Kor J Physiol (1991) 25(1), 61-67

Nephron Heterogeneity of Renin Release in Rat Kidney Slices: Effects of L-Isoproterenol, Angiotensin II and TMB-8

Kyung Hwan Seul, Suhn Hee Kim, Gou Young Koh and Kyung Woo Cho

Department of Physiology. Jeonbug National University Medical School, Jeonju 560-180

= ABSTRACT =

In order to determine possible relationships between the renin-angiotensin system and nephron heterogeneity, we compared the response of renin release and the angiotensin-converting enzyme (ACE) activity from different areas of the rat kidney. We used the renal cortical slices from the capsular surface to the juxtamedullary junction. Slices from outer one-third of the cortex were designated as outer cortical slices (OC), middle one-third as midcortical slices (MC), and inner one-third as inner cortical slices (IC). The renal renin content markedly decreased from OC and MC to IC. The basal renin release was higher in OC than in MC or IC. On the contrary the percent change of renin release in response to L-isoproterenol was significantly higher in MC than in OC or IC. By TMB-8, the renin release in MC by 231±21% was higher than OC by 177±19% or IC by 162±19. Angiotensin II suppressed renin release in OC and MC by 68±2, 71±4% respectively, but only 40±7% in IC. The ACE activity was higher in IC than in OC, MC, medulla and papilla. The present data indicate that renin content and basal renin release gradulally decreased from outer (OC) to inner (IC) cortex. The renin release in response to beta-adrenergic agonist, L-isoproterenol and intracellular calcium antagonist, TMB-8 were higher in MC than in OC and IC, but angiotensin II suppressed renin release less in IC than in OC and MC. It is suggested that juxtaglomerular cells of outer, mid-and inner cortices show a difference in renin release response to the stimuli.

Key Words: Nephron heterogeneity, Renin, Kidney, Renin-angiotensin system, Angiotensin-converting enzyme.

INTRODUCTION

It has long been established that both glomeruli and vasculo-tubular patterns differ from region to region of the kidney (Baines & De Rouffignac, 1969; Beeuwkes & Bonventre, 1975; Horster & Thurau, 1968; Imai, 1984; Munkacsi & Palkovits, 1965). The renal renin content and its release from juxtaglomerular cells gradually decrease from outer cortex to inner cortex (De Rouffignac et al, 1974; Flamenbaum & Hambereger, 1974; Jones et al, 1979). This heterogeneity in the renin release and the vasculo-tubular relationship have led the authors to speculate a different role of the renin-angiotensin system within the kidney in regulation of intrarenal distribu-

Received June 1, 1991; Accepted June 30, 1991

This work was partly supported by a grant from the Korea Science and Engineering Foundation.

tion of blood flow, glomerular filtration rate and tubular reabsorption of sodium.

Endogenous angiotensin II formed in the kidney has a direct vasoconstrictive effect on the efferent arteriole of rat glomeruli (Davalos et al, 1978; Frega et al, 1980). Additionally, angiotensin-converting enzyme is predominantly located in the inner cortex and outer medulla (Morin et al, 1989). But, little has been reported on the heterogeneity of renin release from different areas of the renal cortex.

To determine possible relationships between the renin-angiotensin system and the nephron heterogeneity, we compared the response of renin release in different areas of the rat renal cortex. In vitro renin release was measured in slices obtained from the outer cortex (OC), midcortex (MC), and inner cortex (IC) treated with L-isoproterenol, angiotensin II, or 3,4,5-trimethoxybenzoic acid 8-(diethylamino) octyl ester (TMB-8). Regional distribution of angiotensin-converting enzyme (ACE) in the renal cortex and medulla was also determined.

