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Effect of Furosemide and Dietary Sodium on Kidney and Plasma Big and Small Renin (41287)

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Abstract. Renin was found in mouse plasma in high-molecular-weight forms (big big renin, big renin) and a low-molecular-weight form (small renin). They were measured by a radioimmunoassay procedure for the direct measurement of renin. In the kidney, 89% of total renin was small renin and the rest was big big and big renin. This distribution pattern of renins was not changed when the kidney tissue was homogenized in the presence of protease inhibitors. Low-sodium or high-sodium diets changed renal renin content, but not the distribution pattern of renins in the kidney. Acute stimulation of renin release by furosemide increased small renin but not big big and big renin in plasma. However, dietary sodium depletion for 2 weeks significantly increased big big, big, and small renin in plasma of mice with or without submaxillary glands. In contrast, high-sodium intake significantly decreased big big, big, and small renin in plasma of mice with or without submaxillary glands.

Previous studies on the mechanism of renin release have shown that factors such as the adrenergic nervous system, diuretics, prostaglandins, sodium depletion, and decreased renal perfusion pressure (1–4) increase plasma renin activity (PRA). In those studies, renin was measured indirectly by determining the amount of angiotensin I generated (enzymatic activity) during incubation with renin substrate. Recent studies have shown that renins of different molecular weights exist in several species (5–9). The enzymatic activity of these high-molecular-weight renins was considerably less than that of low-molecular-weight renin (6, 10). Therefore, in studies related to the mechanism of renin release, measurement of renin by its enzymatic activity may not always represent actual renin levels.

In the present study, we investigated the effects of furosemide, low-sodium, and high-sodium intake on plasma big and small renins as well as on the content of these renins in the kidney. These renins were isolated using gel filtration chromatography and then measured individually by a radioimmunoassay procedure developed in our laboratory (11) for the direct measurement of renin. This procedure measures both renal and submaxillary gland renins since the antiserum to submaxillary renin used in these studies was found to cross-react with mouse renal renin (11).

Methods. *Animals.* Adult male Swiss albino mice weighing 30–35 g were used as experimental animals. Intact animals and animals whose submaxillary glands were removed (sialoadenectomized) under pentobarbital anesthesia (60 mg/kg ip) 2 weeks before the experiments were used. The study consisted of two major groups of animals, those treated with furosemide and those given low- or high-sodium diets. Control animals were included in all experiments.

Furosemide-treated animals. In these experiments, animals whose submaxillary glands were removed 2 weeks before were used. They were divided into three groups and 1 μg/g body wt furosemide was injected via the tail vein under pentobarbital anesthesia to all animals except those of the first group. Two hundred microliters of blood was drawn from the inferior vena cava at 0 (first group), 60 (second group), and 120 min (third group). The blood samples were placed in ice-cold polystyrene tubes containing EDTA [(ethylenedinitrilo)tetraacetic acid disodium salt]. The plasma was immediately separated by centrifugation at 4° and stored at −20° until used for renin assay or gel filtration.

Low-sodium and high-sodium diet animals. In this study the animals were divided

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into two groups. The first group were intact animals and the second group were animals sialoadenectomized 2 weeks before the experiment. Each group was divided into three subgroups of animals: Those fed a basal diet (Na = 0.29%, K = 0.46%), those fed a low-sodium diet (Na = 0.05%, K = 0.46%), and those fed a high-sodium diet (N = 3.5%, K = 0.46%), obtained from Ralston Purina Company. After 2 weeks on these diets, blood was drawn from the inferior vena cava of each animal under pentobarbital anesthesia into ice-cold polystyrene tubes containing EDTA. The plasma was separated as above and used for renin assay or gel filtration. The kidneys from intact animals were also removed and used for determination of renin content or for gel filtration of kidney homogenate.

Separation of renins by gel filtration. A gel filtration procedure was used for the separation of renins of different molecular weights in both the kidneys and the plasma.

