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Changes of Renin Isoelectric Heterogeneity after Acute and Chronic Stimulation of Renin Secretion (42337)

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Abstract. Changes in the multiple forms of renin secreted or stored *in vitro* by renal cortical slices were studied in rats made hypertensive with deoxycorticosterone, adrenalectomized rats, and rats fed a high or low salt diet. Renal slices from normal rats were also incubated with angiotensin II, vasopressin, and verapamil. Aliquots of incubation media were subjected to isoelectric focusing, and the six forms of renin were quantified and expressed as a percentage of the total renin activity recovered from the gel. The results showed that chronic and acute stimulation of renin secretion produced a similar modification of the isoelectric focusing profile, consisting of an increased proportion of renin forms with the more acidic isoelectric points. The change in the proportions of the more acidic renin forms was greater with chronic stimulation than that after stimulation with verapamil. However, chronic and acute inhibition or reductions of the rate of renin secretion did not modify the renin profile. We suggest that the progression in the shift of secreted renin forms to those with the more acidic isoelectric points correlates with the intensity or duration of stimulation of renin secretion. These data support the hypothesis that different pools of renin exist and are altered differently by chronic and acute stimulation of renin secretion.
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Renin heterogeneity (active and inactive forms of renin with different molecular weights and isoelectric points) has been reported in different species (1–6). A number of researchers have focused their interest in understanding the role of inactive renin, but few studies have addressed the physiological significance of multiple forms of active renin (7). In the rat, it was found that six forms of active renin with distinct isoelectric points (*p*Is) are stored and released by the kidneys (8–11). Changes in the isoelectric focusing pattern of secreted renin were observed *in vitro* using incubated renal slices from rats fed a low salt diet and slices from normal rats stimulated with isoproterenol (8, 9, 11). Furthermore, an altered profile of stored renin in kidneys of Goldblatt hypertensive rats suggested that some renin forms might be implicated more than others in the regulation of salt and water balance (11). Evidence now exists which demonstrates functional differences between the forms. Experiments on dogs showed that hepatic removal of the more acidic forms was slower than for the more basic ones (12). More recently, we have shown that in the rat each of the six forms of renin has a unique half-life (13). The form with the most basic *p*I has the shortest half-life, and the most acidic one, the longest half-life. These findings have caused us to accept as a working hypothesis that the profile of renin existing in blood may effect function.

In this study we investigated the changes of the isoelectric focusing pattern of renin in response to various chronic and acute stimuli, and inhibitors of renin secretion.

Materials and Methods. Male Sprague–Dawley rats weighing 250–350 g were used in all experiments.

DOCA rats. Rats were anesthetized with sodium pentobarbital (50 mg/kg), uninephrectomized, and given a subcutaneous implant of deoxycorticosterone-acetate (DOCA, 200 mg/kg, Sigma Chemical Co). The DOCA was mixed in Silastic strips (Dow Chemical Corporation, Midland, Mich.), one part DOCA to two parts Silastic (w/w). Following recovery, they were given regular Purina rat chow with 1% NaCl and 0.1% KCl in their drinking water. Six weeks after DOCA implantation, the animals were anesthetized again, and the kidneys were removed, placed in cold saline, and treated as described below.

Adrenalectomized rats. Rats were anesthetized with sodium pentobarbital and bilaterally

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adrenalectomized (ADX). They were given regular Purina rat chow with 1% NaCl in their drinking water. After 1 week, the rats were anesthetized, and the kidneys removed and placed in cold saline.

Control, low, and high sodium diet rats. Rats were fed a low salt diet *ad libitum,* obtained from ICN Nutritional Biochemicals (0.005 meq Na/g of food). Rats on the high sodium diet were given regular Purina rat chow with 1% NaCl added to their drinking water. Control rats were fed regular Purina chow diet (0.07 meq Na/g of food) with tap water to drink. After 8 days, kidneys were removed and placed in cold saline.

