Immunohistochemical Findings of Juxtaglomerular Cells on Acute Phase in Two-Kidney Goldblatt Hypertensive Rats and Spontaneously Hypertensive Rats

Jun YAMAKOSHI, Katsunori OHSHITA, and Reiji TAKAHASHI¹⁾

Research and Development Division, Kikkoman Corporation, 399 Noda, Noda, Chiba 278, ¹⁾Department of Pathology, School of Veterinary Medicine, Azabu University, 1-17-71 Fuchinobe, Sagami-hara, Kanagawa 229, Japan

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ABSTRACT. Juxtaglomerular (JG) cells on the acute phase in two-kidney Goldblatt hypertensive (2KGH) rats and spontaneously hypertensive rats (SHR) were examined immunohistochemically. JG cells in 2KGH rats and SHR were positively stained with anti-renin serum and anti-angiotensin II (A II) serum. In 2KGH rats, the number of renin and AII immunoreactive JG cells in the clipped kidneys increased throughout the observation period. The number of renin and AII immunoreactive JG cells in the unclipped kidneys was almost the same as that in control rats, although immunoreactivity of these cells was weak and they were small in size. These changes in the unclipped kidneys became obvious with the time course after operation. We did not see any changes in these cells in SHR. In 2KGH rats treated with captopril, the number of renin immunoreactive JG cells in the clipped kidneys increased, whereas that of AII immunoreactive JG cells in the bilateral kidney decreased. When captopril was administered to SHR, the number of renin immunoreactive JG cells in the bilateral kidney increased, whereas that of AII immunoreactive JG cells in the bilateral kidney decreased. These results suggested that the JG cell in the bilateral kidney was closely related to the development of hypertension in 2KGH rats, but not in SHR. The increase of renin immunoreactive JG cells in 2KGH rats and SHR treated with captopril was probably due to the removal of negative feedback inhibition of AII on JG cells.—**KEY WORDS:** captopril, immunohistochemistry, juxtaglomerular cell, renal hypertensive rat, SHR.

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It has been well known that hypertensive rats can be divided into 2 types from the view of pathophysiology. One is two-kidney Goldblatt hypertensive (2KGH) rats which develop hypertension through the function of the systemic renin-angiotensin system (RAS) [3], and the other is spontaneously hypertensive rats (SHR) which develop hypertension without the function of the systemic RAS [6, 9].

Recently, a novel concept was emerged that challenged the traditional belief that the RAS was solely an endocrine system. This concept proposed that the endogenous RAS was present in many local tissues and that these systems exerted autocrine and paracrine influences on local tissue functions. Recent data supported the existence of intrarenal RAS by immunohistochemical, biochemical and molecular biological techniques [4, 10].

However, the relation between intrarenal RAS and the development of hypertension has not been examined in hypertensive rats by immunohistochemical technique.

In this paper, we compared the changes of JG cells on the acute phase in 2KGH rats with those in SHR, treated with or without captopril, by immunohistochemical technique.

MATERIALS AND METHODS

Animals: Seventy-six male Crj: Wistar rats, 56 male SHR/NCrj and 20 male WKY/NCrj aged 6 weeks were used in this study. For preparation of 2KGH rats, a silver clip was applied to the left renal artery of 56 Wistar rats to produce an internal diameter of 0.2 mm in width. Twenty Wistar rats, which served as controls, underwent a sham operation. WKY rats did not receive a sham operation.

Experimental design: Rats were divided into the following 6 groups; 2KGH group, 2KGH captopril group, sham operation group, SHR group, SHR captopril group and WKY group (Table 1). Captopril (Sigma Chemical Co.), dissolved in the drinking water at a concentration of 0.5 mg/ml and 0.7 mg/ml, were given to rats of 2KGH captopril group and SHR captopril group, respectively. After anesthetization with ether, each group of rats was killed on day 7, 14, 21 and 28. Experimental design was previously described in detail [14].

Tissue preparation: The removed kidneys were fixed in Bouin's fixative for 3–7 hr and serial paraffin sections of 2 μ m in thickness were prepared.

