THE HEMODYNAMICS OF EARLY STAGES OF SPONTANEOUS HYPERTENSION IN RATS

PART I: MALE STUDY

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Male spontaneously hypertensive rats (SHR) were tested for the cardiac output and organ blood flows at the age 30, 40, 50, 60, 90 and 180 days. The results showed that during the course of developing hypertension in SHR the cardiac output was not elevated. During the prehypertensive period, total peripheral resistance was not elevated, but systolic blood pressure was higher in SHR than in normotensive rats. Increased contractility of the myocardium in SHR was considered to be involved in the pathogenesis of hypertension. Decreased kidney blood flow in SHR after 90 days of age might be related to the high incidence of nephrosclerosis in 180 days old male SHR.

PRELIMINARY experiments¹ have shown that in spontaneously hypertensive rats (SHR) there is a lower pulse rate, higher blood pressure and peripheral resistance than in normals. Values of cardiac output in 50 days old SHR were not substantially different from normotensive controls at the same age. In 9-month old female SHR, cardiac outputs were significantly lower than normotensive controls.²

These results show that the haemodynamics of SHR vary as functions of age and duration of the hypertension. Aoki *et al.*³ subdivided SHR by pathological criteria and blood pressure levels into three stages; prehypertensive, early hypertensive and fully developed and advanced hypertension. It is of interest that Aoki's pre-hypertensive stage ends at age 60 days, i.e. in that period when a number of hemodynamic changes were observed to occur to meet the tissue requirements in rapidly growing normoten-

sive animals. After the age of 60 days the balanced state of adult animal sets in.

It would therefore be justifiable to pose the question — what differences exist between haemodynamic kinetics in the prehypertensive and early hypertensive stages? No such comparison has yet been presented, although high cardiac output (CO) have been reported in the pre-hypertensive stage of rats with renal and salt hypertensions^{4,5} which has been considered to be similar to the initial stages of essential hypertension.

The present results were obtained by the observations on Okamoto-Aoki's SHR strain which had been presented to this laboratory by the Department of Pathology of Kyoto University in 1970.

METHODS

SHR males of the 26th and 27th generation (3rd and 4th generation in this laboratory) of the Okamoto-Aoki strain were used. Haemodynamic measurements were carried out at the age of 30, 40, 50, 60, 90 and 180 days, and were compared with normotensive Wistar strain controls of the same age from our own stock.

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Fig.1. Blood pressure in male rats. SHR=Spontaneously hypertensive, NR=normotensive rats. Description see text.

Surgical procedures and all measurements were carried out under Inactin anaesthesia, 10 mg/kg i.p. Blood pressure was measured through a carotid cannula connected to an Elema

Schönander strain gauge-electromanometer-Mingograph system, which also gave the pulse waves on paper, along with electrically integrated mean values. The record was used to read pulse rate, systolic and diastolic and mean pressures. CO was measured by Evans Blue dye dilution. The injected amounts into jugular vein were 50 μ l of 1% (w/v) solution. Blood samples from the carotid artery were collected on a rapidly rotating fraction collector under free flow conditions, and the dye concentration in the plasma was determined photometrically. These procedures allowed us to construct a dye dilution curve and calculate CO. Organ blood flows were determined by the Sapirstein technique⁶ of ^{86}Rb distribution 60 sec after i.v. injection. Organs used for radioactivity determination were removed post mortem and weighed accurately (± 0.5 gr precision) with a torsion balance.

RESULTS

Fig. 1 shows a comparison of blood pressure in SHR (polka dot area) and normotensive rats (squared area). The lines indicating the upper edge of the areas connect mean values of systolic pressure, and the lower border lines connect mean diastolic values in the separate age groups. The y axis of the area shows the magnitude of the pulse pressure. This graph shows that in both control and SHR mean blood pressure values