Renal cortical slice preparation. After decapsulation, renal cortical slices (0.4 mm thick) were made with a Stadie-Riggs microtome. Two slices were incubated in a physiological salt solution (PSS) for two 10-min periods; the medium was changed at the end of each period as described previously (8, 11). The PSS was maintained at 37°C, pH 7.4, and bubbled with 95% O_2–5% CO_2. The slices were then incubated for 1 hr in 2.5 ml of PSS with 0.5% bovine serum albumin (BSA). The gas phase was layered over the medium in order to prevent renin inactivation (14). Following incubation, the media were centrifuged and the supernatants were frozen at −20°C until assayed. Two other slices were homogenized with cold distilled water containing 0.5% BSA, centrifuged at 1000*g* for 30 min at 4°C, and the supernatants stored at −20°C. This protocol was used for renal slices from control, low, and high salt diet rats and ADX animals. Slices from DOCA rats were directly homogenized because of their low renin content.

The protocol for acute stimulation was as follows: after washing the slices for two 10-min periods in PSS, two slices from normal rats were incubated for 20 min in 2.5 ml of PSS with 0.5% BSA. Twenty-five microliters of the incubation medium were collected and replaced by 25 μl of PSS or one of the following substances: ANG II (final concentration 10^{-6} *M*), AVP (final concentration 10^{-6} *M*), or verapamil (final concentration 5×10^{-4} *M*) (Sigma) dissolved in PSS. The slices were then incubated for 1 hr, and the medium was stored as above.

Isoelectric focusing. Isoelectric focusing was performed in a 5% polyacrylamide gel as described previously (8). Depending on the renin concentration, aliquots of 50–300 μl were applied to the top of the gel. After 20 hr at 4°C, the gel was frozen and serially sliced. Each gel segment was eluted in distilled water with 1% BSA. The pH of the gel segments were determined at 4°C before elution.

Renin activity measurement. Aliquots of incubation media, plasma, homogenates, and gel elutions were combined with substrate prepared from 40 hr nephrectomized rats as described previously (8). After incubation, the angiotensin I (ANG I) generated was measured using a New England Nuclear (Boston, Mass.) ANG I radioimmunoassay kit. Recovery of renin from the isoelectric focusing gel was approximately 85%. The amount of renin secreted in the incubation medium was expressed as ng ANG I/mg of cortical slice/hr/hr (ng ANG I/mg/hr/hr). Each renin form in a focusing gel was expressed as a percentage of the total renin recovered from that particular gel.

Statistical methods. Student's *t* test and paired *t* test were used to compare control and experimental groups. The Mann–Whitney *U* test was used to compare the percentage of renin forms between groups.

Results. The rate of basal release of renin from renal cortical slices, the renal renin content and plasma renin concentrations (PRC) in chronically stimulated or inhibited animals are presented in Table I. There were no significant differences between control and experimental groups in the amount of renin released into the incubation medium. The renal renin content was significantly reduced only in DOCA rats, and the PRC of rats fed on a low Na diet and ADX rats were significantly higher than that of the control group.

The isoelectric focusing pattern of renin released depicts six peaks of renin activity with *p*Is of 5.9, 5.7, 5.4, 5.2, 5.0, and 4.8 (for renin forms one to six, respectively) as described elsewhere (11). Figure 1 represents the different profiles of renin forms stored or secreted *in vitro* by renal slices from the different groups of animals. Kidneys from DOCA hypertensive rats and rats fed a high Na diet had profiles similar to those of control rats. However, the isoelectric focusing profiles of renin from rats fed a low Na diet and ADX rats were significantly modified: the relative proportion of

TABLE I. RENIN ACTIVITY IN CONTROL AND CHRONICALLY STIMULATED ANIMALS

	Control	DOCA	High Na	Low Na	ADX
Incubation medium (ng ANG I/mg/hr/hr)	77.0 ± 7.0 (12)		63.5 ± 11.4 (10)	61.6 ± 10.1 (8)	104 ± 17.1 (5)
Homogenate (ng ANG I/mg/hr)	711 ± 63.1 (12)	139 ± 27.0* (6)	655 ± 50.8 (10)	883 ± 175 (8)	568 ± 121 (9)
Plasma renin concentration (ng ANG I/ml/hr)	10.3 ± 1.3 (12)		10.6 ± 2.2 (10)	33.7 ± 6.6** (8)	197 ± 61.0** (4)

