Increased Plasma Renin Activity in the Spontaneously Hypertensive Rat (36331)

WYBREN DE JONG,[1] WALTER LOVENBERG,[2] AND ALBERT SJOERDSMA

Experimental Therapeutics Branch, National Heart and Lung Institute, National Institutes of Health, Bethesda, Maryland 20014

The spontaneously hypertensive (SH) rat (1, 2) provides a useful animal model for studying hypertension, although the mechanism responsible for the development of the high blood pressure in these animals is still unknown (1, 3, 4). It has been suggested that the renin-angiotensin system does not contribute to the hypertension in the SH rat, since renin content of the kidneys was found to be decreased in SH rats older than 15 weeks of age (5, 6). However, in these latter studies plasma renin activity was not determined. Since plasma renin activity is a more direct indicator of the renin-angiotensin system, we have studied changes in both renal and plasma renin levels. Data will be presented showing that plasma renin activity increases with age in SH rats but not in control Wistar rats. This increase is independent of changes in renin substrate and kidney renin.

Materials and Methods. Male SH rats (NIH, F_{21-24} generations) and appropriate (2, 3) normotensive male Wistar rats (NIH) of the same age were used in the present studies. Animals were maintained on a regular rat pellet diet containing 0.5% NaCl, and demineralized water. At all ages studied the body weight of the SH rats did not differ from that of the respective normotensive controls. Systolic blood pressure of unanesthetized animals was determined using a tail sphygmographic method (7). In order to obtain basal values of plasma renin activity, 2–3 rats per cage were kept overnight in a quiet rat room. Between 8 and 9 AM the following day, the animals were decapitated quickly and their blood was collected. Kidneys were removed and immediately frozen on dry ice, weighed and kept at —16° until renin content was assayed directly by the pressor response of kidney homogenate (20 mg tissue) in nephrectomized rats (8).

Plasma renin activity was measured by the method of Pickens *et al.* (9) with modifications described in detail previously (8, 10). Two ml of plasma were incubated under conditions known to completely inhibit angiotensinase activity (9), for 4 hr at pH 5.5 and 37°. The angiotensin generated from endogenous substrate was then estimated by blood pressure bioassay (Fig. 1) as reported previously (11). Plasma renin substrate was determined by a substrate exhaustion technique as described by Nasjletti *et al.* (12).

Results and Discussion. Blood pressure of the SH rats rapidly increased after the age of 4–5 weeks (Fig. 2) and reached a plateau at 16 weeks. Plasma renin activity of SH rats was significantly ($p < .01$) higher than in controls at 12 weeks of age and this difference remained up to 35 weeks of age (Fig. 2). Several experiments were done to further characterize the pressor substance in the SH plasma incubation samples. The peptide formed during incubation at 37° is similar to angiotensin I (1-asp,5-ile) as determined by paper chromatography (13) and by its blood pressure response. Incubations done at 0° gave no pressor activity indicating that the pressor substance was formed only upon in-

[1] On leave of absence from the Rudolf Magnus Institute for Pharmacology, Vondellaan 6, Utrecht, The Netherlands, supported by the University of Utrecht.

[2] Reprint requests should be addressed to Dr. Walter Lovenberg, Experimental Therapeutics Branch, National Heart and Lung Institute, National Institutes of Health, Bldg. 10, Room 7-N-262, Bethesda, Maryland 20014.

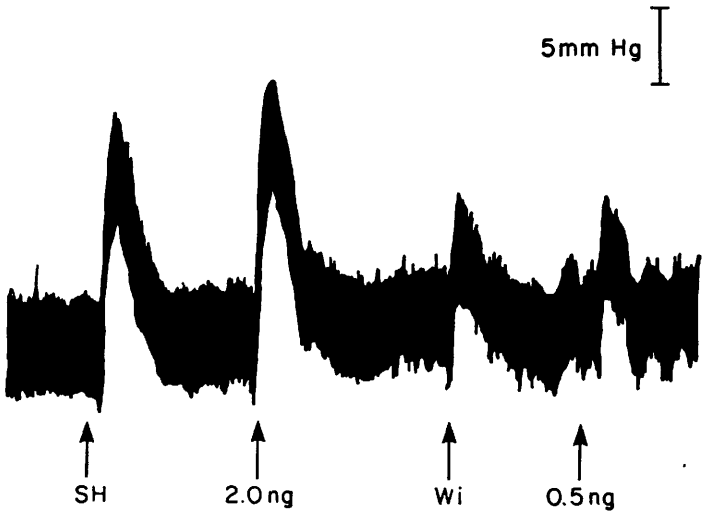

FIG. 1. Blood pressure recording illustrating the bioassay of plasma renin activity of 35-week-old animals. Angiotensin II (2.0 and 0.5 ng) and 0.1 ml of samples for assay from spontaneously hypertensive (SH) and normotensive Wistar (Wi) rats were injected iv at arrows.

cubation and was not present as an endogenous peptide.

