Decreased Adrenal Responsiveness to Angiotensin II: A Defect Present in Spontaneously Hypertensive Rats

A POSSIBLE MODEL OF HUMAN ESSENTIAL HYPERTENSION

GORDON H. WILLIAMS, LYNNE M. BRALEY, and ALPHONSA MENACHERY,
*Endocrine-Hypertension Unit, Department of Medicine, Harvard Medical
School, Brigham, Women's Hospital, Boston, Massachusetts 02115*

ABSTRACT 30% of patients with essential hypertension have a decreased adrenal response to angiotensin II (A II) on a low but not a high sodium intake. They also have a compensatory increase in the activity of the renin-angiotensin system best documented in a sodium-restricted state.

To assess whether such a mechanism could account for the hypertension in genetically hypertensive rats, adrenal responsiveness to A II was determined in three groups of rats; spontaneously hypertensive rats (SHR), normotensive Wistar rats (WKY), and normotensive Sprague-Dawley rats (SDR). Animals in each group were placed on either a low or high sodium diet for 14 d with balance assessed by sodium excretion. The animals were then decapitated, blood was obtained for plasma renin activity (PRA), A II and aldosterone and adrenals isolated for the preparation of purified glomerulosa cells. The cells were incubated in Krebs-Ringer bicarbonate solution, containing bovine serum albumin, for 60 min in the absence and presence of increasing concentrations of A II.

The PRA, basal aldosterone output, and adrenal sensitivity to A II were similar in the three groups of rats on the high sodium diet. On the low sodium diet the SHR had a significantly ($P < 0.01$) higher PRA (25 ± 7 ng/ml per h) than either the WKY (12 ± 2 ng/ml per h) or the SDR (7 ± 1 ng/ml per h) and lower basal aldosterone output (68 ± 17 vs. 154 ± 43 and 197 ± 21 ng/ 10^6 cells per h, respectively). In addition, the slope of the A II dose response curve was more shallow ($P < 0.01$) in the cells from the SHR than those obtained from the WKY and SDR.

Thus, the SHR PRA and aldosterone responses to sodium restriction and aldosterone response to A II were similar to that previously described in a subgroup

of patients with essential hypertension suggesting that the SHR will serve as a model for exploring the mechanism(s) responsible for the hypertension in these patients.

INTRODUCTION

A number of mechanisms are purported to underlie the elevated blood pressure in patients with essential hypertension. One of these is a decreased adrenal response to angiotensin II (A II)¹ resulting in a compensatory increase in renin release (1). Patients manifesting this mechanism, comprising 25–30% of patients with essential hypertension, have angiotensin-dependent hypertension documented by normalization of blood pressure when a competitive antagonist of A II is administered (2). The reason for the altered adrenal response is unclear although there is some evidence that there is a change in the adrenal A II receptor (3). However, a precise definition of the abnormality is difficult using data derived from clinical studies. Thus, the purpose of this study was to determine whether spontaneously hypertensive rats may also have an altered adrenal response to A II and, thereby, serve as a model for this form of essential hypertension. Adrenal responsiveness was assessed in spontaneously hypertensive rats because two recent studies have suggested that (a) their blood pressure may be significantly reduced when an A II antagonist is given (4); and (b) their renin levels may be higher than normotensive animals when assessed during carefully controlled balance conditions (5).

¹ *Abbreviations used in this paper:* A II, angiotensin II; BSA, bovine serum albumin; KRBCA, Krebs-Ringer bicarbonate solution containing 4% BSA, glucose, L-glutamine and minimal Eagle's solution; SDR, Sprague-Dawley rats; SHR, spontaneously hypertensive Kyoto Wistar rats; WKY, normotensive Kyoto Wistar rats.

Received for publication 19 May 1981 and in revised form 5 August 1981.

