

# Control of Aldosterone Secretion in the Spontaneously Hypertensive Rat

By Ronald H. Freeman, James O. Davis, Nora Varsano-Aharon, Stanley Ulick, and Myron H. Weinberger

## ABSTRACT

Adrenal secretion rates of aldosterone, corticosterone, and deoxycorticosterone were studied sequentially in the spontaneously hypertensive rat and the normotensive Kyoto Wistar rat. Steroid secretion was studied at three different ages: 7-8, 11-13, and 22-25 weeks. Also, peripheral plasma levels of aldosterone and plasma renin activity were determined in both the spontaneously hypertensive and the normotensive rats at 7-8 weeks of age. Aldosterone secretion was elevated markedly in dexamethasone-morphine-treated spontaneously hypertensive rats at both 7-8 and 11-13 weeks of age but was not significantly different from control in 22-25-week-old spontaneously hypertensive rats. No statistically significant differences in corticosterone or deoxycorticosterone secretion rates were observed between the spontaneously hypertensive rats and the normotensive Kyoto Wistar controls; however, the data suggested that dexamethasone did not suppress adrenocorticotrophic hormone in the 7-8- and 11-13-week-old spontaneously hypertensive rats to the same extent that it did in the normotensive Kyoto Wistar rats. Therefore, aldosterone secretion was reexamined in acutely hypophysectomized 7-8-week-old rats to eliminate completely the influence of the anterior pituitary; no differences in aldosterone, corticosterone, or deoxycorticosterone secretion rates were observed between hypophysectomized spontaneously hypertensive rats and normotensive Kyoto Wistar rats. Moreover, aldosterone secretion in the hypophysectomized 7-8-week-old spontaneously hypertensive rats was reduced markedly compared with that in the intact 7-8-week-old spontaneously hypertensive rats, thus confirming the importance of the pituitary in these animals. Determinations of peripheral plasma aldosterone concentration and plasma renin activity in unstressed 7-8-week-old spontaneously hypertensive and normotensive rats revealed that both parameters were depressed significantly in the spontaneously hypertensive rats. Thus, the present data indicate that the renin-angiotensin-aldosterone system is suppressed in the spontaneously hypertensive rat but do not suggest that the system is critically involved in the hypertensive process in these animals.

■ The importance of genetic determinants in the pathogenesis of hypertension has been considered for many years, and congenital hypertension has been produced in several strains of rats by selective breeding (1, 2). Although the spontaneously hypertensive rat developed by Okamoto and Aoki (1) has been studied extensively, the precise role of the renin-angiotensin-aldosterone system in the hypertensive process is unknown. Sen et al. (3) have reported that plasma renin activity is elevated in young spontaneously hypertensive rats but is suppressed below normal in older animals (14-16 weeks). de Jong et al. (4) have reported that plasma renin activity is normal in young spontaneously hypertensive rats (8 weeks) but is elevated with increasing age (12 weeks and older). Similarly,

Moll et al. (5) have reported that aldosterone secretion is suppressed in young spontaneously hypertensive rats (8 weeks), but they found no change in aldosterone secretion with increasing age (12 weeks and older). In contrast, Rapp and Dahl (6) have reported that aldosterone secretion is elevated in older spontaneously hypertensive rats (5-7 months).

The present study was undertaken to evaluate aldosterone production during the early pathogenic stages in the spontaneously hypertensive rat. Adrenal secretion of aldosterone, corticosterone, and deoxycorticosterone was studied sequentially in both the spontaneously hypertensive rat and the normotensive Kyoto Wistar rat strain from which the spontaneously hypertensive rats were derived originally by Okamoto and Aoki (1). Rats were studied at three different ages: 7-8, 11-13, and 22-25 weeks. In addition to these steroid secretion studies, peripheral plasma levels of aldosterone and plasma renin activity were determined in both normotensive and spontaneously hypertensive rats at 7-8 weeks of age.

From the University of Missouri School of Medicine, Columbia, Missouri 65201, the Indiana University School of Medicine, Indianapolis, Indiana 46202, and the Veterans Administration Hospital, Bronx, New York 10468.