Kidney renins. The kidneys obtained from the intact animals were homogenized by using a Potter–Elvehjem homogenizer at 0°. The homogenizations were done in five different buffers as follows: 1 ml of 0.05 *M* sodium phosphate buffer, pH 7.4, containing 0.1 *M* sodium chloride and 0.02% sodium azide (buffer A); 1 ml of buffer A with 5 m*M* sodium tetrathionate (buffer B); 1 ml of buffer A with 5 m*M* *N*-ethylmaleimide (buffer C); 1 ml of buffer A with 10 m*M* diisopropyl fluorophosphate (buffer D); 1 ml of buffer A with 10 m*M* EDTA, 2 m*M* iodoacetic acid, 2 m*M* phenylmethanesulfonyl fluoride, 5 m*M* sodium tetrathionate, and 5 m*M* *N*-ethylmaleimide (buffer E) as protease inhibitors. Each of the five separate homogenates was centrifuged for 60 min at 105,000*g* and the supernatants obtained were immediately applied to individual Sephadex G-100 superfine columns (1.6 × 100 cm) at 4°. Each column was equilibrated and eluted with 0.05 *M* sodium phosphate buffer (pH 7.4) containing the same protease inhibitors as used for the corresponding homogenization step. The flow rate was 5 ml/hr. Fractions of 2 ml were collected and used for measurement of protein and renin by the direct radioimmunoassay (11). Each column was standardized with Calibration Kits (Pharmacia Fine Chemicals) and ^{125}I-labeled pure submaxillary renin.

The kidneys from mice on low- or high-sodium diet were homogenized with buffer E and the subsequent procedures were the same as those described above.

Plasma renins. For the separation of plasma renins, 100 to 200 μl of each individual mouse plasma sample were applied to a Sephadex G-100 superfine column (0.6 × 100 cm) at 4°. The column was equilibrated and eluted with 0.05 *M* sodium phosphate buffer, pH 7.4, containing 0.1 *M* sodium chloride and 0.02% sodium azide at a rate of 0.625 ml/hr. Fractions of 0.4 ml were collected into tubes at 4°. In each fraction, protein absorbance was measured at 280 nm, and renin content was determined with the radioimmunoassay for the direct measurement of renin (11). The molecular weights of renin were estimated by the same column fractionation procedure using aldolase, bovine serum albumin, ovalbumin, and chymotrypsinogen A as molecular-weight standards. There were three renins of different molecular weights. The first peak was that of the highest molecular weight (big big renin). The next peak was renin of 68,500 mol. wt. (big renin), and the third peak was that of small renin with molecular weight 38,000 (small renin). The enzymatic activity (angiotensin I-generating activity) of each renin was also determined using plasma from nephrectomized sialoadenectomized mice as renin substrate. By comparing the level of activity of these renins, small renin had strong enzymatic activity, whereas big big renin and big renin showed considerably less enzymatic activity. The high-molecular-weight renins are considered to be renins because they were enzymatically active by generating angiotensin I, and their enzymatic activity was neutralized by renin antibody and pepstatin (12).

Measurement of renin. Renin was measured by the direct radioimmunoassay procedure developed in our laboratory (11). In brief, newly purified mouse submaxillary

gland renin was used to prepare rabbit anti-renin serum. Pure mouse submaxillary renin was iodinated according to the method of Hunter and Greenwood (13) and the immunoreactive fraction was isolated by gel filtration. In preparing incubation mixtures for radioimmunoassay, 100 μl of labeled renin (3000 cpm) and 100 μl of antiserum (1: 30000) were added to 0.8 ml of 0.05 *M* sodium phosphate buffer, pH 7.4, containing 0.5% BSA and either unknowns or standard solutions of renin. After 3 days of incubation at 4°, 100 μl of a 1:4 dilution of goat anti-rabbit gamma globulin serum and 100 μl of a 1:20 dilution of normal rabbit serum (Research Products International Corp.) were added for separation of antibody-bound and free renin. These samples were again incubated for 24 hr at 4°. At the end of the incubation, 1 ml of 0.05 *M* sodium phosphate buffer, pH 7.4, was added and immediately mixed and then centrifuged at 3000 *g* for 30 min. The supernatant and the precipitate were separated by decantation and counted in a well-type gamma counter. Big renin and big big renin were found to displace iodinated submaxillary renin from anti-renin antibody in this radioimmunoassay procedure with the same characteristics as small renin. PRA (angiotensin I generated) was also measured using plasma from nephrectomized sialoadenectomized mice as substrate (14).