METHODS

Male wistar rats weighing about 200g were decapitated, both kidneys were rapidly removed, and kept in cold physiologic saline. After removing the renal capsule, the kidneys were divided transversely into 3 parts, and then the upper and lower one-third were discarded. The middle one-third of the kidney was divided transversely into 3 pieces without medullar portion (Fig. 1b) and the medulla just below the arcuate artery (Fig. 1c) was discarded. The remaining cortical fragments were serially cut in 0.2mm thickness with a Stadie-Riggs microtome from capsular surface to corticomedullary junction. The outermost piece of slices was discarded and the next two slices were considered as OC slices. Discarding a following slice, the next two were taken as MC slices. The next slice was again discarded and next two slices, just above corticomedullary junction, were taken as IC slices (Fig. 1d)

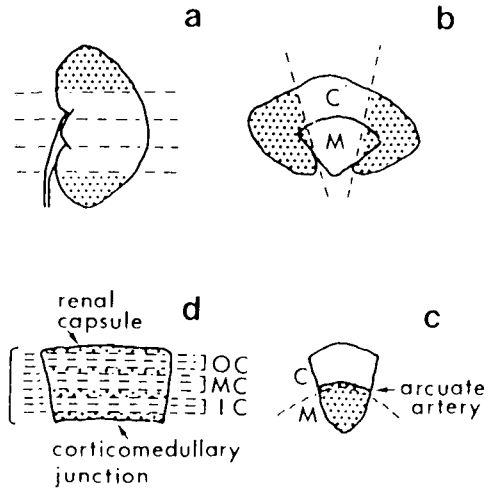

Fig. 1. Fragmantation of kidney. C, Cortex; M, medulla; OC, outer cortex; MC, midcortex; IC, inner cortex. Dotted areas indicate the parts of discarded. Small arrow indicates the arcuate artery.

Each pool of slices from OC, MC, and IC was separately incubated in a vial containing 20ml of physiological salt solution (PSS) bubbled with 95% O_2-5% CO_2 mixture at 37° C in an oscillating incubator. In vitro experiment was carried out as reported previously (Cho & Malvin, 1979) with slight modifications. The composition of PSS was 118mM NaCl, 4.7mM KCl, 2.5mM $CaCl_2$, 1.2mM KH_2PO_4, 1.2mM $MgSO_4$, 2.5mM $NaHCO_3$, 10mM glucose and 0.1% bovine serum albumin (BSA). After incubation for 10 min, two slices (weighing about 10mg of fresh tissues) were placed in a siliconized vial containing 5ml of PSS which had previously been gassified with 95% O_2-5% CO_2 at 37°C. The flask were placed in an oscillating incubator and continously gassified through the experiment. At the end of 20 min preincubation, 200ul of incubation media were withdrawn, and then 50ul of drug solution or vehicle were

added into the flask. L-Isoproterenol, angiotensin II and TMB-8 were diluted in physiologic saline. The final concentrations of L-isoproterenol, angiotensin II and TMB-8 were 10^{-6}, 10^{-6}, and 5×10^{-5} M/L, respectively. Following 60min incubation, 200ul of incubation medium were taken and centrifuged at $10,000\times g$ for 2min at 4°C. Supernatants were frozen until renin activity were determined. Renal slices were blotted to dry, then wei-ghed, and stored at -20°C until analysis of renin content. All experiments were performed in triplicates.

Renin concentration was measured using homologous substrate obtained from 48h nephrectomized rats, as described previously (Cho et al, 1987b). The renin activity of a sample was expressed in nanogram of angiotensin I generated per hour per mg of fresh tissue (ng ANG I/mg of fresh tissue/h). Total renal renin activity was calculated as the sum of the absolute renal renin activity of slices plus total renin activity released into the medium during 80 minutes of incubation.

To calcuate the percent change in renin release induced by L-isoproterenol and TMB-8, the following equation was used;

$$\left[\frac{\text{E80-E20}}{\text{C80-C20}} - 1\right]\times 100(\%)$$

The degree of suppression of renin release by angiotensin II was calculated as follow;

$$\left[1 - \frac{\text{E80-E20}}{\text{C80-C20}}\right]\times 100(\%)$$

where (E80-E20) was the amount of renin released in 60 minutes after the addition of drugs at t=20min and (C80-C20) was the amount of renin released during 60 minutes after the addition of an equivalent volume of vehicle at t=20min. Frozen tissues were thawed at 4°C and then placed in polypropylene vials containing 5ml cold distilled water. Renal tissues were homogenized and subsequently centrifuged at 10,000xg for 2min at 4°C. Five ul of supernatant were added to homologous renin substrate in the same way as described above.

