A GENETIC STUDY OF HYPERTENSION IN OKAMOTO-AOKI SPONTANEOUSLY HYPERTENSIVE RATS

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SUMMARY

Data from crosses between Wistar/Furth rats and spontaneously hypertensive rats indicate that there are a small number of genes (3 to 4) controlling the hypertension trait in the Okamoto-Aoki spontaneously hypertensive rats. However, F_1 rats bred from reciprocal crosses of Sprague-Dawley rats and spontaneously hypertensive rats were hypertensive. F_2 rats bred from these $F_1 \times F_1$ rats showed a 3 to 1 segregation ratio of hypertensive to normotensive rats. Furthermore, male progeny from Sprague-Dawley $\times F_1$ backcross segregated into a 1 : 1 ratio of hypertensive to normotensive rats. These data suggest that among those 3 to 4 genes that control hypertension in the spontaneously hypertensive rats there is at least one major gene. This locus is not sex-linked but its expression is modified by sex differences.

1. INTRODUCTION

A ROLE of heredity in the development of essential hypertension in man was first suggested by Wilhelm Weitz in 1923 (Bauer, 1968) and subsequently documented by other investigators (Bauer, 1968; McKusick, 1960). The mode of inheritance of this syndrome, however, is not yet clear. Hamilton *et al.* (1954) concluded from their studies that blood pressure is a multifactorial trait. Pickering (1961) subsequently maintained that blood pressure is a continuous trait and hypertension is merely the upper end. In contrast, Platt (1959) proposed that the pattern of inheritance may be a result of the action of a single gene with incomplete dominance for essential hypertension. Platt's hypothesis is supported by the studies of Morrison and Morris (1959) and by those of McDonough, Garrison and Hames (1964).

The influence of hereditary factors on the development of essential hypertension in laboratory animals has been summarised by Schlager (1972). In mice, rats, chickens and rabbits the estimate of heritability for the variation in blood pressure is about 20 per cent and the involvement of more than one gene is indicated. Among these species, the Okamoto-Aoki spontaneously hypertensive rats (SHR) are unique in that the incidence of hypertension is 100 per cent (Okamoto and Aoki, 1963). Substantial progress has been made in recent years on the study of these rats (Okamoto, 1972). However, the genetics of these rats is not well understood. During the course of this

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study, reports of two independent studies on the genetics of SHR have appeared (Louis, Tabei, Sjoerdsma and Spector, 1969; Tanase, Suzuki, Ooshima, Yamori and Okamoto, 1970, 1972). In both studies, only Wistar rats, inbred or non-inbred, were used as normotensive rats to mate with SHR. In our study, both inbred Wistar/Furth rats (WFR) and non-inbred Sprague-Dawley rats (SDR) were crossed to SHR. Results of both series of experiments are compared in this report.

2. MATERIAL AND METHODS

Eleven Okamoto-Aoki SHR were kindly supplied by Professor K. Aoki of Nagoya City University, Nagoya, Japan. These rats belonged to the 16th generation of his inbred colony. Two male and two female siblings of these 11 SHR were bred to produce all the first-generation SHR in the Lilly colony. The blood pressure of these four rats was monitored from 25 to 70 weeks of age. That of the two female SHR varied from 168 to 199 mm Hg and the two male SHR, 182 to 196 mm Hg. Inbred WFR were purchased from Microbiological Associates, Walkersville, Maryland. SDR were supplied by Southern Animal Farms, Prattville, Alabama. Purina Laboratory Chow and water were available *ad libitum*. The room was lighted with fluorescent lights from 6 a.m. to 6 p.m. daily.

Systolic blood pressure was measured by the modified method of Friedman and Freed (1949). The validity of this method in measuring the systolic blood pressure of SHR has been reported (Pfeffer, Pfeffer and Frohlich, 1971). The whole animal was warmed for 30 to 45 seconds with a microwave oven set at 32° C. to assure audibility of the systolic pressure in the tail of each rat. The animal was restrained in a Lucite holder after being removed from the microwave oven. The systolic sound was picked up distal to an occlusion cuff, 1 inch in diameter, with a crobon microphone element and was amplified with a high gain audio-amplifier. The blood flow in the tail was occluded by a small membrane cuff that was attached to a sphygmomanometer. An average of three determinations was taken as the systolic blood pressure. All blood pressure data in this report are expressed as mean $\pm$ s.d. in terms of mm Hg.

3. RESULTS

(i) *Crosses between Wistar/Furth and spontaneously hypertensive rats*

The blood pressure of our third-generation SHR, WFR, F_1 ($WFR \times SHR$) and F_2 ($F_1 \times F_1$) rats is shown in table 1. The relationship of their blood pressure can be illustrated in the following mathematical form:

1. Male SHR > female SHR.
2. Male WFR = female WFR.
3. $F_1 < (WFR + SHR)/2$. (Male, $P < 0.05$; female, $P < 0.01$.)
4. $F_2 > F_1$. (Male and female, $P < 0.01$.)
5. Male $F_2 = (\text{male WFR} + \text{male SHR})/2$.
6. Female $F_2 > (\text{female WFR} + \text{female SHR})/2$. ($P < 0.05$.)

