Role of Renin in the Regulation of Angiotensinogen Levels in Plasma[1] (35802)

HIROSHI TATEISHI, ALBERTO NASJLETTI, AND GEORGES M. C. MASSON
Cleveland Clinic Foundation, Research Division, Cleveland, Ohio 44106

The demonstration that under normal conditions plasma renin activity and rate of angiotensin formation are dependent as much on renin substrate (angiotensinogen) as on renin concentration (1), has emphasized the need for further information on the factors regulating angiotensinogen levels. That renin might be one of the factors has been inferred from the negative correlation between renin and its substrate found in nephrectomized and in adrenalectomized animals (2, 3); assuming a constant synthesis, angiotensinogen levels would be determined by the rate of angiotensinogen destruction, hence by renin concentration. There are however other experimental situations where this correlation does not obtain in spite of high or low plasma renin values (2, 4). Furthermore, attempts to influence substrate levels by administration of heterologous renin to normal or nephrectomized animals have led to conflicting results (4), thus suggesting that factors other than renin may be at play.

The purpose of this investigation was to reexamine the role of renin by measuring both plasma renin and angiotensinogen concentration in situations known to be associated with abnormal levels of either component: bilateral ureteral ligation, pregnancy, combination of nephrectomy with either adrenalectomy or pregnancy, and administration of homologous renin to normal or nephrectomized rats.

Methods. Female Sprague-Dawley rats weighing 180–240 g were used. They were fed a commercial chow and given tap water to drink.

First experimental series. Relationship between renin and angiotensinogen in plasma were studied under the following experimental conditions.

1. Adrenalectomy without salt supplement. Animals were killed on day 7.

2. Adrenalectomy plus nephrectomy. Bilateral nephrectomy was performed on day 7 postadrenalectomy. Animals were killed 8 hr later.

3. Pregnancy. Blood samples were taken prior to mating and on 17–18 days of pregnancy or on 4–5 days postdelivery.

4. Pregnancy plus nephrectomy. Bilateral nephrectomy was performed during the last quarter of pregnancy. Animals were killed 24 hr later.

5. Bilateral ureteral ligation. Animals were killed 24 hr later. All surgical procedures were performed during ether anesthesia. Adequate control groups were added to each experiment. Blood samples (0.8 ml) with EDTA as anticoagulant were taken from the jugular vein following light ether anesthesia. Renin substrate was determined by incubating 0.1 ml of plasma with an excess of rat renin and measuring angiotensin formed (3). Results are expressed in nanograms (ng) of angiotensin per milliliter of plasma. Renin concentration was determined by incubating 0.1 ml of plasma in the presence of an excess of rat renin substrate and measuring amounts of angiotensin formed (5). Results are expressed in nanograms per milliliter of plasma per hour of incubation. Bioassay of angiotensin was carried out in pentolinium-treated rats, using angiotensin II amide (Hypertensin, Ciba) as standard.

Second experimental series. These experiments were designed to study the effects of exogenous renin on plasma angiotensinogen and renin concentration in normal and nephrectomized rats. Rats were divided into groups of 6 animals each. They received one subcutaneous injection of 5 or 20 Goldblatt units (GU) of rat renin. In nephrectomized animals the injection was given immediately

[1] Supported by Grant HE-6835 from the National Heart and Lung Institute.

TABLE I. Effects of Nephrectomy Plus Adrenalectomy or Pregnancy on Plasma Renin Substrate and Renin Concentration.

Group	No. of animals	Renin substrate (ng/ml)	Renin conc (ng/ml/hr)
Normal controls	8	300 ± 33	15.4 ± 6.2
Nephrectomy (8 hr)	8	1086 ± 245	0.72 ± 0.4
(24 hr)	4	2160 ± 560	Undetectable
Adrenalectomy (7 days)	8	102 ± 39	450 ± 163
+ nephrectomy (8 hr)	7	1072 ± 235	16.3 ± 12
Pregnancy + nephrectomy (24 hr)	5	1912 ± 347	2.2 ± 1.5
Ureteral ligation (24 hr)	6	1688 ± 537	2.5 ± 1.1

after surgery or 12 hr later. In one group of nephrectomized rats, a crude saline extract of rat kidneys (1 ml of fluid/g of tissue) was administered sc, once at the time of nephrectomy and 3 hr later for a total dose equivalent to 2 kidneys. Sham-operated rats used as controls received one injection of heat-denatured renin or of saline. Following amobarbital anesthesia (9 mg/100 g), blood was withdrawn from the aorta at various intervals up to 6 hr in separate groups of animals.