METHODS

Experimental design. All animals were female obtained from Charles River Breeding Laboratories, Wilmington, Mass., and studied at 12 or 13 wk of age. Three sets of animals were used; spontaneously hypertensive Kyoto Wistar rats originally developed by Okamoto and Aoki (6) (SHR), normotensive Kyoto Wistar rats (WKY), and normotensive Sprague-Dawley rats (SDR). Rats were maintained for 2 wk on either a low (0.5%) or high (1.6%) sodium Purina Rat Chow (Ralston-Purina Co., St. Louis, Mo.). All SHR were hypertensive at the time of study with the range of systolic blood pressure being 175–200 mm Hg. In contrast, the range of systolic blood pressure for the WKY animals was 110–145 mm Hg. Metabolic balance was assessed the day before death by measuring urinary sodium from rats kept individually in metabolic cages. On each diet, 12 rats in each group were studied. After the rats had been on the diet for 2 wk, they were decapitated and blood obtained for PRA, A II, and aldosterone measurements. In addition, at the time of killing, the adrenals were removed and isolated glomerulosa cells were prepared. The cells were stimulated with A II ranging from 25 pM to 25 nM. Basal as well as A II stimulated aldosterone and corticosterone levels were measured by radioimmunoassay.

Preparation of cell suspension. The preparation of dispersed semi-purified rat adrenal glomerulosa cell suspensions has been described (7). In brief, after decapitation, the adrenals are dissected and bisected, and the capsular (glomerulosa) portion separated from the decapsulated (fasciculata-reticularis) portion. The cells are then preincubated in a modified Krebs-Ringer bicarbonate solution containing 4% bovine serum albumin (BSA), 0.2% glucose L-glutamine, an essential amino acid mixture, and a nonessential minimal Eagle's solution with the potassium concentration adjusted to 3.7 meq/liter (KRBGA). Crude collagenase (3.7 mg/ml) and DNAase (0.5 mg/ml) are added to the KRBGA for the preincubation period. After preincubation at 37°C for 50 min, the cells are centrifuged at 4°C for 10 min, the supernatant discarded, and the pellet washed twice with KRBGA. The washed pellet is then resuspended in 4% BSA-KRBGA and the cells incubated for 1 h at 37°C under an atmosphere of 95% O₂–5% CO₂ in a final medium volume of 2 ml. A II (1-aspartic acid 5-isoleucine angiotensin II; Sigma Chemical Co., St. Louis, Mo.) was dissolved in KRBGA and adjusted to pH 4 with HCl and added to the incubates in increasing concentrations.

Laboratory procedures. Renin, A II, corticosterone, and aldosterone levels in the plasma and aldosterone and corticosterone determinations in the incubation medium were performed as described by radioimmunoassay techniques (7–9). Sodium and potassium concentrations in the urine and serum were determined by flame photometry. The data were evaluated statistically by Fisher Exact, Sign or Unpaired *t* tests except for the dose response curves for A II where *t* values for the response of each dose compared with the control were computed on a log transformation of the data when appropriate. Homogeneity of variance was assessed by Bartlett's test (10) and *P* values were obtained from Dunnett's tables (11). The data are presented as the mean±SEM.

RESULTS

In vivo studies. There were no significant differences in the PRA in the three groups on the high sodium intake; although the Sprague-Dawley rats had

the lowest renin levels (Fig. 1). Contrariwise, on the low sodium intake there were significant differences in renin activity in each of the groups of rats with the SHR having significantly ($P < 0.01$) greater renin levels (25 ± 7 ng/ml per h) than either of the other two groups of animals. However, there was also a significant difference ($P < 0.01$) between the renin in the WKY animals (12 ± 2 ng/ml per h) and the SDR (7 ± 1 ng/ml per h). These differences in renin activity could not be accounted for by differences in sodium or potassium balance as they were similar in the three groups of animals on the day of study (Table I).

On the high sodium intake plasma aldosterone levels in the three groups of animals were similar to the renin levels, i.e., there were no significant differences. In contrast, significant differences were observed on the low sodium intake but the pattern of response was the reciprocal of that noted for renin activity. The SHR animals had significantly ($P < 0.01$) lower plasma aldosterone levels than either of the other two groups (Fig. 2). Plasma corticosterone levels were also measured (Table II). Contrary to the plasma aldosterone levels, there were no significant differences in basal corticosterone levels in the three rat strains on either diet, indicating that ACTH was not responsible for the differences observed in the SHR.

FIGURE 1 Plasma renin activity in SHR and two groups of normotensive animals; WKY, and SD. 12 animals in each group were studied on a high and low sodium intake (mean±SEM).