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## Methods

All rats used in this study were bred at the University of Missouri School of Medicine unless otherwise indicated.<sup>1</sup> Breeders for the spontaneously hypertensive rats were from the F33 generation and those for the normotensive Kyoto Wistar rats were from the F6 generation.<sup>2</sup> All rats were bred and housed in a common environment and fed a normal laboratory rat chow; both food and drinking water were available ad libitum. Only male rats were used for measurements of steroids and renin, because the adrenal gland is larger in the female than it is in the male rat and is subject to the influence of estrogens (7).

For the collection of adrenal venous blood, male rats were anesthetized with sodium pentobarbital (50 mg/kg, ip). A tracheotomy was then performed, and the rat was intubated with polyethylene tubing to facilitate respiration. The carotid artery was cannulated (PE 50), and arterial blood pressure was measured via a Statham strain gauge (P23Db) and a Hewlett-Packard recording system (model 7702B). A catheter (PE 90) was inserted into the left femoral vein and advanced through the abdominal vena cava to the level of the left renal vein. A laparotomy was then performed to permit visualization and manipulation of the catheter tip into the left adrenal vein. The tip of the catheter was advanced as far as possible into the adrenal vein. Care was taken to minimize disturbance of the renal vein and the kidney during the procedure. After placement of the catheter tip, the rat was given 200 units of aqueous sodium heparin (0.2 ml of 1000 units/ml, iv), and adrenal venous blood collection was begun. During the collection period, arterial blood pressure was maintained by infusion of whole blood through a catheter (PE 10) placed in the external jugular vein. Fresh donor blood was obtained from female rats of the same age and strain as the recipient males. All rats were given dexamethasone phosphate (1.0 mg/kg, im, Decadron, Merck, Sharp & Dohme) 2–4 hours prior to experimentation and morphine sulfate (1.25 mg/100 g body weight, im) 10 minutes following pentobarbital anesthesia unless otherwise stated.

Following the timed collection of approximately 2–3 ml of adrenal vein blood, the exact volume was recorded, the sample was centrifuged at 4°C, and the plasma was stored at –20°C until the time of analysis. The adrenal venous plasma concentrations of aldosterone, corticosterone, and deoxycorticosterone were determined by the double isotope derivative method of Kliman and Peterson (8). Adrenal steroid secretion rates were calculated as the product of adrenal plasma flow and the concentration of the steroid in the adrenal venous plasma. Blood samples for determination of plasma electrolytes and hematocrit were taken from the adrenal vein catheter. Plasma sodium and plasma potassium were determined by flame photometry.

Adrenal steroid secretion was determined in four different experimental groups of spontaneously hypertensive rats and normotensive Kyoto Wistar rats. Group 1 consisted of rats 7–8 weeks of age. Group 2 rats were

11–13 weeks old. Group 3 rats were 22–25 weeks old. In group 4, rats 7–8 weeks old were hypophysectomized 2–4 hours prior to beginning the experiment; the hypophysectomized rats were not given dexamethasone and morphine. Cortisone acetate (4.0 mg, im) was given to group 4 rats 1 hour prior to the time of hypophysectomy to maintain arterial blood pressure. Hypophysectomy was performed via the external ear canal with a stereotaxic device (Hoffman-Reiter hypophysectomy apparatus) under light ether anesthesia. Following hypophysectomy, the rats were allowed to recover for 2–4 hours prior to the administration of sodium pentobarbital and subsequent surgery for the collection of adrenal venous blood. Completeness of the hypophysectomy was determined by the rate of corticosterone secretion.

In addition to the adrenal steroid secretion experiments, peripheral levels of aldosterone and plasma renin activity were measured in a fifth group of spontaneously hypertensive rats which were 7–8 weeks old. Untreated conscious rats were decapitated with a small animal guillotine, and whole blood (approximately 3–5 ml) was collected in 0.05 ml of 10% disodium ethylenediaminetetraacetate. The blood was centrifuged at 4°C, and the plasma was stored at –20°C until the time of analysis. The determination of plasma concentrations of aldosterone required the sensitivity of radioimmunoassay. Two methods were used: one was based on immunologic purification (9) and the other on chemical purification (10). The tests were performed in two different laboratories by different groups, because the first results with the double antibody method showed an unexpected lower value for the hypertensive rats. Plasma renin activity was determined by radioimmunoassay with a commercially available kit (Squibb). Plasma samples were prepared for radioimmunoassay by adjusting the pH to 5.3 via dialysis against a phosphate buffer for 18 hours (four changes) and adding diisopropylfluorophosphate to inhibit angiotensinase activity. Plasma samples were incubated for 1 hour at 37°C and then placed in ice water to stop the reaction. Preliminary studies indicated that the generation of angiotensin I was linear under these conditions.