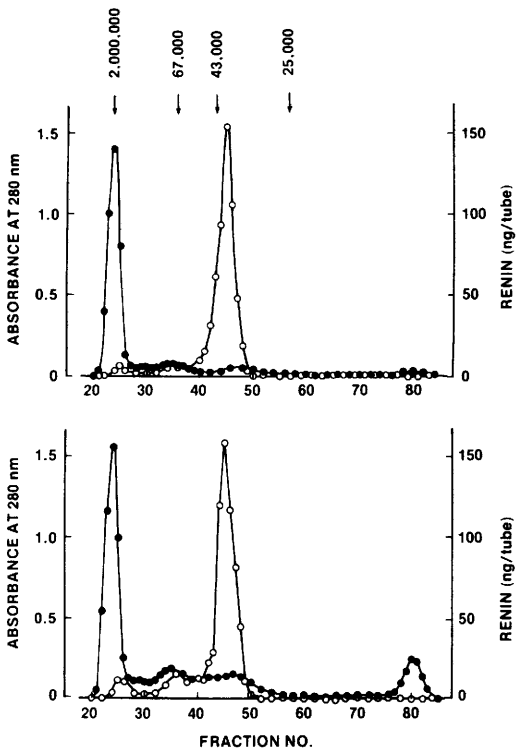

FIG. 1. Gel filtration of intact mouse kidney homogenate on Sephadex G-100 superfine column (1.6 × 100 cm) with or without protease inhibitors. Volumes collected were approximately 2 ml/tube. The flow rate was 5 ml/hr. The closed circles (●) indicate protein absorbance at 280 nm. The open circles (○) indicate renin measured by direct radioimmunoassay. The numbers and arrows at the top indicate molecular weight. The upper panel of the figure is the gel filtration of kidney homogenate with buffer A which did not contain protease inhibitors. The lower panel of the figure is the gel filtration of kidney homogenate with buffer E.

Results. *Kidney homogenate.* The results of gel filtration of renins from kidney homogenate are shown in Fig. 1. In each fraction, renin concentration was determined by the direct radioimmunoassay of renin and protein absorbance was measured at 280 nm.

The results of gel filtration of kidney homogenate with buffer A, which did not contain protease inhibitors, showed that there was a very high peak of small renin. Higher-molecular-weight renins were also present but in considerably lesser amounts. In gel filtration of kidney homogenate with buffer B, C, D or E, which contained protease inhibitors, the results were very similar to the elution pattern of gel filtration of kidney homogenate with buffer A.

Enzymatic activity of each fraction was also determined. Small renin had a greater enzymatic activity, whereas big big renin and big renin showed considerably less activity. The enzymatic activity was neutralized by renin antibody and pepstatin (Fig. 2).

In intact animals on sodium diet the results of gel filtration of kidney homogenate were similar to the results shown in Fig. 1. There was a very high peak of small renin, however, there were smaller peaks of higher-molecular-weight renins in the basal

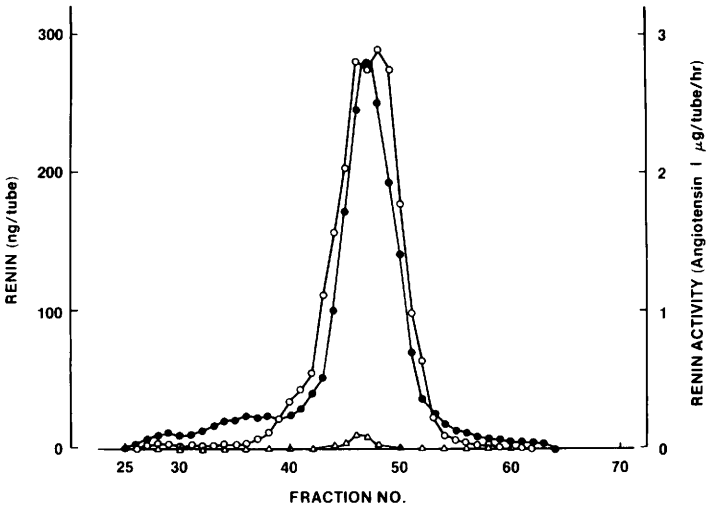

FIG. 2. Gel filtration of intact mouse kidney homogenate on Sephadex G-100 superfine column (1.6 × 100 cm) with buffer D. Volumes collected were approximately 2 ml/tube and the flow rate was 5 ml/hr. The closed circles (●) indicate renin measured by direct radioimmunoassay. The open circles (○) indicate enzymatic activity of renin with mouse plasma as renin substrate which was drawn from sialoadenectomized and nephrectomized mice. The triangles (△) indicate the neutralization of enzymatic activity of renin with renin antiserum or pepstatin.

diet group, the low-sodium diet group and the high-sodium diet group. The percentages of big big renin, big renin, and small renin against the total renin amount were not changed by low-sodium or high-sodium diet compared to the basal diet group (Table I). Renal renin content measured by the renin radioimmunoassay procedure, was significantly increased by low-sodium diet and decreased by high-sodium diet (Table I).