Note. Values are means ± SE. Number of experiments are indicated in parentheses. Comparison between control and experimental groups were significant at *$P < 0.01$; **$P < 0.001$.

renin form 2 decreased ($P < 0.01$, $P < 0.004$, respectively) and those of forms 3, 4, 5, and 6 increased ($P < 0.01$, $P < 0.004$) (Fig. 1).

Table II contains the results of acute experiments. The amount of renin released into the incubation medium was significantly reduced after ANG II (49 ± 9% decrease) and AVP (52 ± 6% decrease) administration, and significantly increased after verapamil (177 ± 16% increase). However, the isoelectric focusing profile of the secreted renin was not modified by ANG II or AVP (Fig. 2). In contrast, renal slices stimulated with verapamil released a modified renin profile: the proportion of renin form 2 decreased ($P < 0.05$) and those of forms 3 and 4 significantly increased compared with those of control slices ($P < 0.05$, $P < 0.01$).

Discussion. In this study, we observed a modification of the isoelectric focusing profile of renin after either chronic or acute stimulation of renin secretion. Conversely, no changes were found after acute or chronic inhibition of renin secretion. These data are in agreement with the modification of the profile found in the Goldblatt hypertensive rats and after isoproterenol stimulation of *in vitro* renal slices (8, 11). The pattern of change was characterized by a shift to the renin forms with the more acidic *p*Is. The change in the renin profile secreted by renal slices from rats fed a low Na diet and ADX rats was similar to that of acute stimulation with verapamil, a Ca^{2+} channel blocker. However, the increase of the more acidic renin forms was greater in chronically stimulated animals (Figs. 1, 2). We have previously shown that the renin profile stored in the kidney also changes with chronic stimulation of renin secretion (9–11) and that the renin profile secreted into the incubation medium is similar to that stored (11). Based on these facts, i.e., a difference in profiles between acute and chronic stimulation of renin secretion, we hypothesize that a stimulus of renin secretion induces a release of the more acidic forms of renin from the kidney and that a prolonged stimulus changes the profile of stored renin as well as that released.

Although the stimuli for the modification of the renin profile is still unknown, it could

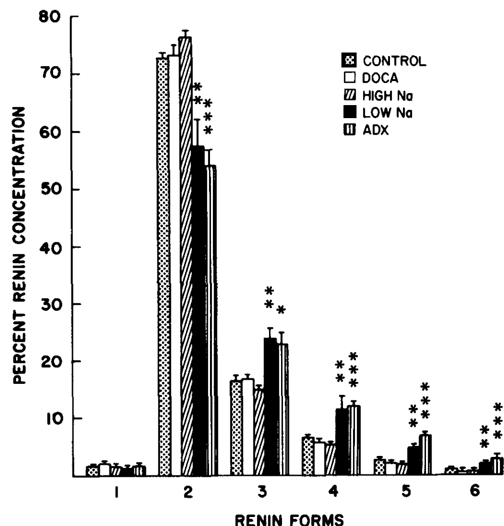

FIG. 1. Renin profile after chronic stimulation. Cortical renal slices from DOCA hypertensive rats (n = 6) and animals on a high Na intake (n = 6) showed an isoelectric focusing pattern of renin similar to that of control rats (n = 6). In ADX (n = 5) and low sodium diet rats (n = 4), the proportions of renin forms were significantly modified. Values are means ± SE. Comparisons with control animals were significant at *$P < 0.05$; **$P < 0.01$; ***$P < 0.004$.