Student's *t*-test for group comparison was used for statistical analysis of the data. Values are presented as means  $\pm$  SE.

## Results

*Experiment 1: Adrenal Steroid Secretion and Arterial Blood Pressure in Spontaneously Hypertensive Rats (N = 8) and Normotensive Kyoto Wistar Rats (N = 9) 7–8 Weeks of Age.*—The results obtained in group 1 rats are presented in Table 1. Both aldosterone secretion and mean arterial blood pressure were markedly elevated ( $P < 0.01$  and 0.005, respectively) in the hypertensive rats compared with the normotensive Kyoto Wistar controls. No significant differences were observed between the two groups in the secretion of either corticosterone or deoxycorticosterone, adrenal plasma flow, or the plasma concentrations of sodium and potassium.

<sup>1</sup>Breeding was supervised by R. A. Patterson, D. V. M.

<sup>2</sup>Breeder rats were generously donated by Dr. C. T. Hansen, National Institutes of Health, Bethesda, Maryland.

TABLE 1

*Adrenal Steroid Secretion and Arterial Blood Pressure in Spontaneously Hypertensive Rats (SHR) and Normotensive Kyoto Wistar Rats (KW)*

	Aldosterone secretion (ng/min)	Corticosterone secretion (ng/min)	Deoxycorticosterone secretion (ng/min)	Mean arterial blood pressure (mm Hg)	Adrenal plasma flow (ml/min)	Plasma [Na] (mEq/liter)	Plasma [K] (mEq/liter)
Group 1							
SHR (8)	0.84 ± 0.14*	412 ± 63	5.7 ± 1.3	160 ± 2†	0.042 ± 0.005	140 ± 1	4.1 ± 0.2
KW (9)	0.42 ± 0.13	278 ± 77	5.1 ± 1.7	119 ± 2	0.041 ± 0.005	143 ± 2	4.0 ± 0.2
Group 2							
SHR (7)	0.65 ± 0.09‡	302 ± 64	6.6 ± 2.0	174 ± 4§	0.038 ± 0.003	140 ± 1	4.1 ± 0.2
KW (7)	0.35 ± 0.06	195 ± 77	5.3 ± 2.5	112 ± 1	0.043 ± 0.004	141 ± 1	3.9 ± 0.1
Group 3							
SHR (8)	1.04 ± 0.17	501 ± 54	13.7 ± 1.3	186 ± 3§	0.045 ± 0.001*	145 ± 2	4.7 ± 0.4
KW (8)	0.85 ± 0.16	477 ± 79	17.8 ± 4.3	138 ± 4	0.055 ± 0.002	144 ± 1	3.7 ± 0.2
Group 4							
SHR (6)	0.34 ± 0.10	28 ± 15	1.8 ± 0.6	143 ± 3†	0.061 ± 0.001	146 ± 2	3.7 ± 0.1
KW (7)	0.45 ± 0.12	7 ± 2	1.3 ± 0.2	95 ± 4	0.074 ± 0.009	149 ± 3	3.9 ± 0.2

Rats in groups 1 (7–8 weeks), 2 (11–13 weeks), and 3 (22–25 weeks) received dexamethasone and morphine. Group 4 rats (7–8 weeks) were hypophysectomized 2–4 hours prior to the experiment. The number of rats tested is given in parentheses. All values are means ± se. All *P* values are for comparisons between spontaneously hypertensive and normotensive rats in the same group.

\* *P* < 0.01.

† *P* < 0.005.

‡ *P* < 0.025.

§ *P* < 0.001.

|| *P* < 0.05.