The elevated plasma renin activity seems to be independent of a higher renin substrate level. Plasma renin substrate increased in SH rats but not until 16 weeks of age (Table I). A marked decrease in kidney renin content as measured by the pressor response was observed in SH rats at 35 weeks of age (Table I). There appears, therefore, to be no direct relationship between the increase in renin substrate and the decrease in kidney renin content or the increase in plasma renin activity.

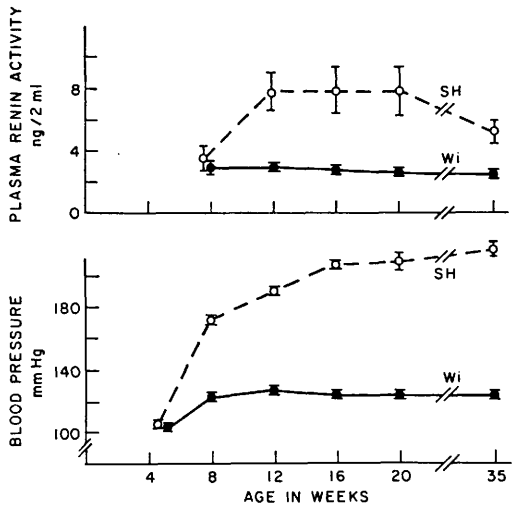

FIG. 2. Systolic blood pressure and plasma renin activity at different ages in spontaneously hypertensive (SH, o- - - -o) and normotensive Wistar (Wi, ●—●) rats. Six or more animals were used for each point (see Table I). Bars depict SEM.

The finding that plasma renin activity in peripheral blood in the SH rats is elevated after the rise in blood pressure had already occurred is similar to findings in experimental renal hypertension in the rat induced by a unilaterally applied renal artery clip (14). During the early stage of this renal hypertension, an elevation in plasma renin activity of renal venous blood obtained from the clipped kidney occurs following pentobarbital anesthesia, although at this time no change in plasma renin activity in peripheral blood is detectable (14). Experiments were undertaken to see if a rise in plasma renin activity could be detected in renal venous blood before that in the peripheral blood in 8- and 12-week-old SH rats under pentobarbital anesthesia. Controls and SH rats were given pentobarbital (35 mg/kg) and heparin (200 units/kg) iv, and renal venous blood was collected 5 min later. A small volume (*ca.* 1 ml) was taken during approximately 1 min in order to minimize the possible effects of

TABLE I. Plasma Renin Substrate, Kidney Weight and Kidney Renin Content of Spontaneously Hypertensive (SH) and Normotensive Wistar (Wi) Rats.

Age (weeks)		No.	Renin substrate (ng/ml)	Kidney weight (g)	Kidney extract pressor response (mm Hg)
8	Wi	15	459 ± 16[a]	1.2 ± 0.05	51 ± 2
	SH	18	472 ± 13	1.4 ± 0.04**	50 ± 2
12	Wi	15	554 ± 23	1.5 ± 0.11	59 ± 2
	SH	14	561 ± 17	1.8 ± 0.04*	49 ± 2*
16	Wi	7	695 ± 20	1.8 ± 0.04	44 ± 3
	SH	9	855 ± 34**	2.1 ± 0.05**	48 ± 2
20	Wi	6	665 ± 18	1.8 ± 0.06	39 ± 3
	SH	6	905 ± 43**	2.2 ± 0.10**	39 ± 3
35	Wi	7	475 ± 24	1.8 ± 0.06	47 ± 2
	SH	7	576 ± 36*	2.4 ± 0.06**	29 ± 3**

[a] Mean ± SEM; no. indicates number of animals.
* $p < 0.05$.
** $p < 0.01$.

blood loss on renin release. Incubations were performed in the usual volume of 2 ml plasma by adding 0.5 ml renal venous plasma to 1.5 ml plasma from male Sprague–Dawley rats which had been nephrectomized 24 hr previously. As shown in Table II, there was no difference in plasma renin activity of renal venous blood at either 8 or 12 weeks of age. The higher level of renin activity in renal venous blood is attributed in part to a renal renin release by pentobarbital.

In another effort to detect changes in peripheral plasma renin activity during the rapid phase of the development of hypertension (8 weeks), this activity was measured at 5 PM. This is the time of peak activity during the normal diurnal rhythm (Leenen, de Jong and de Wied: unpublished observations). No difference in plasma renin activity was seen. Values in SH and control rats were 5.5 ± 0.6 and 5.9 ± 0.6 ng/2 ml/4 hr, respectively. The numbers given are means ± SEM with 8 and 7 animals per group.