Effect of furosemide on total plasma renin. The effect of furosemide on total plasma renin is shown in Table II. The total plasma renin, which was measured by the direct renin radioimmunoassay, increased after furosemide injection and nearly reached the maximum response at 120 min.

Effect of furosemide on big big, big, and small renin in plasma. To find out the effect of furosemide on renins of different molecular weights, plasma samples were fractionated and renins were isolated as described before. As shown in Fig. 3, furosemide significantly increased small renin at 60 and 120 min, but big big and big renin were not changed significantly.

In animals which did not receive furo-

TABLE I. EFFECT OF LOW-SODIUM DIET AND HIGH-SODIUM DIET ON BIG BIG, BIG, AND SMALL RENIN ON KIDNEY HOMOGENATE AND ON RENAL RENIN CONTENT IN INTACT ANIMALS

	Big big renin/ total renin[a] (%)	Big Renin/ total renin[a] (%)	Small renin/ total renin[a] (%)	Renin content[b] (ng/mg tissue)
Basal diet	3.3 ± 0.87	7.8 ± 1.78	88.9 ± 2.56	10.11 ± 0.48
Low-sodium diet	4.3 ± 0.74	8.8 ± 0.45	86.8 ± 1.18	32.07 ± 3.05*
High-sodium diet	3.5 ± 0.81	7.6 ± 0.32	88.8 ± 0.49	5.34 ± 0.49*

Note. Results are expressed as mean ± SEM. Big big renin/total renin, big renin/total renin, or small renin/total renin = the amount of each renin was divided by the amount of total renin and expressed as a percentage.

[a] Four animals.

[b] Twenty animals.

* $P < 0.005$ compared with basal diet group.

TABLE II. EFFECT OF FUROSEMIDE ON TOTAL PLASMA RENIN IN SIALOADENECTOMIZED MICE

Time (min)	Total plasma renin (ng/ml)
0 (N = 18)	43.4 ± 3.2
60 (N = 16)	62.3 ± 6.3*
120 (N = 10)	71.3 ± 6.3*

Note. Results are expressed as mean ± SEM. N = number of animals.
* $P < 0.01$ compared with 0-min value.

TABLE III. EFFECT OF HIGH-SODIUM DIET AND LOW-SODIUM DIET ON TOTAL PLASMA RENIN IN INTACT OR SIALOADENECTOMIZED MICE

	Total plasma renin (ng/ml)
Intact mice	
Basal diet (N = 15)	50.0 ± 2.9
High-sodium diet (N = 19)	28.8 ± 2.8*
Low-sodium diet (N = 21)	90.4 ± 12.8*
Sialoadenectomized mice	
Basal diet (N = 20)	51.8 ± 3.3
High-sodium diet (N = 20)	22.9 ± 2.0*
Low-sodium diet (N = 19)	75.5 ± 8.0*

Note. Results are expressed as mean ± SEM. N = number of animals.
* $P < 0.005$ compared with basal diet in each group.

semide (control group), the plasma level of big renin was higher than that of small renin. However, in furosemide-treated animals which had blood drawn 60 and 120 min after furosemide administration, the levels were reversed and a greater increment of small renin was found as compared to big renin. The elution characteristics of the various forms of renin from plasma of nontreated mice has been reported previously (12).

Effect of high-sodium and low-sodium diet on plasma renins. As shown in Table III, total plasma renin was significantly decreased by high-sodium diet and increased by low-sodium diet in either the intact group or the sialoadenectomized group.