Antiserum: Anti-renin serum was obtained from rabbits immunized with renin which was purified

Table 1. The total numbers of renin or angiotensin II (AII) immunoreactive cells in 25 juxtaglomerular bodies/kidney of 2KGH rats and SHR

Experimental period (days)	Experimental group (number of rats)	Renin		AII	
		RK ^{a)}	LK ^{b)}	RK ^{a)}	LK ^{b)}
7 days	2KGH(7)	160± 6 ^{c)}	172±16	130±17	147±16
	2KGH captopril(7)	165± 6	189±23	118±17	122±11
	Sham operation(5)	165±10	165± 8	131±16	130±11
	SHR(7)	162±15	166± 7	117±11*	118±12
	SHR captopril(7)	189±20 ^{*d)}	179±17	116±14	115±16
	WKY(5)	167± 9	162±17	129± 3	123±16
14 days	2KGH(7)	163± 7	195±23*	125±17	145±17
	2KGH captopril(7)	168± 7	189±20*	111±15	107±21*
	Sham operation(5)	161± 7	165± 7	127± 5	131±12
	SHR(7)	160± 7*	167± 9	127±12	124±14
	SHR captopril(7)	189±18	195±12 ^{**d)}	116±15	112±15
	WKY(5)	172± 7	166± 6	125±14	127± 4
21 days	2KGH(7)	161±16	187± 7**	115±20	153±28*
	2KGH captopril(7)	172±17	214±25**	96±22*	101±26
	Sham operation(5)	169± 8	164±11	135±23	124± 6
	SHR(7)	162±12	159± 9	120± 8	123± 6
	SHR captopril(7)	193±20	202±17**	117±16	114±22
	WKY(5)	172±11	168±13	120± 5	128±16
28 days	2KGH(7)	157± 6	194± 7**	116± 9	150±25
	2KGH captopril(7)	175±12	220±29*	91±12**	100±12**
	Sham operation(5)	163±11	164± 4	126±19	127± 7
	SHR(7)	163±13	159± 8	123±11	118±16
	SHR captopril(7)	200± 9**	206±24**	102±10**	104±13*
	WKY(5)	161± 9	166±10	127± 4	120±10

a) Right kidney, b) Left kidney (clips applied to the left renal artery in 2KGH group and 2KGH captopril group), c) Mean±standard deviation (n=5 or 7), d) *, ** Significant difference from sham operation group or WKY group at p<0.05, p<0.01, respectively.

from mouse submandibular gland [5], and was used for immunohistochemical staining at 1:6000 diluted with phosphate-buffered saline (PBS, pH 7.4). Anti-AII rabbit serum (Peninsula Lab. Inc.) was also used at 1:200 diluted with PBS.

Immunohistochemical staining: Serial sections were stained immunohistochemically by the strept-avidin-peroxidase conjugate (SP) method [8]. First, sections were covered by a drop of diluted antiserum and incubated for 20 hr at 4°C, followed by the biotinylation with anti-rabbit IgG goat serum for 20 min at room temperature. Then they were covered with peroxidase conjugated streptavidin for 20 min at room temperature. After each incubation step, the sections were washed twice with PBS. The immunoreactive substances were stained using di-amino-benzidine and H₂O₂. Control sections were treated similarly with non-immune rabbit serum instead of either anti-renin serum or anti-AII serum.

Semiquantitative evaluation: The total number of renin or AII immunoreactive cells in 25 JG bodies/kidney were counted by the method of juxtaglomerular cell count (JGCC) [12]. Results were expressed

as the mean ± standard deviation. The following 6 couples of groups were examined statistically by Student's *t* test; 2KGH group and sham operation group, 2KGH captopril group and sham operation group, SHR group and WKY group, and SHR captopril group and WKY group of each observation period.

RESULTS

The JG cells in the kidneys obtained from rats of each group were positively stained with both anti-renin serum and anti-AII serum. The total numbers of renin or AII immunoreactive cells in 25 JG bodies/kidney are shown in Table 1.