**Experiment 2: Adrenal Steroid Secretion and Arterial Blood Pressure in Spontaneously Hypertensive Rats (*N* = 7) and Normotensive Kyoto Wistar Rats (*N* = 7) 11–13 Weeks of Age.**—The results obtained in group 2 rats are presented in Table 1. Again, both aldosterone secretion and mean arterial blood pressure were markedly elevated (*P* < 0.025 and 0.001, respectively) in the hypertensive group compared with the normotensive Kyoto Wistar rats. No significant differences were observed between the two groups in any of the other parameters measured.

**Experiment 3: Adrenal Steroid Secretion and Arterial Blood Pressure in Spontaneously Hypertensive Rats (*N* = 8) and Normotensive Kyoto Wistar Rats (*N* = 8) 22–25 Weeks of Age.**—The results obtained in group 3 rats are presented in Table 1. Although mean arterial blood pressure was still elevated markedly (*P* < 0.001) in the spontaneously hypertensive rats, no significant differences were observed between the spontaneously hypertensive rats and the normotensive Kyoto Wistar rats in the secretion rates of aldosterone, corticosterone, and deoxycorticosterone. Plasma potassium concentration was elevated in the hypertensive rats, whereas adrenal plasma flow was higher in the Kyoto Wistar controls. It should be noted that arterial blood pressure and steroid

secretion rates increased with age in both the spontaneously hypertensive rats and the Kyoto Wistar rats; arterial blood pressure increased by approximately 20–25 mm Hg between the ages of 7–8 weeks and 22–25 weeks in both groups of rats.

**Experiment 4: Adrenal Steroid Secretion and Arterial Blood Pressure in Acutely Hypophysectomized Spontaneously Hypertensive Rats (*N* = 6) and Normotensive Kyoto Wistar Rats (*N* = 7) 7–8 Weeks of Age.**—The effects of acute hypophysectomy (2–4 hours) on adrenal steroid secretion and arterial blood pressure in rats 7–8 weeks of age are presented in Table 1. Mean arterial blood pressure was still elevated markedly (*P* < 0.005) in the hypophysectomized spontaneously hypertensive rats compared with the hypophysectomized normotensive Kyoto Wistar rats; however, no significant differences were observed between the two groups in the secretion rates of aldosterone, corticosterone, and deoxycorticosterone. Following acute hypophysectomy, secretion rates of aldosterone (0.34 ± 0.10 ng/min), corticosterone (28 ± 15 ng/min), and deoxycorticosterone (1.8 ± 0.6 ng/min) in spontaneously hypertensive rats 7–8 weeks of age were significantly decreased (*P* < 0.025 for all three steroids) compared with secretion rates of aldosterone (0.84 ± 0.14 ng/min), corticosterone (412 ± 63 ng/min), and deoxycorticosterone (5.7 ± 1.3 ng/

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min) in 7–8-week-old spontaneously hypertensive rats with intact pituitaries. Acute hypophysectomy failed to decrease aldosterone secretion in the 7–8-week-old normotensive Kyoto Wistar rats but did decrease significantly ( $P < 0.025$  for both steroids) the secretion rates of corticosterone and deoxycorticosterone.

**Experiment 5: Peripheral Plasma Levels of Aldosterone and Plasma Renin Activity in Unstressed Decapitated Spontaneously Hypertensive Rats and Normotensive Kyoto Wistar Rats 7–8 Weeks of Age.**—The data are presented in Table 2. Peripheral levels of plasma aldosterone and plasma renin activity were significantly less ( $P < 0.025$ ) in 7–8-week-old spontaneously hypertensive rats than they were in 7–8-week-old normotensive Kyoto Wistar rats. These findings probably represent true basal levels of circulating aldosterone and plasma renin activity, since the rats were unstressed prior to death and the collection of blood.

### Discussion

The present study was undertaken to evaluate aldosterone production in the spontaneously hypertensive rat and to identify those factors which are important in its regulation. Because of a strong, overriding influence of adrenocorticotrophic hormone (ACTH) on aldosterone production during stressful maneuvers such as laparotomy (11), the strategy employed in this study was to minimize the influence of acute stress due to surgical procedures and anesthesia. It was hoped that such an approach might provide new information for the assessment of the role of aldosterone in the hypertensive process in the spontaneously hypertensive rat.