TABLE I
Sodium and Potassium Balance Data on the Day of (for Serum Levels) and Day before Sacrifice

	Serum		Urine	
	Sodium	Potassium	Sodium	Potassium
	meq/liter		meq/24 h	
	High sodium intake			
Sprague-Dawley	139±2	4.7±0.3	4.1±1.3	2.2±0.5
Wistar-Kyoto	139±2	4.6±0.2	4.6±0.4	2.1±0.2
Spontaneously hypertensive	137±3	5.1±0.3	5.0±0.4	2.2±0.2
	Low sodium intake			
Sprague-Dawley	140±1	4.3±0.4	0.03±0.01	2.1±0.4
Wistar-Kyoto	140±1	4.6±0.3	0.04±0.01	1.4±0.1
Spontaneously hypertensive	140±1	5.1±0.5	0.03±0.01	1.2±0.2

No significant differences existed between the groups (mean±SEM).

A II levels were determined in the SHR and the SD animals. The antibody used to measure A II levels cross-reacts 100% with angiotensin III (A III). Thus, the immunoassayable angiotensin measurements reflect the total angiotensin adrenal stimulating activity in the circulation, i.e., both A II and A III concentration. On the high sodium intake, there were no significant differences in the angiotensin levels in the two groups of animals, similar to what was observed for renin activity. In contrast, on the low sodium diet, the SHR group had significantly ($P < 0.05$) higher angiotensin levels (Fig. 3). The difference in plasma angiotensin concentration could not be accounted for by cross reactivity of angiotensin I with the A II antibody, as at even the highest circulating angiotensin I level measured (5 ng/ml) this would measure as only 5 pg in the A II assay.

In vitro studies. The aldosterone production by the isolated glomerulosa cells from the three groups of animals on the two diets had a pattern similar to that observed for plasma aldosterone levels (Fig. 4). On the high sodium diet, there were no significant differences between the aldosterone production rates in any of the three groups of animals. On the low sodium intake, again, the SHR group had significantly ($P < 0.01$) lower aldosterone production (68 ± 17 ng/ 10^6 cells per h) than either the WKY (154 ± 43 ng/ 10^6 cells per h) or the SDR (197 ± 21 ng/ 10^6 cells per h). The aldosterone production between the SDR and WKY animals was also significantly different ($P < 0.01$).

Although there was a trend for the SHR groups to have a lower aldosterone response to A II in comparison with the other two groups on the high sodium intake, this was not statistically significant (Fig. 5). In

contrast, on the low sodium intake, dose-response curves generated from each of the three groups were significantly different both in slope and peak response

FIGURE 2 Plasma aldosterone levels in SHR and two groups of normotensive animals; WKY, and SD. 12 animals in each group were studied on a high and low sodium intake (mean±SEM).

TABLE II
Basal Plasma Corticosterone Levels (micrograms/100 milliliters)
in the Three Rat Strains on Low and High Sodium Intakes

	SD	WKY	SHR
High sodium diet	96.1 ± 20.0	102.6 ± 13.8	82.0 ± 6.2
Low sodium diet	95.0 ± 10.4	102.7 ± 16.4	90.8 ± 8.9

There were no significant differences observed in any strain on either diet (mean ± SEM).

($P < 0.01$). The slope of the dose-response curve was nearly five times steeper in the SDR than in the SHR ($P < 0.01$) with the WKY animals occupying an intermediate position, the slope being approximately two-fold greater than the SHR and 2.5-fold less than the SDR. These differences were significant at a P value of < 0.01 , as was the maximum response between the three groups of animals (Fig. 6). In addition, A II stimulated corticosterone levels reflected a similar pattern. At the highest A II dose added, corticosterone levels were significantly ($P < 0.01$) greater in the SD (646 ± 101 ng/ 10^6 cells) than in the WKY (282 ± 64 ng/ 10^6 cells), or the SHR (151 ± 43 ng/ 10^6 cells) on a low sodium diet.

As an important indicator of the activity of the late pathway of aldosterone biosynthesis (the conversion of corticosterone to aldosterone) the ratio of aldosterone to corticosterone output was assessed in the control incubates. On the high salt diet there were no consistent differences although the SHR group tended to have a lower ratio. Contrariwise, on the low sodium

intake the SHR ratio (0.10 ± 0.02), was significantly less ($P < 0.02$) than in either the WKY (0.17 ± 0.02) or SDR (0.24 ± 0.02).