The distribution of blood pressure of these four types of rats is presented in fig. 1. The blood pressure of F_2 rats had a wider spread than that of F_1 rats ($P < 0.01$ for males and $P < 0.05$ for females).

TABLE 1

Mean blood pressure (mm Hg) of Wistar/Furth rats (WFR), spontaneously hypertensive rats (SHR) and rats derived from their matings

	Age (weeks)	Male		Female	
		N	$\bar{X} \pm \text{s.d.}$	N	$\bar{X} \pm \text{s.d.}$
WFR	14-15	49	142.5 $\pm$ 6.6	50	143.7 $\pm$ 4.9
SHR*	17-20	29	196.3 $\pm$ 13.0	15	176.1 $\pm$ 7.6
F ₁ (WFR $\times$ SHR)	18	38	165.1 $\pm$ 6.2	37	154.2 $\pm$ 7.5
F ₂ (F ₁ $\times$ F ₁)	17-18	72	171.0 $\pm$ 11.6	61	163.4 $\pm$ 10.2

* Third-generation SHR of the Lilly colony.

FIG. 1.—Distribution of mean blood pressure of Wistar/Furth (W) and spontaneously hypertensive (SH) rats and rats derived from their matings. F₁ rats were from female Wistar/Furth $\times$ male SH and F₂ rats were from F₁ $\times$ F₁. The length of the horizontal bars represents the number of rats with the shortest length equivalent to one rat. The triangle on the left of each column indicates the mean blood pressure of that group of rats. The number of rats per type is the same as that shown in table 1.

TABLE 2

Mean blood pressure (mm Hg) of Sprague-Dawley rats (SDR), spontaneously hypertensive rats (SHR) and rats derived from their matings

	Age (weeks)	Male		Female	
		N	$\bar{X} \pm \text{s.d.}$	N	$\bar{X} \pm \text{s.d.}$
SDR	16	50	146.7 $\pm$ 6.9	50	136.9 $\pm$ 7.3
SHR*	17-21	22	184.0 $\pm$ 10.1	18	178.7 $\pm$ 11.3
F ₁ † (SDR $\times$ SHR)	18-19	64	177.6 $\pm$ 7.2	67	166.9 $\pm$ 8.2
F ₂ (F ₁ $\times$ F ₁)	16-18	44	172.0 $\pm$ 10.6	54	157.0 $\pm$ 9.5
BC (SDR $\times$ F ₁)	15-16	19	161.0 $\pm$ 16.5	32	143.4 $\pm$ 10.4

* First-generation SHR of the Lilly colony.

† The blood pressure of F₁ rats from SDR $\times$ SHR was not significantly different from that of F₁ rats from SHR $\times$ SDR. Therefore, the data on F₁ rats in this table are pooled from reciprocal crosses.

(ii) *Crosses between Sprague-Dawley and spontaneously hypertensive rats*

The blood pressure of our first-generation SHR (table 2) was lower than that of our third-generation SHR (table 1). The rise of the blood pressure of the third-generation SHR could be due to the stabilisation of the environment. Reciprocal crosses were done between SHR and SDR. The blood pressure of F_1 rats from SHR $\times$ SDR was not different from that of F_1 rats from SDR $\times$ SHR. Thus, the mean blood pressure of all F_1 rats is shown in table 2. The relationship of blood pressure of five types of rats shown in table 2 can be illustrated in the following mathematical form:

1. Male SHR > female SHR.
2. Male SDR > female SDR.
3. $F_1 > (\text{SDR} + \text{SHR})/2$. (Male and female, $P < 0.01$.)
4. $F_1 > F_2$. (Male and female, $P < 0.01$.)
5. $F_2 = (\text{SDR} + \text{SHR})/2$.
6. Male BC = (male F_1 + male SDR)/2.
7. Female BC < (female F_1 + female SDR)/2. ($P < 0.01$.)

The distribution of the blood pressure of these various types of rats is shown in fig. 2A and B. Both male F_2 rats and rats from (SDR $\times$ F_1) backcrosses had a wide distribution of blood pressure ranging from normotension to hypertension.

In order to classify F_2 rats into normo- and hypertensive, a discriminant function was obtained for SDR and hypertensive rats. In this case, the hypertensive rats were composed of both SHR and F_1 rats. Since the blood pressure of SHR and that of F_1 rats overlap extensively (fig. 2A and B), it is difficult to keep F_1 rats in a separate category. The discriminant function is:

$$z = 17.5333 - 0.9948 X_1 - 0.1015 X_2, \quad (1)$$

where X_1 = sex ($X_1 = 1$ for males and $X_1 = 2$ for females); and X_2 = blood pressure at 16-21 weeks of age.

For each rat, a discriminant score was thus computed. The distribution of discriminant scores for normal rats and hypertensive rats (both SHR and F_1 rats) is given in fig. 3. The cut-off point for classifying F_2 rats into normal or hypertensive is set at 0.228, which establishes the probability for misclassification in either direction at 0.023. From our results, four of the 100 SDR would be classified as hypertensive and two of 171 SHR and F_1 rats would be classified as normotensive. Thus, only six out of a total of 271 rats would be misclassified. It should be noted that at $z = 0.228$, X_2 (blood pressure) = 160.7 for males and 150.9 for females (equation (1)).