TABLE 2

*Peripheral Plasma Levels of Aldosterone and Plasma Renin Activity in Decapitated Spontaneously Hypertensive Rats (SHR) and Normotensive Kyoto Wistar Rats (KW) 7–8 Weeks of Age*

Group	Plasma aldosterone (ng/100 ml)		Plasma renin activity (ng/ml hour <sup>-1</sup> )
	Method 1	Method 2	
SHR	7.4 ± 1.8* (6)	14.2 ± 3.4† (8)	3.5 ± 0.6† (6)
KW	49.3 ± 9.0 (6)	33.5 ± 7.2 (7)	5.6 ± 0.5 (6)

Method 1 for the determination of plasma aldosterone is that of Gomez-Sanchez et al. (9), and method 2 is that of Varsano-Aharon and Ulick (10). Values are means ± SE. The number of rats in each group is given in parenthesis.

\*  $P < 0.005$  compared with the corresponding value for the Kyoto Wistar controls.

†  $P < 0.025$  compared with the corresponding value for the Kyoto Wistar controls.

The sequential analysis of aldosterone secretion in the present study showed that it was elevated markedly in the 7–8- and 11–13-week-old spontaneously hypertensive rats but was not significantly different from control in the 22–25-week-old rats. However, although not statistically different, the data for corticosterone secretion suggested that the dexamethasone–morphine sulfate regimen did not suppress ACTH in the 7–8- and 11–13-week-old spontaneously hypertensive rats to the same extent that it did in the normotensive Kyoto Wistar rats (Table 1). Therefore, aldosterone secretion was reexamined in acutely hypophysectomized 7–8-week-old rats to eliminate completely the influence of the anterior pituitary. In these hypophysectomized rats no differences in aldosterone, corticosterone, or deoxycorticosterone secretion rates were observed between the hypertensive and the normotensive control rats; moreover, aldosterone secretion in the hypophysectomized 7–8-week-old hypertensive rats were reduced markedly compared with that in the intact 7–8-week-old hypertensive rats, thus confirming the importance of the pituitary in the control of aldosterone secretion in these animals. This finding may indicate the existence of an abnormal pituitary-adrenal axis in the young spontaneously hypertensive rat. However, hypophysectomy failed to decrease aldosterone secretion in 7–8-week-old Kyoto Wistar rats (hypophysectomized  $0.45 \pm 0.12$  ng/min, intact  $0.42 \pm 0.13$  ng/min). This finding suggests that additional factors may have prevented the expected fall in aldosterone secretion in these Kyoto Wistar controls. Determinations of peripheral plasma aldosterone concentration and plasma renin activity in unstressed 7–8-week-old spontaneously hypertensive rats and Kyoto Wistar rats revealed that both parameters were depressed significantly in the hypertensive rats (Table 2). Thus, it seems likely that aldosterone secretion failed to fall in the hypophysectomized 7–8-week-old Kyoto Wistar rats because the renin-angiotensin system maintained secretion at a higher level; plasma renin activity was higher in control rats than it was in hypertensive rats. Moreover, arterial blood pressure fell to  $95 \pm 4$  mm Hg in the hypophysectomized Kyoto Wistar rats (Table 1), and additional activation of the renin-angiotensin system could have occurred under these conditions. Thus, the data of the present study indicate that the renin-angiotensin-aldosterone system is suppressed in the spontaneously hypertensive rat, but the findings do not suggest that the system is critically involved in the hypertensive process in these ani-

mals. An overactive pituitary-adrenal axis may be present in the hypertensive rats.