Determination of ACE activities were performed as previously (Cho at al, 1987a).

I-125-angiotensin I was obtained from New England Nuclear (Boston, MA, USA). L-isoproterenol, angiotensin I, angiotensin II, TMB-8, BSA, Trizma base, neomycin sulfate, 8-hydroxyquinoline, phenylmethylsulfonyl fluoride and charcoal were purchased from Sigma Chemical (St. Louis, MO, USA). Dextran T-70 was from Pharmacia Fine Chemicals (Sweden). All other chemicals were of the analytical grade from commercial sources.

Results are expressed as means±SE. Student's paired t-test for significance was performed.

RESULTS

As shown in Figure 2A, renal renin contents of outer and midcortices were higher than that of inner cortex; 1702.92±176.12, 1465.57±155.82 vs. 981.54±98.64 ng ANG I/mg of fresh tissue/h ($p<0.01$). But there was no significant difference in the renin content between the outer and midcortices. The amounts of renin released into incubation medium during 60 min incubation have a decreasing gradient from OC to IC as follows; OC 176.44±13.88, MC 118.15±9.03, and IC 55.04±4.53 ng ANG I/mg of fresh tissue/h ($n=23$, $p<0.01$). The renin release, expressed as a percentage of the renin content of the slices, was higher in outer cortex, 6.68±0.81 than that of midcortex, 4.82±0.76 ($p<0.05$) and inner cortex, 4.14±0.35% ($p<0.01$) (Figure 2B).

The response of renin release expressed as a percent change of renin release to L-isoproterenol, 10^{-6} M/L, was higher in the midcortex, 221±15% than in the outer cortex, 156±11% ($p<0.01$) and in the inner cortex, 169±10% ($p<0.05$), although the renal renin content and basal renin release were higher in the outer cortex (Figure 3A). The percent change of renin release by TMB-8, 5×10^{-5} M/L, was

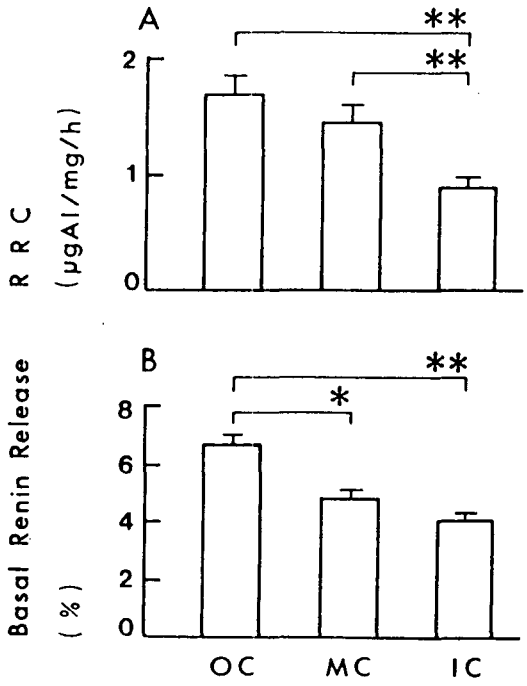

*Fig. 2. Renal renin content (RRC) and basal renin release of rat kidney slices from the outer (OC), mid (MC) and inner (IC) cortices. *, **, Statistically significant difference, $p<0.05$ and $p<0.01$, respectively. The percent change in renin release was calculated as shown in Materials and Methods.*

higher in midcortex, 231±21% than that of outer cortex, 177±19% ($p<0.01$) and that of inner cortex, 162±19% ($p<0.01$), as shown in Figure 3B. The percent suppression of renin release by angiotensin II, 10^{-6} M/L in the inner cortex, 40±7% was less than that of outer and midcortex, 68±2%, 71±4%, respectively ($p<0.01$). No differences in response between outer and midcortices was observed (Fig. 3C).

The ACE activity in the inner cortex was the highest and that of the outer cortex was lower than any other region of renal tissues (Fig. 4).