The effect of high-sodium and low-sodium diet on big big, big, and small renin levels in plasma is shown in Figs. 4 and 5. Figure 4 shows the results on intact animals and Fig. 5 shows the results on sialoadenectomized animals. In both groups, big big renin, big renin, and small renin were significantly decreased by high-sodium diet and increased by low-sodium diet. The presence of submaxillary glands had no effect on the plasma levels of big big, big, and small renin in high- or low-sodium diet fed animals.

Discussion. In the pig, dog, rabbit, and rat, inactive renin or high-molecular-weight renin is reported to exist in the kidney, and it is suggested that high-molecular-weight

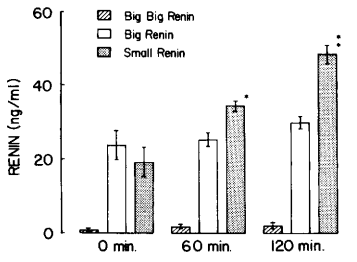

FIG. 3. Effect of furosemide on big big renin, big renin, and small renin of plasma in sialoadenectomized animals. Plasma samples were eluted on a Sephadex G-100 superfine column (0.6 × 100 cm) and the amount of renin in each tube was measured by direct radioimmunoassay. Vertical lines represent SEM. 0 min = control group (N = 6); 60 min = 60 min postfurosemide injection (1 μg/g body wt) the animals were sacrificed (N = 5). At 120 min postfurosemide injection the animals were sacrificed (N = 7). *$P < 0.01$ compared with 0-min group. **$P < 0.005$ compared with 0-min group.

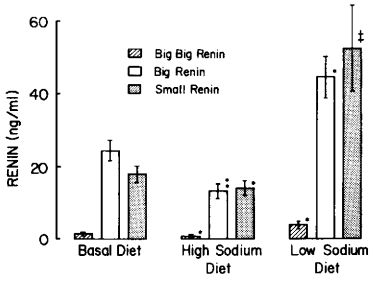

FIG. 4. Effect of high-sodium diet and low-sodium diet on plasma big big renin, big renin, and small renin in intact animals. Basal Diet = animals were fed a basal diet (Na = 0.29%, K = 0.46%) for 2 weeks before being sacrificed (N = 5). High Sodium Diet = animals were fed a high-sodium diet (Na = 3.5%, K = 0.46%) for 2 weeks before being sacrificed (N = 6). Low Sodium Diet = animals were fed a low-sodium diet (Na = 0.05%, K = 0.46%) for 2 weeks before being sacrificed (N = 5). ‡$P < 0.05$ compared with basal diet value. *$P < 0.025$ compared with basal diet. **$P < 0.005$ compared with basal diet.

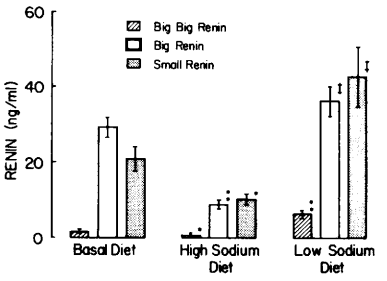

FIG. 5. Effect of high-sodium diet and low-sodium diet on plasma big big renin, big renin, and small renin in sialoadenectomized animals. Basal Diet, High Sodium Diet, or Low Sodium Diet = animals were fed a basal diet ($N = 5$), a high-sodium diet ($N = 5$), or a low-sodium diet ($N = 9$) for 2 weeks before being sacrificed, respectively. ‡$P < 0.05$ compared with basal diet. *$P < 0.025$ compared with basal diet. **$P < 0.005$ compared with basal diet.

renin may be a precursor of renin (6–8, 15, 16). It has been suggested that in mouse submaxillary glands, renin is synthesized as a precursor with a molecular weight of 50,000 and stored as fully active renin with a molecular weight of 40,000 (17). In the present experiments, gel filtration showed that mouse kidney homogenate contains mainly small renin, however, small amounts of high-molecular-weight renins were also present (Fig. 1). The pattern of gel filtration was not changed with or without protease inhibitors or dietary sodium treatment. It is possible that in the mouse kidneys and submaxillary glands, renin is stored as low-molecular-weight renin (17). Another possibility is that the protease inhibitors did not inhibit the conversion of high- to low-molecular-weight renin. If the latter is true, then either the conversion was very rapid during homogenization of the kidney tissue or the proteases involved in the conversion are different from those of other species.

