Influence on Blood Pressure of Renal Isografts between Spontaneously Hypertensive and Normotensive Rats, Utilizing the F₁ Hybrids

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SUMMARY

The F₁ hybrids (F₁) of spontaneously hypertensive rats (SHR) and Wistar rats (W), whose kidneys were transplanted from SHR at the age of 10 weeks and 20 weeks, showed significant elevation of blood pressure (BP) for 11 weeks after the transplantation. In F₁ with W or F₁ kidneys BP was decreased near to the normal level. F₁ whose kidneys were transplanted from SHR or W showed low renin activity both in plasma and the kidney. It is suggested that BP of SHR is probably determined by the renal pro-hypertensive factor(s) other than renin influencing on sympathetic nerves through central nervous systems.

Additional Indexing Words :

Renal transplantation Plasma renin activity Kidney renin activity
Denervation of the kidney

RENAL origin of the hypertensive cardiovascular disease has long been considered by many investigators. According to this concept hypertension may be caused by some dysfunction of the kidney. In hypertension associated with renal diseases, or caused by manipulation of the kidney, no one can doubt its renal origin. Similarly, spontaneous hypertension in rats, as well as essential hypertension in humans, might be induced by some renal dysfunction, although no responsible dysfunction has yet been clarified.

Utilizing the renal transplantation studies, Bianchi et al¹⁾ and Dahl, Heine, and Thompson²⁾ disclosed that the kidney of their own strain appeared to determine the level of blood pressure or the susceptibility to hypertension. However, in the spontaneously hypertensive rat (SHR) developed by Okamoto and Aoki,³⁾ the evidences presently available have not yet been conclusive. In SHR, nervous systems have been vigorously investigated and the increased sympathetic nerve activity has been stated to be the primary cause

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of the hypertension of SHR.⁴⁾⁻⁶⁾

Renal factor in SHR still remains unclear. Glomerular filtration rate (GFR) was varied among the colonies of SHR,⁷⁾ changes of urine volume and sodium balance have not been confirmed.^{8),9)} On the other hand, the recent reports on sodium and water metabolism showed that exaggerated natriuresis was recognized at least in some phase of hypertension of SHR.^{10),11)} Decreased excretion of sodium was shown in anesthetized SHR.¹²⁾ According to the theory of Guyton et al,¹³⁾ this latter data are explained by the fall of blood pressure (BP) during anesthesia. The BP decrease results in the smaller urinary output in the hypertensive individuals than the normotensives, because there is renal excretory dysfunction in the hypertensives.

The purpose of this study is to elucidate the role of the kidney in the pathogenesis of SHR by exchanging the kidney between the SHR and normotensive Wistar rats (W), utilizing the F₁ hybrids.

MATERIALS AND METHODS

Male SHR, the offspring (F₃₈₋₄₂) of original Kyoto SHR, inbred W (F₉₋₁₃), the colony from Wistar Kyoto, and their hybrids (F₁), raised in Biological Research Laboratories, Central Research Division, Takeda Chemical Industries, were used. The rats were transferred to our laboratory at 6 weeks of age and fed with regular laboratory chow and tap water *ad lib*.

Blood pressure: Blood pressure (BP) of the rats was determined by the plethysmographic tail method. Measurements were made in triplicate to the nearest 10 mmHg. BP was determined weekly until 10 weeks after the transplantation, then the BP was determined directly through the polyethylene tube inserted into the abdominal aorta.¹⁴⁾

Renal transplantation: All the renal transplantations were performed when the rats were at 10 or 20 weeks of age. To prevent allograft reaction, transplantation of the kidney was performed from SHR to F₁, and from W to F₁ instead of the direct transplantation from SHR to W or *vice versa*. Surgery was performed with the rats under ether anesthesia, utilizing the modified microsurgical technique of Lee.¹⁵⁾ All anastomoses were sutured with 7/0 atraumatic needles. The total ischemia time varied between 30 and 50 min. Heparinized saline, 1 to 2 ml, was flushed through the donors' right kidneys immediately before their removal. The recipients were subjected to bilateral nephrectomy at the time of transplantation.

Renin activity: Plasma renin activity (PRA) was determined by the method of Shiono and Sokabe.¹⁶⁾ About 1 ml of blood was collected through polyethylene tube inserted into the aorta at the termination of the experiments. Then the kidneys were removed to determine the kidney renin activity (KRA) by the modified method Nishimura and Sokabe.¹⁷⁾ Renin activity was expressed as the first reaction constant, K, calculated from the following equation:

$$K = \{2 - \log_{10}[100(M-X/M)]\} \cdot 2.3/t$$

where M is the angiotensinogen content of the plasma and X is the angiotensin

formed during an incubation time, t .

Blood urea nitrogen: Blood urea nitrogen (BUN) was determined with Azostix®, a reagent strip for BUN.¹⁸⁾ Results were provided directly in mg urea nitrogen per 100 ml on comparison with color blocks of 10, 20, 40, and 60 mg urea nitrogen per 100 ml. If color produced fell between the 2 color blocks, the results were interpolated.