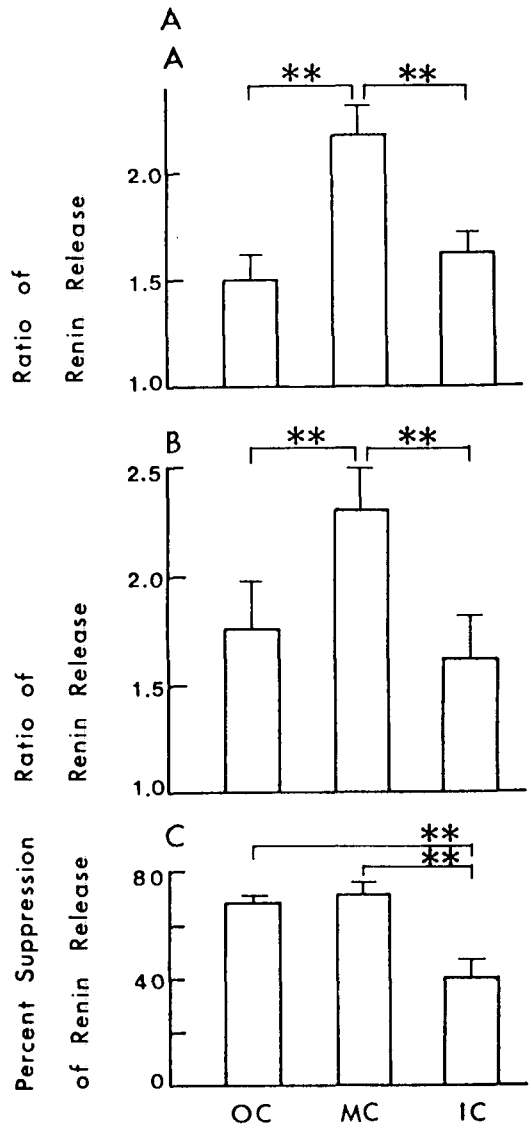

Fig. 3. Responses to L-isoproterenol (A), TMB-8 (B) and angiotensin II (C) in the outer, mid-and inner cortices. Legends are the same as in Fig. 2.

DISCUSSION

Because of differences in the method of slicing the renal cortex, a designation of outer

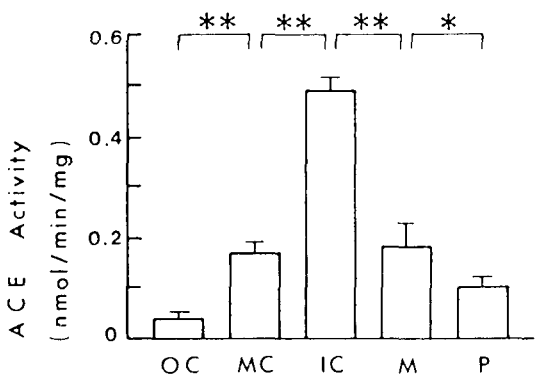

Fig. 4. Regional differences of the angiotensin I-converting enzyme activity in the rat kidney. ACE, angiotensin I-converting enzyme; P, papilla. Other legends are the smae as in Fig. 2.

cortical slices differs from experimentor to experimentor (Capponi & Vallotton, 1976; Churchill et al, 1983; De Rouffignac et al, 1974; Flamenbaum & Hamberger, 1974; Jones et al, 1979). Flamenbaum and Hamberger (1974) considered the outermost 2 or 3 slices as outer cortex comparable to that of OC in this experiment. De Rouffignac et al. (1974) considered upper two-third of the renal cortex as outer cortex comparable to those of both OC and MC in the present experiment, and De Vito et al. (1970) used both cortex and medulla. Therefore, the response of the renin release of midcortex has been overlooked, mixed or omitted in the past experiments although it has been suggested that OC, MC and IC nephron have different vasculo-tubular patterns.

The present study confirmed that the renin content of outer cortex was higher than that of the inner cortex. The rate of renin release was correlated with the tissue renin content.