DISCUSSION

Recent reports have indicated that altered aldosterone responsiveness may explain the altered renin responsiveness found in human hypertension. Thus, low renin essential hypertension may be secondary to an enhanced adrenal response to A II (12-13), and high renin essential hypertension may result from a decreased adrenal response to A II (1-3). Additional studies have suggested that this latter defect may not be limited to those patients with high renin essential hypertension, but also can be detected in patients with normal renin essential hypertension and may contribute to the elevated blood pressure in as many as 30% of patients with essential hypertension (1).

The patients with decreased adrenal responsiveness have four characteristics that distinguished them from normotensive subjects and patients with hypertension who do not have this abnormality: (a) their renin response to an acute challenge, e.g., upright posture on a low sodium intake, is enhanced; (b) their basal aldosterone levels in a sodium restricted state tend to be lower with subnormal aldosterone increments with upright posture; (c) their adrenal response to exogenously administered A II is decreased; (d) renin and aldosterone levels and responsiveness to A II in a normal or high sodium state usually are normal. The re-

FIGURE 3 Plasma angiotensin II levels in SHR and SD. 12 animals in each group were studied on a high and low sodium intake (mean ± SEM).

FIGURE 4 Basal aldosterone output from isolated glomerulosa cells obtained either from SHR, WKY, or SD animals. 12 experiments were conducted simultaneously in each group with animals maintained on a high or low sodium intake before death (mean ± SEM).

FIGURE 5 Response of isolated glomerulosa cells to angiotensin II in three groups of animals maintained on a high sodium intake before death (mean $\pm$ SEM of 12 experiments).

sults of the present study would suggest that similar alterations may be present in the SHR.

Controversy has existed as to the status of the renin-angiotensin system in SHR. Early results suggested that the level of renin activity was low with the degree of renin suppression age dependent, i.e., the older the animals, the lower the renin (14–16). A recent study using the WKY as a control has not confirmed these findings (5, 17, 18). Invariably, the renin levels have been greater in the SHR animals, the only disagreement being whether or not with aging they fall and the significance of the elevated renins. Those investigators who have noted an increased level before an increase in blood pressure have suggested that renin may be participating in the development of the hypertension (18). Others have suggested that the high renin levels observed after the hypertension has been established may simply be a manifestation of hypertension-induced nephrosclerosis (5).

Whether the elevated renin levels are participating in the maintenance of the elevated blood pressure has been difficult to establish. Captopril, a converting enzyme inhibitor, administered from birth reduced the rate of increase of blood pressure in SHR with reversal of the antihypertensive effect within 10 d of discontinuing treatment, suggesting that A II may be involved (19). However, it is unclear that the hypotensive response to converting enzyme inhibitors is solely due to a change in A II formation (20). Competitive

antagonists of A II have also been used to evaluate angiotensin's role in the maintenance of blood pressure in these animals. Pals et al. (21) reported that A II blockade had no effect on blood pressure in conscious rats leading them to conclude that the renin-angiotensin system is not involved in genetic hypertension. However, Munoz-Ramirez have reported a hypotensive response to a different A II antagonist given to sodium-depleted rats (4). Additional studies have suggested that the antihypertensive effect of A II blockade may be mediated by modifying the action of brain A II (22). Unfortunately, these studies are often difficult to interpret because either improper or no control animals were studied and/or dietary intake was not controlled and/or assessed.

The results of the present study, in agreement with several previous ones, would suggest that on a high sodium intake there is no significant difference in the renin levels in the SHR compared to WKY or SD animals. However, when the renin-angiotensin system is activated by sodium restriction, the increase in PRA and A II levels in the SHR is significantly greater ($P < 0.01$) than in either the WKY or SD animals. Of interest, the SD had the lowest renin increment with sodium restriction. The present paper does not provide data to resolve the conflict concerning changes in renin levels or responsiveness with age as all animals were studied at 12 to 13 wk of age, at the beginning of the established phase of hypertension.