The Student t -test was used for statistical analysis. Values given represent the mean and standard error of the mean.

RESULTS

Course of blood pressure in animals used in this experiment was demon-

Fig. 1. BP of SHR (●), W (○), and F_1 (◐), determined by the plethysmographic tail method. BP (#) was determined at the age of 31 weeks through the polyethylene tube inserted into the abdominal aorta. Mean $\pm$ S.E.

Fig. 2. BP of the bilaterally nephrectomized recipients after the transplantation of the kidneys from the SHR (SHR $\rightarrow$ F₁), W(W $\rightarrow$ F₁), and F₁(F₁ $\rightarrow$ F₁). Transplantation was performed when the donors and recipients were at the age of 10 weeks. BP of F₁ before the transplantation and after the unilateral nephrectomy (F₁-UN) were also shown. Symbols are the same as Fig. 1.

strated in Fig. 1. When the kidneys from SHR aged 10 weeks were transplanted into the F_1 , the recipients developed significantly higher BP than the F_1 transplanted with F_1 kidneys ($p < 0.001$), or the unilaterally nephrectomized F_1 (F_1 -UN) ($p < 0.001$). When the kidneys from normotensive W were transplanted into the F_1 , BP fell to lower level than F_1 -UN ($p < 0.03$) (Fig. 2). In F_1 who received the F_1 kidneys (syngrafts) the BP also reduced to normal (Fig. 2). No statistical difference was noted between the BP of F_1 transplanted with F_1 and W kidneys (Fig. 2).

These changes in BP after the renal transplantation were essentially the same when the surgery was performed at the age of 20 weeks (Fig. 3). The BP of the F_1 with SHR kidneys was higher than that of F_1 with F_1 kidneys ($p < 0.002$) and that of F_1 -UN ($p < 0.015$). In F_1 with W kidneys the BP to reduced also to the lower level than F_1 -UN ($p < 0.001$) (Fig. 3).

PRA was low in SHR and in F_1 . PRA of the F_1 whose kidneys had been transplanted from SHR, F_1 , or W was lower than that of F_1 -UN at the age of 20 weeks, but there were no significant differences between the F_1 with SHR, F_1 , or W kidneys (Tables I, II). KRA was also low in SHR but was

Fig. 3. BP of F_1 operated when the rats were at the age of 20 weeks. Details are the same as Fig. 2.

Table I. PRA and KRA in W, SHR, and F_1

Rats	Age (wk)	PRA* ($K \times 10^{-5}$)	KRA* ($K \times 10^{-2}$)
W	20	3.69 ± 0.70 (8)	2.80 ± 0.51 (8)
	30	2.09 ± 0.67 (8)	3.05 ± 0.28 (8)
F_1	20	2.87 ± 0.72 (7)	2.73 ± 0.35 (7)
	30	0.80 ± 0.07 (7)	2.39 ± 0.26 (7)
SHR	20	2.11 ± 0.20 (8)	1.75 ± 0.17 (8)
	30	1.89 ± 0.29 (7)	2.40 ± 0.26 (8)

* Number of determinations is in parentheses.

Table II. PRA and KRA in Transplanted F_1 with F_1 , W, and SHR Kidneys

Rats	Age* (wk)	PRA** ($K \times 10^{-5}$)	KRA** ($K \times 10^{-2}$)
Transplantation			
$F_1 \rightarrow F_1$	20	1.51 ± 0.38 (5)	1.18 ± 0.42 (5)
	30	1.22 ± 0.36 (5)	1.02 ± 0.35 (5)
$W \rightarrow F_1$	20	1.69 ± 0.55 (7)	0.84 ± 0.17 (7)
	30	0.49 ± 0.07 (7)	1.00 ± 0.07 (7)
$SHR \rightarrow F_1$	20	1.45 ± 0.59 (7)	1.05 ± 0.11 (7)
	30	0.64 ± 0.17 (7)	0.96 ± 0.17 (7)
Control			
F_1 -UN	20	2.88 ± 0.37 (7)	1.22 ± 0.07 (7)
	30	1.20 ± 0.14 (7)	2.84 ± 0.37 (7)

* All rats are at the 10th week after renal transplantation.

** Number of determinations is in parentheses.

Table III. BUN after Renal Transplantation

Rats	Age* (wk)	No.	BUN (mg%)
Transplantation			
$F_1 \rightarrow F_1$	20	5	16.0 ± 1.0
	30	5	19.0 ± 1.0
$W \rightarrow F_1$	20	7	27.1 ± 6.7
	30	6	30.8 ± 8.2
$SHR \rightarrow F_1$	20	7	19.3 ± 3.2
	30	7	40.0 ± 4.0
Control			
F_1 -UN	20	7	10 ± 0
	30	4	17.5 ± 0.25

* All rats are at the 10th week after renal transplantation.

insignificant in F_1 , and was decreased in transplanted kidneys (Tables I, II) BUN was elevated in F_1 with SHR or W kidneys. But F_1 with renal syngrafts did not show any high BUN (Table III).

