and interestingly, salt-feeding to the SP parents results in pups with certain physiological differences from those born to non-salt fed SP parents in the early postnatal period, especially. For example, at day 1 postnatal, body weights and heart weights of SF are less, while the heart weight to body weight ratio is higher than those of SP (Table 1). Also, though the DNA concentration of the SF heart is not significantly different than that of the SP (Fig. 1.), both the RNA and protein concentrations (Figs. 3 and 4) were significantly less in the SF. Thus, the course of cardiomegaly in the SP pups in the early postnatal period can be modified environmentally, i.e.. By excess salt intake by the parent animals.

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