Previous studies by other investigators have found both an increase (6) and a decrease (5) in the rate of aldosterone secretion in the spontaneously hypertensive rat compared with that in the normotensive Wistar rat. Both of these groups of investigators have also reported a decrease in the secretion of deoxycorticosterone (5, 6). Moll et al. (5) found that secretion rates of 18-hydroxy-11-deoxycorticosterone and corticosterone were suppressed in the spontaneously hypertensive rat; these investigators suggested that there may be an enzymatic defect prior to pregnenolone in the biosynthetic pathway, since they observed no major differences between hypertensive and Wistar control adrenal glands in the conversion of pregnenolone to aldosterone, corticosterone, deoxycorticosterone, and 18-hydroxy-11-deoxycorticosterone in vitro (5). The steroid secretion data of the present study are not comparable to those of either Rapp and Dahl (6) or Moll et al. (5), because neither of these groups gave dexamethasone and morphine to suppress ACTH in their rats prior to surgical procedures and adrenal vein catheterization. In addition, both Rapp and Dahl (6) and Moll et al. (5) used ordinary normotensive Wistar rats as their control animal, whereas the normotensive Kyoto Wistar rat was the control animal in the present study. Conceivably, ordinary Wistar rats and Kyoto Wistar rats may differ to an important extent with regard to response to stress, adrenal sensitivity, and factors which influence steroidogenesis.

The present data on peripheral plasma aldosterone concentration in the spontaneously hypertensive rat are the first of their kind. These data probably represent true basal levels of plasma aldosterone, because the rats were unstressed prior to death and the collection of blood. Moreover, the quantitative differences between the plasma aldosterone values of spontaneously hypertensive rats obtained by the method of Gomez-Sanchez et al. (9) and by the method of Varsano-Aharon and Ulick (10) are probably methodologic in origin, since both methods assayed plasma collected in the same manner from rats bred and raised in the same colony under identical conditions. However, both methods showed that the peripheral plasma levels of aldosterone were suppressed below normal in the spontaneously hypertensive rat. It is not possible from the present data to tell whether low peripheral levels of aldosterone in the spontaneously hyper-

tensive rat are due to a low secretion rate or a high metabolic clearance rate of aldosterone.

Previous studies by other investigators have indicated that plasma renin activity is elevated in young spontaneously hypertensive rats but suppressed in older rats (3) or is normal in young spontaneously hypertensive rats but elevated in older rats (4) compared with the levels in regular normotensive Wistar rats. In contrast, Forman and Mulrow (12) have reported recently that plasma renin activity in spontaneously hypertensive rats remains normal and does not change with age (4 weeks to 11 months) compared with the levels in regular normotensive Wistar rats. Again, it must be emphasized that these previous studies (3, 4, 12) are not strictly comparable to the present experiments, because the regular normotensive Wistar rat was the control animal in these earlier studies. Moreover, plasma renin activity was determined by bioassay in two of these reports (3, 4), whereas radioimmunoassay of angiotensin I was employed in the present study and in the work by Forman and Mulrow (12). Also, Sen et al. (3) obtained blood samples for the determination of plasma renin activity from anesthetized rats which have elevated levels of plasma renin activity (13). Forman and Mulrow (12) collected blood by decapitation and determined plasma renin activity by radioimmunoassay; thus, their plasma renin activity data for the spontaneously hypertensive rat should be comparable to those of the present study. In their experiments, basal plasma renin activity averaged approximately  $2 \text{ ng/ml hour}^{-1}$  (range 0.9 to  $3.5 \text{ ng/ml hour}^{-1}$ ), which is remarkably similar to the present value of  $3.5 \pm 0.6 \text{ ng/ml hour}^{-1}$  for spontaneously hypertensive rats. The observed suppression of plasma renin activity in the spontaneously hypertensive rats of the present study might be secondary to the elevation in blood pressure.

The results of the present study suggest strongly that the renin-angiotensin-aldosterone system is suppressed in the spontaneously hypertensive rat; they do not indicate involvement of this system in the hypertensive process. Other studies are consistent with this conclusion. Neither a converting enzyme inhibitor (14) nor a specific competitive antagonist of angiotensin II (15) significantly lowers arterial blood pressure in the spontaneously hypertensive rat; this finding suggests that the renin-angiotensin system does not maintain the blood pressure in these animals. Also, Baer et al. (16) have reported that young bilaterally

adrenalectomized spontaneously hypertensive rats maintained on 1% saline continue to develop hypertension, although the time course of the development is slower than it is in rats with their adrenal glands intact. This finding suggests that aldosterone is not critically involved in the hypertensive process in these rats, but a secondary role of aldosterone as a consequence of transient elevations in ACTH cannot be excluded in the developing phase of hypertension.

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