Fray (1978) found that renin content and renin release of the salt-depleted animals is higher than that of the salt-loaded animals. He suggested that the rate of renin release from outer and inner cortex might be changed differently in response to similiar stimuli. Torretti et al. (1978) previously reported that outer cortical slices but not the inner cortex respond to sympathetic neurotransmitter, norepinephrine, in the cat. The present results indicate that the midcortex has higher responsiveness to L-isoproterenol compared to the other parts.

It has been known that an increase in intra- or extracellular calcium ion inhibits the renin release from the juxtaglomerular cells (Churchill, 1985). Using cortical and juxtamedullary glomeruli of the human kidney, Martin-Dupont (1984) recently reported that only the cortical glomeruli became vasodilated in response to the calcium inhibitor, verapamil. To study the renin response to calcium antagonist in the different region of the cortex, we used TMB-8, an intracellular calcium antagonist. Our results showing the highest response of midcortex to TMB-8 suggest that the regulation of renin release by intracellular calcium ion in the juxtaglomerular cells of the midcortex may be different from that of the outer and inner cortices.

According to Vander et al. (1965), angiotensin appears to inhibit the renin release at the level of juxtaglomerular cells. It has been reported that glomeruli have the receptor for angiotensin II (Torretti & Weinberger, 1978). By employing the technique of scanning electron microscopy, Hornych et al. (1972) have reported that an intravenous infusion of angiotensin in rats causes a contraction of the glomerular capillaries in the outer zone of the cortex, but an enlargement of the juxtamedullary glomeruli. Thus, they concluded that the redistribution of intrarenal blood flow by the infusion of angiotensin II was due to constriction of the efferent arterioles of the juxtamedullary glomeruli. The fact that the renin release of inner cortex was less suppressed than that of outer and mid-cortices by angiotensin II supports the above observations. If the renin release of IC glomeruli were less suppressed by angiotensin II delivered systemically, the rate of angiotensin II formed within those will be relatively higher than that

of OC and MC glomeruli.

The ACE activity in the inner cortex was higher than in the outer-, midcortex, medulla and papilla. In the present study, the renin release of the inner cortex was also less suppressed by angiotensin II. Following 3-day dehydration, the rate of renin release in inner cortex stimulated by L-isoroterenol was significantly increased compared with outer-and midcortices (data not shown). Thus this difference in the control of renin-angiotensin system by nephron heterogeneity may account for the renal adaptation to sodium restriction, which showed a 38% decrease in the filtration rate of superficial nephrons, but a 250% decrease in that of juxtamedullary nephrons (Ericson et al, 1982). If the in vivo responses of renin release are different from region to region within the kidney in vivo as was shown in the present experiments in vitro, the amount of angiotensin II generated in the kidney may differ by nephron heterogeneity. Thus our results provide some possible explanations for the intrarenal roles of the renin-angiotenisn system via different responses of renin release and ACE activities in the different region of renal cortex.

In summary, the responses of renin release to L-isoproterenol and TMB-8 were greater in the midcortex, and that to angiotensin II was lower in the inner cortex than others. These specific responses of renin release to different stimuli may influence intrarenal distribution of blood flow, glomerular filtration rate, and the tubular reabsorption of sodium via regulation of angiotensin II formed endogenously within the kidney.

ACTNOWLEDGEMENT

We thank Kyung Hwa Nam and Eun Jin Nah for their technical assistance.

REFERENCES

Baines AD & De Rouffingnac C (1969) Functional heterogeneity of nephrons. II. Filtration rates, intraluminal flow velocities and fractional water reabsorption. *Pflügers Arch* **308,** 260-276

Beeuwkes III R & Bonventre JV (1975) Tubular organization and vascular-tubular relations in the dog kidney. *Am J Physiol* **229,** 695-713

Capponi AM & Vallotton MB (1976) Renin release by rat kidney slices incubated in vitro: Role of sodium and alpha and beta adrenergic receptors, and effect of vincristine. *Circ Res* **39,** 200-203

Cho KW, Kim SZ, Kim SH, Koh GY & Seul KH (1987a) Characterization of angiotensin I-converting enzyme activity in the freshwater turtle, Amyda japonica. *Comp Biochem Physiol* **87A,** 645-648