Angiotensinogen was determined as in the first series and renin concentration by an adaptation of the Gould procedure (6).

The rat renin used for injection was prepared according to the method of Haas *et al.* (7). It contained 50 GU/ml. All results are expressed as means ± standard deviation.

Results. First experimental series (Tables I and II). Nephrectomy caused an increase in plasma substrate and a gradual disappearance of plasma renin. Ureteral ligation had similar effects, except that the decrease in renin was not as marked. By contrast, both adrenalectomy and pregnancy caused a decrease in substrate and an increase in renin. Following delivery, substrate remained at the levels seen during pregnancy while renin decreased to near normal levels. These results were not affected by removal of the litters. When adrenalectomized or pregnant animals were nephrectomized, substrate increased to the levels found in nephrectomized controls, and renin decreased but not to the same extent as after nephrectomy alone.

Second experimental series. 1. Effects of renin in normal rats (Fig. 1). Administration of 5 GU of renin did not cause any significant change in renin substrate levels although plasma renin showed a 2-fold increase on the first hour followed by a gradual return to normal. Administration of 20 GU of renin caused a significant decrease in substrate during the first 4 hr, and about a sixfold

TABLE II. Effects of Pregnancy on Plasma Renin Substrate and Renin Concentration.

Groups	Renin substrate (ng/ml)		Renin conc (ng/ml/hr)	
	A[a]	B[b]	A[a]	B[b]
Pregnancy	299 ± 44	207 ± 40 ($p < 0.01$)	12.7 ± 2.9	39.7 ± 19 ($p < 0.025$)
Postdelivery	304 ± 33	197 ± 18.7 ($p < 0.001$)	11.6 ± 2.1	15 ± 1.7 ($p < 0.05$)

[a] Values prior to mating.

[b] Values on day 17 or 18 of pregnancy or 4–5 days after delivery.

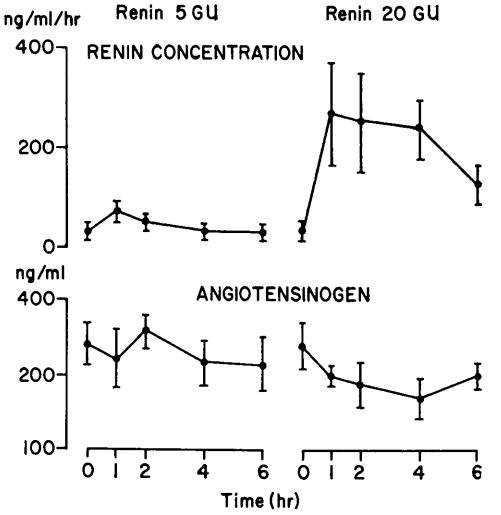

FIG. 1. Effects of rat renin on plasma renin and angiotensinogen in normal rats. Transverse bars indicate 1 SD.

increase in renin on the fourth hour. Measurements of urinary renin after injection of 20 GU showed a peak of excretion between 2 and 4 hr. Total amounts excreted during the first 4 hr corresponded to 1740 ng of angiotensin as compared with 7 ng in normal rats during the same period. These values are equivalent respectively to 0.095 and 0.00041 GU of renin.

2. Effects of renin in nephrectomized rats (Fig. 2). Administration of 5 GU of renin slightly delayed the rise in substrate but, at the fifth hour, values were not significantly different from those obtained after nephrectomy alone; plasma renin levels were significantly elevated. These effects were more marked with a dose of 20 GU. The rise in substrate was delayed until the fourth hour and was not as great on the sixth hour as after nephrectomy alone. Plasma renin increased about 20 times. Injection of crude kidney extracts did not prevent the increase in substrate caused by nephrectomy alone, although renin levels were several times higher than normal. At the sixth hour, substrate averaged 885 ± 74 ng/ml compared with 700 ± 65 ng/ml in nephrectomized controls while plasma renin, which was undetectable in the controls, averaged 125 ± 4 ng/ml/hr. When administration of renin was delayed until 12 hr after nephrectomy (Table III), the dose of 5 GU had no significant effect on substrate when values were compared with those obtained 12 hr after nephrectomy; however they were significantly lower than those obtained 18 hr after nephrectomy ($p<0.025$). Substrate was significantly lowered by 20 GU of renin. Renin levels were 3 times higher with 20 GU than with 5 GU.