TABLE II. RENIN ACTIVITY IN CONTROL AND ACUTELY STIMULATED RENAL SLICES

	Control	ANG II	AVP	Verapamil
Incubation medium (ng ANG I/mg/hr/hr)	168.2 ± 54.1 (6)	81.7 ± 27.1* (6)		
	176.5 ± 52.5 (6)		78.9 ± 17.4* (6)	
	108.7 ± 7.7 (6)			293.5 ± 18.8** (6)

Note. Values are means ± SE. Number of experiments are indicated in parentheses. Comparison with control values were significant at $^*P < 0.05$; $^{**}P < 0.001$.

be related to PRC, blood pressure, or aldosterone level. However, since the renin profile was not changed in the DOCA rats, high blood pressure is eliminated as the unique cause of the modification of the renin profile. (Blood pressure of DOCA rats was 205 ± 15 mm Hg and control was 116 ± 5 mm Hg.) Aldosterone changes also do not appear to be related to the changes in the renin profile; the profile was similar in rats fed a low salt food and ADX rats despite their differences in plasma aldosterone levels.

Factors which did correlate with the shift of the renin profile to the more acidic forms are PRC and the duration of the stimulation applied to the kidney. This suggests that changes in renin forms appear when renin synthesis and secretion are increased. This hypothesis is supported by the finding that the profile of renin stored and released in the clipped kidney of Goldblatt hypertensive rats is shifted to the more acidic forms, and the degree of this shift is greater 90 days following clipping as compared with that of 45 days following clipping (11). This pattern of change in the renin profile also correlated with the increase of PRC (11). Moreover, this hypothesis is consistent with the finding that the shift of renin profile to the more acidic forms is also observed in the spontaneously hypertensive stroke prone rats (SHRSP) (15). It was previously reported that SHRSP have an increased PRC, which is associated with malignant vascular lesions in the kidneys and brain (16, 17).

It has been shown that the renin forms with the more acidic *p*Is are degraded more slowly by the liver (12, 13). Thus, the shift of renin profile to the more acidic forms in chronically stimulated animals might reflect an adaptation to a more economic system, in terms of energy expended for the synthesis of the enzyme. Long term stimuli lead to a proportionate increase in the more acidic renin forms; the ones with the longer half-lives. Short term stimuli cause a reduced release of those forms with the longer half-life. Thus, in the later case the on/off response is more rapid than in the former. If long stimuli lead to a shift in renin profile toward those forms with a longer half-life, there would be an energetic saving; i.e., for a given plasma concentration, the secretory

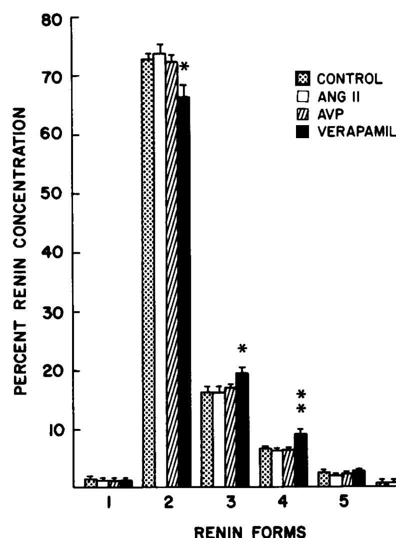

FIG. 2. Renin profile after acute stimulation. Application of angiotensin II ($n = 5$) or vasopressin ($n = 4$) did not change the renin profile secreted *in vitro*. Verapamil ($n = 6$) significantly increased the proportions of renin forms 3 and 4, and decreased the proportion of form 2. Values are means ± SE. Comparisons with control animals were significant at $^*P < 0.05$; $^{**}P < 0.01$.

rate would be less than if short half-life forms were secreted. In order to explain the increase of more acidic renin forms being related to the intensity and duration of stimulation, the existence of multiple pools of renin has been hypothesized (11, 18–20). The modification of the profile of renin forms during stimulation could reflect a different response of these multiple pools to stimulation. This hypothesis also suggests the possibility that some of the renin forms have different physiological functions. However, this point is quite speculative.

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