In the dog, furosemide administration caused a rise in PRA and exposure of the same plasma to pH 4.5 did not result in a further increase in PRA (18). In the pig and human furosemide caused an increase in plasma concentration of active and inactive renin (19–21). In the present experiments furosemide increased plasma small renin levels significantly, and big renin levels only to an insignificant extent. Our data are similar to those of other studies (20) in the human, even though a different method for determination of renin was used.

To find out whether there was any correlation between the presence or absence of submaxillary glands and the effect of dietary sodium on plasma renin levels, intact and sialoadenectomized mice were studied. In both groups, big big, big, and small renins were significantly decreased in high-sodium and increased in low-sodium diets fed animals. These data suggest that the presence or absence of submaxillary glands had no effect on the plasma big big, big, and small renin levels in high- or low-sodium diet fed animals.

In our experiments big big, big, and small renin plasma levels were decreased in mice which were fed high-sodium diets for 14 days. In human subjects, after saline infusion, plasma renin concentration was suppressed, but inactive renin and total renin concentration did not decrease (19). The difference between these data could be due to the duration of stimulus to renin release. It is possible that the response of active renin to sodium load is faster than that of big renin.

Low-sodium diet fed to mice for 14 days caused an increase in big big, big, and small renin plasma levels. Similar experiments were performed in the human and the rabbit. Millar *et al.* (20) reported that 4 days dietary sodium restriction caused an increase in active renin and total renin, but not in inactive renin concentration in human plasma. In the rabbit during dietary sodium depletion active renin and total renin were increased, but inactive renin levels initially fell to zero and then increased until after 13 days when inactive renin was again 10% of total renin levels, a proportion comparable to the control values (22). As to the time course of these studies, our data obtained from mice are similar in pattern to the rabbit data when the animals were fed low-sodium diet for a longer period.

Hsueh *et al.* (23) reported that in normal human, dietary changes in salt intake from high (400 m*M*) to low (10 m*M*) increased both active and inactive plasma renin but

the portion of total renin existing in the inactive form had fallen from 82 to 65%. Also both big and small renin can exist as inactive or active enzyme in normal human plasma (23). In our studies the portions of total plasma renin existing as big big, big, and small renin were similar in both the low-sodium diet and the high-sodium diet groups (Figs. 4 and 5). It has also been reported previously that acidification did not activate big and small renin in mouse plasma (10). It is possible that the discrepancy in the results of studies with human and mouse could be due to species difference.

The more important difference between the present study and those of other reports is the methodology used for renin measurement. Whereas in other studies renin was determined by the enzymatic method which determines the amount of angiotensin I generated by renin, in the present study it was measured by a radioimmunoassay procedure for the direct measurement of renin (11, 12). Previous studies (10) have shown that the validity of the currently used term "total" renin as the enzymatic renin activity after acid activation is questionable. Therefore, it may not be adequate to compare our results to those of other investigators who used acid activation of renin because we used the renin radioimmunoassay procedure that measures renin itself (11, 12).

Acute stimulation of renin release by furosemide, isoproterenol, tilting, or diazoxide increased active or small renin in human (20, 24), pig (21), dog (18), and mouse (12). Inactive or big renin was not changed in dog by furosemide (18), slightly increased in human by furosemide and isoproterenol (20, 24), and greatly increased in pig by furosemide and isoproterenol (21), and in mouse by isoproterenol (12). In addition, when active or small renin was significantly increased by isoproterenol in pig and mouse, inactive or big renin was also greatly increased (12, 21). In our present data furosemide as compared to isoproterenol did not cause much of an increase in big big and big renin plasma levels. However, low- or high-sodium diet for 2 weeks caused significant changes in big big, big and small renin in both the intact and the sialoadenectomized animals (Figs. 4 and 5) but the effect on the difference between the amount of small renin and the amount of big renin was less. It is possible that the levels of big renin in plasma may be influenced by the amount of small renin present during the time course of the experiment.

Other studies in man (25–28) have shown that dietary sodium depletion for several days results in an increase in both active and inactive renin, whereas acute dietary or pharmacologic depletion only results in an increase in active renin. This is in accordance with our results reported here.

Based on the results of our experiments regarding big and small renin levels in kidney homogenate and in plasma, at least two possibilities for the origin of plasma big renin may be proposed: Small renin may be released by the juxtaglomerular cells in response to stimulation and combined with tissue components (29) or with plasma components (30) to become big renin. Big and small renin may be released from the kidney into the circulation in response to stimulation. In the latter case the released amounts of big or small renin may be dependent on the nature of the stimulation.