The number of renin immunoreactive JG cells in the clipped kidney of 2KGH rats increased (Fig. 1-a), compared with control rats, and that in the clipped kidney of 2KGH rats treated with captopril also increased (Fig. 1-b). In contrast, the number of the immunoreactive JG cells of SHR did not increase (Fig. 1-c) being almost the same as that of control rats (Fig. 1-f), although that in SHR treated

Fig. 1. The JG cells in the kidneys obtained from rats of 2KGH and SHR group on day 28 (SP-staining with anti-renin serum). $\times 200$. a: 2KGH rat. Immunoreactive JG cells in the clipped kidney increased. b: 2KGH rat treated with captopril. Immunoreactive cells in the clipped kidney markedly increased. c: SHR. The number of immunoreactive cells was almost the same as that of control rat (f). d: SHR treated with captopril. Immunoreactive cells increased. e: 2KGH rat. Immunoreactivity of JG cells in the unclipped kidney was weak and they were small in size. f: Control rat (WKY).

with captopril increased (Fig. 1-d).

The number of AII immunoreactive JG cells in the clipped kidney of 2KGH rats increased (Fig. 2-a), whereas that in the clipped kidney of 2KGH rats treated with captopril decreased (Fig. 2-b) as compared with control rats. In contrast, the number of the immunoreactive JG cells of SHR was constant (Fig. 2-c) and almost the same to control rats (Fig. 2-f). On the other hand, the number of the immunoreactive cells of SHR treated with captopril decreased (Fig. 2-d).

Furthermore, renin and AII immunoreactive JG cells in the unclipped kidney of 2KGH rats were almost the same in number as that of control rats, but immunoreactivity of these cells was weak and they were small in size. These changes of JG cells appeared from day 7 after operation, and became obvious with the time course after operation, especially on day 28 (Fig. 1-e, 2-e). In the control sections applied nonimmune rabbit serum as a primary antibody, no reactive cells were observed.

DISCUSSION

In this paper, we compared the time course of changes of JG cells on the acute phase between 2KGH rats and SHR, treated with or without captopril, by the immunohistochemical technique.

It was found in 2KGH rats that the number of renin and AII immunoreactive JG cells in the clipped kidney increased from day 7 after operation and that these cells showed weak immunoreactivity and were small in size. These changes of JG cells became obvious with an increase in blood pressure and plasma renin activity with the time after operation [14]. This suggested that the JG cell in the bilateral kidney was closely related to the development of hypertension in 2KGH rats. These morphological changes of JG cells may be explained by baroreceptor and macula densa hypotheses on the regulation of renin secretion [1, 2, 13]. Namely, morphological changes of JG cells in the clipped kidney may be occurred by the activation of baroreceptor and decrease of sodium delivery to the

Fig. 2. The JG cells in the kidneys obtained from rats of each group on day 28 (SP-staining with anti-AII serum). $\times 200$. a: 2KGH rat. Immunoreactive JG cells in the clipped kidney increased. b: 2KGH rat treated with captopril. Immunoreactive cells in the clipped kidney decreased. c: SHR. The number of immunoreactive cells was almost the same as that of control rat (f). d: SHR treated with captopril. Immunoreactive cells decreased. e: 2KGH rat. Immunoreactivity of JG cells in the unclipped kidney was weak and they were small in size. f: Control rat (WKY).

macula densa due to reduction of renal arterial pressure. On the contrary, morphological changes of JG cells in the unclipped kidney may be due to the inactivation of baroreceptor and increase of sodium delivery to the macula densa due to the increment of renal arterial pressure. It was considered that hypertension may be associated with a correlation between JG cells in the clipped kidney and those in the unclipped kidney in 2KGH rats.

On the other hand, Taugner *et al.* showed that JG-index for renin and AII increased in the clipped kidney and that decreased in the unclipped kidney of 2KGH rats, although the plasma level of AII was elevated on day 21 after operation. Hence they assumed that immunocytochemically detectable AII in the juxtaglomerular apparatus region was intrarenal origin rather than systemic origin [11]. Further examinations of AII in JG cells were needed in our study.

We did not see any changes in the number of JG cells in SHR. This suggested that the JG cell was not

related to the development of hypertension in SHR.

The increase in number of renin immunoreactive cells in 2KGH rats and SHR treated with captopril was probably due to the removal of negative feedback inhibition of AII on JG cells [7].

Further investigations are required to examine the interaction between the intrarenal RAS and the systemic RAS in 2KGH rats and SHR.

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