Cho KW, Kim SH & Koh GY (1987b) Radioimmunoassay and Characterization of renin-angiotensin system in the fresh-water turtle. *J Exp Zool* **242,** 255-262

Cho KW & Malvin RL (1979) Renin inactivation during in vitro experiment. *Am J Physiol* **236,** F501-F504

Churchill PC (1985) Second messengers in renin secretion. *Am J Physiol* **249,** F175-F184

Churchil MC, Churchill PC & McDonald FD (1983) Comparison of the effects of rubidium and potassium on renin secretion from rat kidney slices. *Endocrinology* **112,** 777-781

Davalos M, Frega NS, Saker B & Leaf A (1978) Effect of exogenous and endogenous angiotensin II in the isolated perfused rat kidney. *Am J Physiol* **235,** F605-F610

Davis JO & Freeman RH (1976) Mechanisms regulating renin release. *Physiol Rev* **56,** 1-56

De Rouffignac C, Bonvalet JP, Menard J (1974) Renin content in superficial and deep glomeruli of normal and salt-loaded rats. *Am J Physiol* **226,** 150-174

De Vito E, Gordon SM, Cabrera RR & Fasciolo JC (1970) Release of renin by rat kidney slices. *Am J Physiol* **219,** 1036-1041

Ericson AC, Sjoquist M & Ulfendahl HR (1982) Heterogeneity in regulation of glomeruli function. *Acta Physiol Scand* **114,** 203-209

Flamenbaum W & Hamberger RJ (1974) Superficial and deep juxtaglomerular apparatus renin activity of the rat kidney. *Clin Res* **54,**

1373-1381

Fray JCS (1978) Mechanism of increased renin release during sodium deprivation. *Am J Physiol* **234,** F376-F380

Frega NS, Davalos M & Leaf A91980) Effect of endogenous angiotensin on the efferent glomerular arteriole of rat kidney. *Kidney Int* **18,** 323-327

Hornych H, Beaufils M & Richet G (1972) The effect of exogenous angiotensin on superficial and deep glomeruli in the rat kidney. *Kidney Int* **2,** 336-343

Horster M & Thurau K (1968) Micropuncture studies on the filtration rate of single superficial and juxtamedullary glomeruli in the rat kidney. *Pflügers Arch* **301,** 162-181

Imai M (1984) Functional heterogeneity of the descending limbs of Henle's loop (II): Interspecies differences among rabbits, rats, and hamsters. *Pflügers Arch* **402,** 393-401

Jones SM, Torretti J, Williams JH & Weinberger SF (1979) Regional renin release by the cat kidney in vitro and in vivo. *Am J Physiol* **237,** F188-F195

Martin-Dupont P, Cambar J & Brothier (1984) Effect of verapamil calcium inhibitoron the vasomotor response of cortical and juxtamedullary glomeruli of the human kidney. *Renal Physiol* **7,** 72-77

Morin JP, Moulin B, Borghi H, Grise P & Fillastre JP (1989) Comparative regional distribution of angiotensin I-converting enzyme in the rat, rabbit, dog, monkey and human kidneys. *Renal Physiol Biochem* **12,** 96-103

Munkacsi I & Palkovits M (1965) Volumetric analysis of glomerular size in kidneys of mammalians living in desert, semidesert or water-rich environment in the Sudan. *Cirs Res* **17,** 303-311

Sraer JD, Sraer J, Ardaillou R & Mimoune O (1974) Evidence for renal glomerular receptors for angiotensin II. *Kidney Int* **6,** 241-246

Torretti J & Weinberger SF (1978) Renin release by outer and inner cortical slices of cat kidney. Differences in the rate of release and in the response to norepinephrine. *Kidney Int* **14,** 704 (a)

Vander AJ & Geelhoed GW (1965) Inhibition of renin secretion by angiotensin II. *Proc Soc Exp Biol Med* **120,** 399-403

Zimmerhackl B, Steinhausen M & Parekh N (1981) Variation in single glomerular loop flow by angiotensin measured with fluorescent erythrocytes. *Pflügers Arch* **39,** R19