Based on the discriminant function given in (1), a discriminant score ($\hat{z}$) was computed for each F_2 rat. The rat was classified as hypertensive if its discriminant score was less than or equal to 0.228; otherwise, it was classified as normotensive. The results of classification are given in table 3. In fitting the number of hypertensive rats and that of normotensive rats to a 2 to 1 ratio, a χ^2 value of 0.217 is obtained with Yate's correction. The probability level for this χ^2 is between 0.6 to 0.7.

FIG. 2.—Distribution of mean blood pressure of Sprague-Dawley (SD) rats, spontaneously hypertensive rats (SH) and rats derived from their matings. F₁ rats were from reciprocal crosses of SD × SH. F₂ rats were from F₁ × F₁. The backcross rats (BC) were from SD × F₁. The length of the horizontal bars represents the number of rats with the shortest length equivalent to one rat. The triangle on the left of each column indicates the mean blood pressure of that group of rats. The number of rats per type is the same as that indicated in table 2. (2A) Male rats. (2B) Female rats.

FIG. 3.—The distribution of discriminant scores for normal rats and hypertensive rats (both SHR and F_1 rats from reciprocal crosses of SDR $\times$ SHR). See text for the computation of discriminant scores. $Z = 0.228$ is used to classify F_2 rats as normotensive or hypertensive to make the probability for misclassification in either direction 0.023.

TABLE 3
Classification of F_2 rats

	No. of F_2 rats	Expected No. for a 3 : 1 ratio
Normal	22	24.5
Hypertensive	76	73.5
Total	98	98

$$\chi^2 \text{ (Yates Correction)} = 0.217 \quad 0.6 < P < 0.7.$$

4. DISCUSSION

Our data from crosses between WFR and SHR are compatible with those of Tanase *et al.* (1970, 1972). The blood pressure of F_1 rats was slightly lower than the average blood pressure of the parental rats (table 1 and fig. 1). The same was observed by Tanase *et al.* on both male and female F_1 rats from SHR $\times$ Wistar-Kyoto and on female F_1 rats from SHR $\times$ Wistar-Mishima (Tanase, 1970). Also, neither we (fig. 1) nor Tanase *et al.* (1970) observed any single gene segregation among the F_2 rats. This suggests that there is more than one gene involved in controlling the hypertension trait in these rats. Based on the data from WFR $\times$ SHR, our estimate of number of loci according to the method of Wright (1952) indicates that there are 2.75 (from data on female rats) to 3.76 (from data on male rats) genes involved. This estimate agrees well with that made by Tanase *et al.* (1970).

On the other hand, crosses between SDR and SHR suggest that among

these three to four genes, there is at least one major gene. This is indicated by the 3 to 1 segregation ratio among the F_2 rats (table 3). Furthermore, among the 19 backcross males, 10 could be classified as hypertensive and 9 normotensive according to their discriminant scores as calculated according to equation (1), thus fitting a 1 to 1 ratio. The classification of the backcross female into a hypertensive group and a normotensive group does not fit a 1 to 1 ratio. The reason for that is discussed in the next paragraph.

In this study, the blood pressure of all male rats was higher than that of corresponding types of female rats except for WFR. However, the blood pressure of F_1 rats from reciprocal crosses of $SDR \times SHR$ was not significantly different, suggesting that this major gene is not sex-linked. Also, we have not observed any maternal or paternal influence as reported by Tanase *et al.* (1970, 1972). Since bilateral gonadectomy did not affect the blood pressure of SHR (Aoki, 1963), the different balance of sex hormones, gonadotrophins and corticosteroids in males and females does not seem to account for the differential expression of this major gene in male and female rats in this particular strain. Pregnancy, however, did affect the blood pressure in the last third of the gestation period (Takeda, 1964). Applying the criterion of hypertension in this study, the onset of hypertension coincides with puberty. It thus seems reasonable to conclude that although sex hormones are not directly involved in the expression of this major gene, its expression is affected by some subtle sex difference. Such sex difference may underlie the difficulty in interpreting the blood pressure of female rats such as those of backcross females from $SDR \times F_1$ (table 2).

The observation that there is a single locus that is capable of controlling blood pressure by itself certainly does not exclude the possibility of the presence of other loci that could affect blood pressure independently or in conjunction with this locus. The existence of other genes is certainly indicated by crosses between WFR and SHR in our study and by the studies of Tanase *et al.* (1970, 1972). One analogy to this is the identification of the genes *agouti*, *obese* and *diabetes* in mice (Green, 1966). All of them can independently modify body weight. The possible existence of such a locus, among other genes, that controls blood pressure prompted us to isolate this gene by introducing it into the genome of the inbred Wistar/Lewis strain. Thus far, we have been able to maintain the hypertensive trait in the progeny from the third backcross. Tanase *et al.* (1972) recently reported that they had undertaken a similar effort. It will be very interesting to compare the results of these two independent studies in the future.

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