TABLE II. Renal Venous Plasma Renin Activity (PRA) of Spontaneously Hypertensive (SH) and Normotensive Wistar (Wi) Rats Anesthetized with Pentobarbital (35 mg/kg).

Age (weeks)		No. of rats	Renal venous PRA (ng/0.5 ml)[a]
8	Wi	10	13.7 ± 0.6[b]
	SH	11	14.2 ± 1.4
12	Wi	9	14.7 ± 1.2
	SH	6	12.7 ± 1.1

[a] 0.5 ml renal venous plasma was incubated with 1.5 ml plasma of nephrectomized rats.
[b] Mean ± SEM.

An important question in studies on genetically hypertensive rats is the use of adequate control rats (2, 3, 15). Using the same method, we previously measured plasma renin activity in another strain of Wistar rats up to the age of 40 weeks (unpublished), and in the current work used normotensive Sprague–Dawley males (12–16 weeks) as additional controls. Plasma renin levels in both of these other normotensive strains were in the same range (1.5–4.5 ng/2 ml/4 hr) as those for Wistar controls in this report. The elevated plasma renin activity thus appears to be unique for the SH rat.

Our data confirm the earlier findings (5, 6) showing that in older SH rats renin content of the kidneys may decrease. The factors governing renal renin concentration are still poorly understood and it is not clear what causes the observed decline of kidney renin. Although high blood pressure by itself is considered to decrease renin content of the kidneys (6), the previous studies (5, 6) as well

as the present data show that this decrease is rather slow, and occurs after several weeks of severe hypertension.

Preliminary studies indicated that the elevated plasma renin activity in SH rats may, at least in part, arise from an extrarenal source, since it is still present in the plasma of these rats (12–16 weeks of age) 18 hr postnephrectomy. Further experiments indicated that as in certain mice strains (16, 17) the submaxillary salivary glands of SH rats contain a considerable amount of a renin-like enzyme. We are inclined to interpret the present data as indicating that the increase in plasma renin activity with age is specific for the SH rat, although no direct relationship between this parameter and hypertension has been established.

Summary. Plasma renin activity increased after the development of hypertension in spontaneously hypertensive (SH) rats, while kidney renin content diminished. However, no difference could be detected in renin activity in renal venous blood of 8–12-week-old SH rats compared to normotensive Wistar rats.

1. Okamoto, K., Int. Rev. Exp. Path. **7**, 227 (1969).

2. Louis, W. J., Tabei, R., Spector, S., and Sjoerdsma, A., Circ. Res. **24** (Suppl. I), I-93 (1969).

3. Louis, W. J., Krauss, K. R., Kopin, I. J., and Sjoerdsma, A., Circ. Res. **27**, 589 (1970).

4. Yamori, Y., Lovenberg, W., and Sjoerdsma, A., Science **170**, 544 (1970).

5. Sokabe, H., Nature (London) **205**, 90 (1965).

6. Sokabe, H., Japan J. Physiol. **16**, 380 (1966).

7. Williams, J. R., Harrison, T. R., and Grollman, A., J. Clin. Invest. **18**, 373 (1939).

8. de Jong, W., Proc. Soc. Exp. Biol. Med. **130**, 85 (1969).

9. Pickens, P. T., Bumpus, F. M., Lloyd, A. M., Smeby, R. R., and Page, I. H., Circ. Res. **17**, 438 (1965).

10. Palkovits, M., de Jong, W., van der Wal, B., and de Wied, D., J. Endocrinol. **47**, 243 (1970).

11. de Jong, W., Eur. J. Pharm. **3**, 93 (1968).

12. Nasjletti, A., Matsunaga, M., and Masson, G. M. C., Endocrinology **85**, 967 (1969).

13. Ziegler, M., Riniker, B., and Gross, F., Biochem. J. **102**, 28 (1967).

14. Leenen, F. H. H., and de Jong, W., J. Endocrinol. **48**, IXXV (1970).

15. Clineschmidt, B. V., Geller, R. G., Govier, W. C., and Sjoerdsma, A., Eur. J. Pharm. **10**, 45 (1970).

16. Werle, E., Trautschold, I., and Schmal, A., Z. Physiol. Chem. **332**, 79 (1963).

17. Bing, J., and Färup, P., Acta Pathol. Microbiol. Scand. **64**, 203 (1965).

Received Sept. 16, 1971. P.S.E.B.M., 1972, Vol. 139.