Discussion. Results from renin administration demonstrate that only high concentrations of circulating renin affect substrate levels, hence suggest that in normal animals renin plays a minor role in the regulation of substrate. A 2-fold increase in plasma renin concentration did not significantly alter substrate levels, while a 6-fold increase reduced substrate by one third. These results are in accord with those of Munoz *et al.* (8) and of Goldblatt *et al.* (9) who showed that in dogs doses between 0.5 and 1 GU/min of renin elevating blood pressure by about 70 mm Hg were necessary to cause a significant fall in angiotensinogen. They are, however, at variance with those obtained in rats by Carretero and Gross (4) who found a significant in-

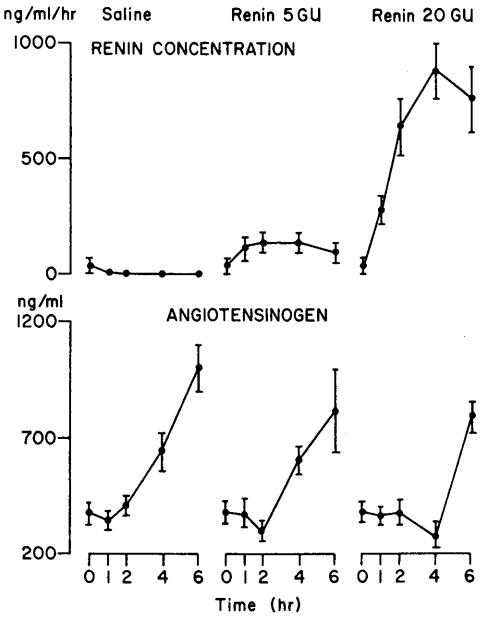

FIG. 2. Effects of rat renin on plasma renin and angiotensinogen in bilaterally nephrectomized rats. Transverse bars indicate 1 SD.

TABLE III. Effects of Exogenous Renin on Plasma Renin Substrate and Renin in 12-hr Nephrectomized Rats.

Groups	Renin substrate (ng/ml)	Renin conc (ng/ml/hr)
12-hr Nephrectomy	2242 ± 737	Undetectable
+ saline[a]	3057 ± 697	Undetectable
+ renin, 5 GU[a]	2038 ± 255	277 ± 36
20 GU[a]	1216 ± 262	990 ± 18

[a] Animals were killed 6 hr after injection.

crease in substrate with a dose of 0.004 U/min and no effect with 0.016 U. Absence of data on plasma renin concentration or on pressor responses does not permit any explanation for this discrepancy.

Nehprectomy alone caused a sharp increase in substrate up to a plateau which is reached 8–10 hr later (4). This is associated with a rapid disappearance of plasma renin. Administration of renin at the time of nephrectomy caused only a delay in the rise of plasma substrate, which was dose related, while administration of renin 12 hr later caused a fall in substrate which was also dose related. This difference in response depending on the time of injection, may be dependent on the rate of substrate formation, which presumably is higher during the rise than during the plateau or on the rate of substrate consumption (4). Thus 12-hr nephrectomized, like normal animals, responded to renin by a fall in substrate. That this fall was greater in the former than in the latter may be due to the levels of plasma renin. The cause for the high and sustained levels of renin in nephrectomized animals is not clear. Rates of metabolic clearance of exogenous and endogenous renin have been reported to be similar in both normal and nephrectomized animals (10, 11), with renin leaving plasma rapidly into the extracellular space. Differences in methods of administration and in dosage may account for the discrepancy. There is, however, agreement on one point. We confirm that urinary renin represents an infinitesimal part of the amounts injected, hence that the kidney plays a minor role in removing renin from the circulation (12).