Relatively few studies have examined adrenal steroidogenesis in the SHR (23–27). While most have re-

FIGURE 6 Response of isolated glomerulosa cells to angiotensin II in three groups of animals maintained on a low sodium intake before death (mean $\pm$ SEM of 12 experiments).

ported no significant changes in basal levels of aldosterone, some have suggested that adrenal steroidogenesis may be decreased (24). The results of the present study document that in the high sodium state aldosterone output from isolated glomerulosa cells is similar in the three groups of animals. On the other hand, in the sodium-restricted state, aldosterone output is significantly less in the SHR than in the normotensive groups. Adrenal responsiveness to A II has not been reported previously in these animals. However, if the animals were studied on a normal or high sodium intake it is unlikely that any significant differences would have been observed, as none were noted in the present study. Contrariwise, with sodium restriction, the adrenal responsiveness to A II in the SHR animals was significantly less than in the other two groups. This was true both for the slope of the dose response curve as well as the maximum output which was two- to fivefold greater in the normotensive than in the hypertensive animals.

There was also a difference in the adrenal responsiveness of the two groups of normotensive animals. The SDR had a greater adrenal responsiveness to A II than did the WKY animals. This was not secondary to a spurious increased responsiveness of the SDR used in this study as their responses were similar to that previously reported (7). This is of particular interest since the spontaneous hypertensive and WKY animals are both of the same breed. Thus, the potential exists that the development of hypertension in the animal strain was possible because of the already present genetic potential for a reduced adrenal responsiveness to A II. Additional studies with back breeding will be necessary in order to evaluate this potential.

In conclusion, a subgroup of patients with essential hypertension and SHR share a common triad of alterations in adrenal steroidogenesis. In response to sodium restriction, there is an increased renin and A II responsiveness, and a decreased adrenal responsiveness. There is also a reduced adrenal *in vitro* response to exogenously administered A II in the sodium restricted state. The mechanism underlying the reduced responsiveness is uncertain. One possible explanation could be a reduced activity of the 18-hydroxylase step since *in vitro* the ratio of the aldosterone to corticosterone levels was decreased in the SHR. Whether the reduced adrenal responsiveness to A II is producing the elevated blood pressure remains to be determined. However, the striking similarity in the responses of the SHR and 30% of patients with essential hypertension raises the likely possibility that these animals will serve as an excellent model for unravelling the pathophysiology of this subgroup of patients.

ACKNOWLEDGMENTS

We gratefully acknowledge the excellent assistance provided by Gary Bradwin, Tham Yao, and Diane Rioux in the preparation of this manuscript.

The study was supported in part by grant 5-R01-HL-16821 from the National Institutes of Health, Heart, Lung and Blood Institute.

REFERENCES

1. Moore, T. J., G. H. Williams, R. G. Dluhy, S. Z. Bavli, T. Himathongkam, and M. Greenfield. 1977. Altered renin-angiotensin-aldosterone relationships in normal-renin essential hypertension. *Circ. Res.* 41: 167-171.
2. Dluhy, R. G., S. Z. Bavli, F. K. Leung, H. S. Solomon, T. J. Moore, N. K. Hollenberg, and G. H. Williams. 1979. Abnormal adrenal responsiveness and angiotensin II dependency in high-renin essential hypertension. *J. Clin. Invest.* 64: 1270-1276.
3. Williams, G. H., N. K. Hollenberg, T. J. Moore, S. L. Swartz, and R. G. Dluhy. 1979. The adrenal receptor for angiotensin II is altered in essential hypertension. *J. Clin. Invest.* 63: 419-427.
4. Munoz-Ramirez, H., M. C. Khosla, F. M. Bumpus, and P. A. Khairallah. 1978. Hypotensive effect of [sar¹, thr⁸] angiotensin II in spontaneously hypertensive sodium-deplete rats. *Am. J. Physiol.* 234: H-447-H-453.
5. Bagby, S. P., W. J. McDonald, and R. D. Mass. 1979. Serial renin-angiotensin studies in spontaneously hypertensive and Wistar-Kyoto normotensive rats. *Hypertension* 1: 347-354.
6. Okamoto, K., and K. Aoki. 1963. Development of a strain of spontaneously hypertensive rats. *Jpn. Circ. J.* 27: 282-293.
7. Williams, G. H., N. K. Hollenberg, and L. M. Braley. 1976. Influence of sodium intake on vascular and adrenal angiotensin II receptors. *Endocrinology* 98: 1343-1350.
8. Emanuel, R. L., J. P. Cain, and G. H. Williams. 1973. Double antibody radioimmunoassay of renin activity and angiotensin II in human peripheral plasma. *J. Lab. Clin. Med.* 81: 632-640.
9. Williams, G. H., L. M. McDonnell, S. A. S. Tait, and J. F. Tait. 1972. The effect of medium composition and *in vitro* stimuli on the conversion of corticosterone to aldosterone in rat glomerulosa tissue. *Endocrinology* 91: 948-960.
10. Snedecor, G. W. and E. C. Cochran. 1967. Statistical Methods, 6th Edition, Ames Iowa State University Press. 296-298.
11. Dunnett, C. W. 1964. New tables for multiple comparisons with a control. *Biometrics* 20: 482-491.
12. Kisch, E. S., R. G. Dluhy, and G. H. Williams. 1976. Enhanced aldosterone response to angiotensin II in human hypertension. *Circ. Res.* 38: 502-505.
13. Wisgerhof, M. and R. D. Brown. 1978. Increased adrenal sensitivity to angiotensin II in low renin essential hypertension. *J. Clin. Invest.* 61: 1456-1464.
14. Sokabe, H. 1966. Renin activity of the kidney in the spontaneously hypertensive rat. *Jpn. J. Physiol.* 16: 380-388.
15. Koletsky, S., P. Shook, and J. Rivera-Velez. 1970. Lack of increased renin-angiotensin activity in rats with spon-