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1. Vander, A. J., Amer. J. Physiol. **209**, 659 (1965).
2. Meyer P., Menard, J., Papanicolaou, N., Alexandre, J. M., Devaux, C., and Milliez, P., Amer. J. Physiol. **215**, 908 (1968).
3. Blaine, E. H., and Davis, J. O., Circ. Res. **28–29** (Suppl. II), II-118 (1971).
4. Dunn, M. J., and Hood, V. L., Amer. J. Physiol. **233**, F169 (1977).
5. Day, R. P., Luetscher, J. A., and Gonzales, C. M., J. Clin. Endocrinol. Metab. **40**, 1078 (1975).
6. Inagami, T., and Murakami, K., Circ. Res. **41**, (Suppl. II), II-11 (1977).
7. Potter, D. M., McDonald, W. J., Dunn, P. M., and Metcalfe, J., Circ. Res. **43**, 832 (1978).

8. Leckie, B. J., and McConnell, A., Circ. Res. **36**, 513 (1975).
9. Boyd, G. W., *in* "Hypertension '72" (J. Genest and E. Koiw, eds.), p. 161. Springer-Verlag, New York (1972).
10. Nielsen, A. H., Malling, C., and Poulsen, K. Biochim. Biophs. Acta. **534**, 246 (1978).
11. Michelakis, A. M., Yoshida, H., Menzie, J., Murakami, K., and Inagami, T., Endocrinology **94**, 1101 (1974).
12. Iwao, H., Lin, C. S., and Michelakis, A. M., Amer. J. Physiol. **238E**, 416 (1980).
13. Hunter, W. M., and Greenwood, F. C., Nature (London) **194**, 495 (1962).
14. Haber, E., Koerner, T., Page, L., Kliman, B., and Purnode, A., J. Clin. Endocrinol. Metab. **291**, 349 (1969).
15. Inagami, T., Hirose, S., Murakami, K., and Matoba, T., J. Biol. Chem. **252**, 7733 (1977).
16. de Senarclens, C. F., Pricam, C. E., Banichahi, F. D., and Vallotton, M. B., Kidney Int. **11**, 161 (1977).
17. Poulsen, K., Clin. Sci. **57**, 1095 (1979).
18. James, S. K., and Hall, R. C., Pfluegers Arch. **347**, 323 (1974).
19. Weinberger, M., Aoi, W., and Grim, C., Circ. Res. **41** (Suppl. II), II-21 (1977).
20. Millar, J. A., Leckie, B. J., Semple, P. F., Morton, J. J., Sonkodi, S., and Robertson, I. S., Circ. Res. **43**, (Suppl. I), I-120 (1978).
21. Bailie, M. D., Derkx, F. M. H., and Schalekamp, M. A. D. H., Circ. Res. **44**, 32 (1979).
22. Grace, S. A., Munday, K. A., Noble, A. R., and Richards, H. K., J. Physiol. **292**, 421 (1979).
23. Huseh, W. A., Carlson, E. J., Luetscher, J. A., and Grislis, G., Lancet **1**, 1281 (1978).
24. Derkx, F. H. M., Wenting, G. J., Man in't Veld, A. J., van Gool, J. M. G., Verhoeven, R. P., and Schalekamp, M. A. D. H., Lancet **2**, 496 (1976).
25. Sealey, J. E., and Laragh, J. H., Circ. Res. **36/37**, Suppl. 1, 10 (1975).
26. Atlas, S. A., Sealey, J. E., Laragh, J. H., and Moon, C., Lancet **2**, 785 (1977).
27. Mackenzie, J. K., and Reisin, E., Clin. Sci. Mol. Med. **55**, 151s (1978).
28. Kappelgaard, A. M., Giese, J., Ibsen, H., Nielsen, M. D., and Rabol, A., Clin. Sci. Mol. Med. **55**, 143s (1978).
29. Funakawa, S., Funaw, Y., and Yamamoto, K., Biochem. J. **176**, 977 (1978).
30. Poulsen, K., Krøll, J., Nielsen, A. H., Jensenius, J., and Malling, C., Biochim. Biophys. Acta. **577**, 1 (1979).

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