In unilaterally nephrectomized SHR the heart weight/100 Gm body weight was significantly higher than in W at 20 weeks of age ($p < 0.001$), and at 30 weeks of age ($p < 0.05$). In the F_1 with SHR kidneys, hearts were more hypertrophic at 20 weeks of age ($p < 0.02$), and at 30 weeks of age ($p < 0.001$) than in F_1 transplanted with F_1 kidneys. These increase in heart weight was parallel with the elevation of BP (Table IV). No marked change in body weight and kidney weight was noted after renal transplantation.

Table IV. Heart Weight in W, F₁, SHR, and F₁—UN and Heart Weight in Transplanted F₁ with F₁, W and SHR Kidneys

Rats	Age (wk)	No.	Heart Weight (Gm/100 Gm body weight)
Control			
W	20	8	0.236±0.010
	30	8	0.252±0.031
F ₁	20	7	0.248±0.003
	30	7	0.328±0.014
SHR	20	8	0.305±0.006*
	30	8	0.337±0.012***
F ₁ —UN	20	7	0.230±0.03
	30	7	0.342±0.025
Transplantation			
F ₁ → F ₁	20	5	0.250±0.002
	30	5	0.234±0.009
W → F ₁	20	6	0.256±0.010
	30	7	0.270±0.007
SHR → F ₁	20	7	0.286±0.011**
	30	7	0.340±0.016*

* p<0.001, ** p<0.02, *** p<0.05

DISCUSSION

The present experiment clearly showed that the kidneys from SHR aged 10 and 20 weeks had the ability to elevate the BP of recipients (F₁). In contrast, in F₁ which were slightly hypertensive the BP reduced to normal when the kidneys from F₁ (syngrafts) or from W were transplanted.

The anti-hypertensive action of normal kidney was not apparent in this experiment. The observed BP decrease may be due to denervation of the kidney and resulted renal vasodilatation. Renal denervation delayed BP increase in SHR.¹⁹⁾ We determined daily urine volume after the transplantation, but any definite tendency was not found because of large variation. In the previous study on renal infarction hypertension,²⁰⁾ anti-hypertensive action of normal kidney was not apparent before the infarcted kidney was removed. The pro-hypertensive action of the kidney in SHR was equally effective at the age of 10 and 20 weeks. In the infarcted kidney, the action lasted for about 2 weeks after the manipulation.²⁰⁾

By the methods of renal transplantation used in this series of experiment, denervation was considered as complete.²¹⁾ Since the effective plasma flow is well preserved in the isografts,²²⁾ the elevation of BP of F₁ transplanted with SHR kidneys was not caused by decreased renal blood flow. But slight hy-

dronephrotic change and minor histoincompatibility between F_1 and SHR or W might cause the increased BUN, although the BP level did not correlate with the disturbance of renal function.

Bianchi et al.¹¹ found that the hypertension had developed when the kidneys from spontaneously hypertensive rats of their own strain were transplanted into the normotensive ones, while the kidneys from the normotensive rats normalized the BP of the hypertensive rats. Similarly, Dahl, Heine, and Thompson²¹ observed that inclination or resistance to hypertension was transferred following the renal transplantation between salt-sensitive, hypertensive-prone and salt-resistant rats. Thus the kidney decides the level of BP in these strains, possibly through some changes in salt and water metabolism by the kidney.²³ On high salt diet, the transplanted normal kidneys lost their anti-hypertensive action in the studies of both Bianchi et al.¹¹ and Dahl et al.²¹

In the present study PRA in SHR and F_1 was low in accordance with the previous report.¹⁶ PRA and KRA were low in F_1 with renal grafts, because of the renal denervation.²⁴ In any event renin did not appear to determine the level of BP.

Our results together with previous transplantation experiments quoted above indicate that the hypertensive kidney releases some pro-hypertensive factor(s) other than renin. In SHR the factor may influence the central nervous system and increase the sympathetic nervous activity, as has been reported.⁴⁾⁻⁶⁾ This unknown factor can be sodium chloride, on the other hand. The SHR or other hypertensive kidneys have some excretory defect, and retain sodium chloride in the body. Thus BP increases to compensate this excretory defect by the mechanism proposed by Guyton et al.¹³ In fact SHR excreted less water and sodium in an anesthetized condition,¹³ in which the systemic BP was decreased.¹⁶ However, much remained to be clarified on renal excretory function of SHR.

Renal excretory functional disturbance in SHR may be congenital. Coleman et al, in contrast, suggested that the increased sympathetic nervous activity compromised the renal function in relatively early life.²⁵ If so, it must be completed before 10 weeks of age, because the renal denervation was complete in our methods.²¹

The present study supports the view to consider human essential hypertension of renal origin.²⁶ No essential differences between SHR and essential hypertension have yet been found until today.

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