TABLE I BLOOD PRESSURE IN SPONTANEOUSLY HYPERTENSIVE AND NORMOTENSIVE MALE RATS

SHR					Normotensive rats			
Systolic pressure	Diastolic pressure	Mean pressure	Pulse pressure	Age group	Systolic pressure	Diastolic pressure	Mean pressure	Pulse pressure
141,57 (22,18)	87,43 (16,58)	112,29 (18,51)	53,43 (7,18)	30	104,00 (6,04)	68,23 (6,10)	83,40 (10,57)	35,80 (4,21)
127,00 (11,63)	85,75 (13,96)	105,85 (12,45)	41,25 (6,08)	40	115,40 (7,33)	83,40 (5,08)	101,00 (5,96)	31,80 (4,44)
155,63 (20,02)	107,00 (13,85)	129,50 (15,62)	48,75 (7,38)	50	105,50 (4,12)	80,00 (2,71)	93,00 (3,46)	26,60 (3,32)
186,17 (13,41)	134,83 (12,29)	159,67 (14,22)	51,50 (3,27)	60	118,50 (6,95)	89,75 (7,76)	106,50 (4,51)	28,75 (2,87)
231,25 (16,19)	173,75 (11,38)	195,75 (14,38)	56,75 (5,56)	90	133,20 (15,83)	105,60 (12,46)	118,00 (10,07)	27,40 (5,50)
221,00 (31,27)	164,50 (18,38)	189,50 (25,96)	56,50 (16,11)	180	143,00 (9,00)	112,00 (5,29)	126,00 (6,00)	31,00 (4,36)

Units: Blood pressures in mm Hg
Figures in parentheses: S.D. of the mean

Fig. 2. Development of the total peripheral resistance and cardiac output in spontaneously hypertensive (SHR) and normotensive (NR) male rats. Cardiac output: open circles=normotensive, filled circles=hypertensive rats. Solid lines indicate the development of total peripheral resistance in two groups of animals. Significant differences: double arrows = $p < 0.01$.

started to rise between ages 40 and 60 days, and this increase continued to age 90 days. In both groups there were no further rises between ages 90 and 180 days. In SHR there was a higher pulse pressure, mainly due to higher systolic pressure. In the controls, pulse pressure decreased from age 50 days and then increased again only slightly. Mean blood pressure values are given in Table I. The significant differences between controls (NR) and SHR in systolic (SBP), diastolic (DBP) and mean (MBP) values were noted.

As shown in Fig. 2, CO (ml/100 gr body wt) was decreased in SHR from the age of 30 days (filled circles). The most rapid rate of fall was observed between 50 and 60 days. As compared with controls (NR, open circles), CO of SHR was significantly low by the age of 60 days. There was no difference in CO between NR and SHR at the ages of 90 and 180 days. In the age range 30–40 days there was no difference in total peripheral resistance (TPR) between SHR and NR. A rapid rise in TPR was observed in the SHR from 40 to 60 days, and a further increase from

TABLE II CARDIAC OUTPUT, HEART RATE AND TOTAL PERIPHERAL RESISTANCE IN SHR AND NORMOTENSIVE RATS

SHR					Normotensive rats			
Cardiac output	CO/100g of b.w.	Heart rate	TPR	Age group	Cardiac output	CO/100g of b.w.	Heart rate	TPR
40,90 (5,98)	51,21 (6,63)	331,00 (23,52)	178,80 (48,12)	30	44,60 (8,93)	47,60 (6,17)	380,40 (18,64)	141,10 (30,61)
56,10 (6,03)	47,13 (6,81)	332,00 (33,74)	184,10 (46,56)	40	68,58 (11,95)	46,34 (6,29)	401,20 (13,90)	180,26 (20,33)
65,71 (9,32)	41,57 (5,55)	334,75 (29,75)	256,64 (40,97)	50	80,75 (5,18)	44,88 (2,35)	347,50 (24,01)	165,92 (13,26)
47,35 (7,36)	26,33 (4,36)	347,00 (34,80)	482,08 (106,88)	60	91,85 (4,14)	36,60 (1,89)	387,00 (37,15)	266,65 (9,18)
71,33 (5,99)	34,82 (2,63)	362,75 (23,20)	450,35 (40,63)	90	99,66 (10,71)	32,24 (3,49)	376,00 (24,51)	277,26 (45,46)
86,93 (13,28)	25,72 (2,70)	290,00 (9,09)	589,95 (71,31)	180	97,50 (13,16)	21,03 (1,89)	350,67 (22,03)	482,27 (59,89)

Units: cardiac output ml/min
cardiac output ml/min/100g of b.w.
per 100g. b.w.
Heart rate beats/min
total 10^3 dyn.sec. cm^{-5} /100g of
peripheral body weight
resistance (TPR)

Figures in parentheses: S.D. of the mean

Fig.3. Heart weight, heart-weight/body-weight ratio, and heart blood flow in spontaneously hypertensive (filled circles) and normotensive (open circles) male rats. For explanation see text. Significant differences indicated by arrows: Single arrow= $p < 0.05$, double arrows= $p < 0.01$.

90 to 180 days. The rise in TPR in the NR was most marked between 50 and 60 days, but after the age of 50 days TPR of the NR remained significantly lower than in the SHR.

As shown in Fig. 3, the absolute weight of the myocardium of SHR (middle portion of the graph) was significantly higher at the age of 90 days and thereafter. The relative weight of the myocardium in NR decreased from the age of 30 days, and by the age of 40 days it was significantly lower than in SHR. The relative weight of the myocardium in SHR between 30 and 60 days of age was not significantly altered, but began to increase somewhat after 60 days.