- taneous hypertension. *Proc. Soc. Exp. Biol. Med.* 134: 1187-1190.
16. Shiono, K., and H. Sokabe. 1976. Renin-angiotensin system in spontaneously hypertensive rats. *Am. J. Physiol.* 231: 1295-1299.
  17. DeJong, W., W. Lovenberg, and A. Sjoerdsma. 1972. Increased plasma renin activity in the spontaneously hypertensive rat. *Proc. Exp. Biol. Med.* 139: 1213-1216.
  18. Sen, S., R. R. Smeby, and F. M. Bumpus. 1972. Renin in rats with spontaneous hypertension. *Circ. Res.* 31: 876-880.
  19. Muirhead E. E., R. L. Prewitt, Jr., B. Brooks, and W. L. Brosius, Jr. 1978. Antihypertensive action of the orally active converting enzyme inhibitor (SQ 14,225) in spontaneously hypertensive rats. *Circ. Res.* 43(Suppl. I): I-53-I-59.
  20. Swartz, S. L., G. H. Williams, N. K. Hollenberg, T. J. Moore, and R. G. Dluhy. 1979. Converting enzyme inhibition in essential hypertension: the hypotensive response does not reflect only reduced angiotensin II formation. *Hypertension*. 1: 106-111.
  21. Pals, D. T., F. D. Mascussi, G. S. Denning, F. Sipos, and D. C. Fessler. 1971. Role of the pressor action of A II in experimental hypertension. *Circ. Res.* 29: 673-681.
  22. Mann, J. F. E., M. I. Phillips, R. Dietz, H. Haebara, and D. Ganten. 1978. Effects of central and peripheral angiotensin blockade in hypertensive rats. *Am. J. Physiol.* 234: H-629-H-637.
  23. Moll, D., S. L. Dale and J. C. Melby. 1975. Adrenal steroidogenesis in the spontaneously hypertensive rat (SHR). *Endocrinology*. 96: 416-420.
  24. Freeman, R. H., J. O. Davis, N. Varsano-Aharon, S. Ulick, and M. H. Weinberger. 1975. Control of aldosterone secretion in the spontaneously hypertensive rat. *Circ. Res.* 37: 66-71.
  25. Karppanen, H., S. Lahovaara, P. Mannisto, and H. Vapaatalo. 1975. Plasma renin activity and *in vitro* synthesis of aldosterone by adrenal glands of rats with spontaneous, renal, or pinealectomy-induced hypertension. *Acta Physiol. Scand.* 94: 184-188.
  26. Iams, S. G., J. P. McMurtry, and B. C. Wexler. 1979. Aldosterone, desoxycorticosterone, corticosterone, and prolactin changes during the life time of chronically spontaneously hypertensive rats. *Endocrinology*. 104: 1357-1363.
  27. Sowers, J. R., E. G. Sollars, M. L. Tuck, and N. D. Asp. 1980. Dopaminergic modulation of renin activity and aldosterone and prolactin secretion in the spontaneously hypertensive rat. *Proc. Exp. Biol. Med.* 164: 598-603.