There is as yet no satisfactory explanation for the rise in substrate after bilateral nephrectomy (13). The logical explanation that substrate accumulates because of a decrease in consumption does not seem to be supported by facts. First, maintenance of plasma renin at near normal or slightly elevated levels following renin administration does not prevent the rise in substrate. Secondly, we confirm that ureteral ligation causes the same rise in substrate as nephrectomy, in spite of elevated plasma renin (2, 14), and thirdly, we show that adrenalectomy and pregnancy which are associated with marked increases in plasma renin do not interfere with the rise in substrate after nephrectomy. Uremia can also be ruled out, since ureterocaval anastamosis caused only small increases in angiotensinogen as compared with acute renal tubular damage (15). The hypothesis that substrate synthesis may somewhat be dependent on glomerular filtration and tubular activity remains to be defined (15). As shown here, attempts to test the possibility that the normal kidney contains some inhibitor of substrate synthesis have been unsuccessful.

The effects of adrenals on plasma substrate and renin levels are known (16). Adrenalectomy caused a 30-fold increase in renin and a 2/3 decrease in substrate. This decrease in substrate could be explained by an increase in consumption. However the following observations do not support such an explanation: cortisol acetate, corticosterone, and even 1% saline (oral) normalized substrate but not renin level (3). The same comments may be applied to the inverse relationship found during pregnancy, at least in the rat (16), since after parturition substrate remained low while renin returned to near normal level. It then appears that renin levels do not play a major role in the regulation of substrate

levels. This is in accord with other observations suggesting that substrate may be mainly regulated by factors other than renin, and that whenever an inverse relationship occurs it is more coincidental than the result of changes in substrate consumption (4, 13).

Summary. The role of renin in the regulation of its substrate was evaluated after administration of homologous renin. In normal rats, increases in plasma renin were associated with decreases in substrate. In nephrectomized rats, renin delayed but did not prevent the rise in substrate seen in nephrectomized controls, in spite of the high levels of plasma renin. An inverse relationship between renin and substrate levels was found after adrenalectomy and during pregnancy, but not after delivery. It is concluded that substrate consumption by renin plays a minor role in regulating substrate levels.

The authors thank Mrs. Kathleen Rice for her expert technical assistance.

1. Blaquier, P., Amer. J. Physiol. **208**, 1083 (1965).

2. Bing, J., and Poulsen, K., Acta Pathol. Microbiol. Scand. **77**, 389 (1969).

3. Nasjletti, A., and Masson, G. M. C., Amer. J. Physiol. **217**, 1396 (1969).

4. Carretero, O., and Gross, F., Circ. Res. **21**, 115 (1967).

5. Boucher, R., Menard, J., and Genest, J., Can. J. Physiol. Pharmacol. **45**, 881 (1967).

6. Nasjletti, A., and Masson, G. M. C., Proc. Soc. Exp. Biol. Med. **136**, 344 (1971).

7. Haas, E., Lamfrom, H., and Goldblatt, H., Arch. Biochem. **48**, 256 (1954).

8. Munoz, J. M., Braun-Menendez, E., Fasciolo, J. C., and Leloir, L. F., Amer. J. Med. Sci. **200**, 608 (1940).

9. Goldblatt, H., Lamfrom, H., and Haas, E., Amer. J. Physiol. **175**, 75 (1953).

10. Schaechtelin, G., Regoli, D., and Gross, F., Amer. J. Physiol. **206**, 1361 (1964).

11. Schneider, E. G., Rostorfer, H. H., and Nash, F. D., Amer. J. Physiol. **215**, 1115 (1968).

12. Houssay, B. A., Braun-Menendez, E., and Dexter, L., Ann. Intern. Med. **17**, 461 (1942).

13. Bing, J., and Poulsen, K., Acta Pathol. Microbiol. Scand. **78**, 669 (1970).

14. Stoerk, H. C., Laragh, J. H., Acets, R. M., and Budzilovich, T., Amer. J. Pathol. **58**, 51 (1970).

15. Blaine, E. H., Davis, J. O., and Baumber, J. S., Proc. Soc. Exp. Biol. Med. **136**, 21 (1971).

16. Page, I. H., and McCubbin, J. W., "Renal Hypertension." Year Book Med. Pub. Chicago (1968).

Received Apr. 1, 1971. P.S.E.B.M., 1971, Vol. 137.