Fig.4. Blood flow through the skin, skeletal muscle and kidneys in spontaneously hypertensive (filled circles) and normotensive (open circles) male rats. Significant differences indicated by arrows.

The myocardial fraction of CO (upper portion, lower pair of curves) rapidly increased between 50 and 60 days in SHR, with a further but slower increase up to 90 days and a slight decrease again at the age of 180 days. After the age of 60 days these differences were significant. Myocardial blood flow per unit tissue weight (upper portion, upper pair of curves) did not differ between SHR and NR – with the exception of age 50 days. Therefore, we can state that the cardiac hypertrophy of SHR is not associated with myocardial hypoxia.

As shown in Fig. 4, during development of the NR skin blood flow (upper portion) increased between 30 and 50 days of age and then decreased to values only slightly lower than had been at the age of 30 days. In the SHR at the age of 30–60 days there was a progressive decrease in flow – after age 90 days the flow was the same as in the NR. Muscle blood flow (middle portion) – the “white” muscle from the forelimbs was considered as representative – showed a) decreasing tendency in NR and SHR, and b) no difference between the two groups.

Renal blood flow (RBF) (lower portion of Fig. 4) as a fraction of CO (lower two curves) increased in NR from 30 to 90 days and did not change thereafter. In SHR at the age of 50 days RBF was lower, at 60 days only slightly higher than NR, but after 90 days of age SHR values were consistently lower than NR. RBF expressed as ml/gm tissue per min. (upper pair of curves) was lower in SHR after the age of 50 days and remained low so far as observed for 180 days. There were no intergroup differences in the absolute and relative weights of kidney, except the age of 180 days when the SHR showed lower values than NR.

DISCUSSION

In the prehypertensive period of SHR — 30 — 60 days of age — there occur haemodynamic changes similar in character to those observed in normotensive rats. Blood pressure and total peripheral resistance both increased and CO/100 gr body weight decreased. All of these parameters are clearly adapting to the demands of the growing organism. The maximum rate of change is found during 40 and 60 days of age. In SHR these changes are more pronounced, so that the final balanced state of the adult animal is shifted beyond that found in normals. The following questions can be put;

- 1) What is the locus of the high total peripheral resistance (TPR) in SHR?
- 2) What causes the increase in resistance?
- 3) What is the basis of the time course of normal changes in NR and pathological change in SHR?
- 4) Is there any correlation between the time of onset of the hypertension and maturation of any particular organ system in the animal?

The answer to the first question appears so far to be simple. In male SHR the high TPR is not localized to either skin or muscle — the main source of the increase would appear to be the kidneys, which may correspond to the high incidence of nephrosclerosis in SHR at the age of 180 days. The other source of high TPR can be sought in the mesenteric bed, in which blood flow data are not yet available but post mortem analysis has shown a higher incidence of periarteritis nodosa in SHR. Since both gut and kidneys take up a large fraction of CO, a small change of resistance in these organs would make a large systemic difference.

The second question goes to the root of the pathogenesis of hypertension in this model, and

can only be answered hypothetically. It is clear that the answer is related to the pre-hypertensive period, since TPR in NR and SHR at age 30–40 days is the same. Diastolic pressure of SHR is slightly increased at the age of 30 days, but the same at the age of 40 days. However, systolic pressure soon begins to rise in SHR. Therefore, pulse pressure is a differentiating factor. The increased systolic pressure would be clearly related to ejection force from the left ventricle, and so we can focus our attention on the myocardium. Calculated diastolic volumes do not suggest that this parameter is increased in SHR. The increased TPR could then be interpreted as an autoregulatory reaction of the arteriolar musculature to a raised pulse pressure. The third question of time correlations to the hypertension suggests that attention should be given to the maturation of sex organs and to a rapid rate of growth in general. In the previous work⁷ the effect of castration and testosterone administration on the developmental haemodynamic changes was tested. The results did not suggest the role of the gonads in the hypertensive reaction. Active growth of body mass — along with growth of the vascular system — in combination with the protein — anabolic effect of testosterone, could, however, play an important role.

The fourth question represents a search for the reasons for an altered myocardial contractility. *Okamoto et al.*⁸ showed that esterase isozyme patterns of SHR on starch gel electrophoresis were different from those of NR, and that this difference was hereditary along with the hypertensive tendency. It could not be excluded, therefore, that we are dealing with an inborn enzyme abnormality which results in myocardial hyperactivity. Again, this is a hypothesis which requires further studies.

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