Failure of feedback suppression of renin release in the spontaneously hypertensive rat

LEO B. CZYZEWSKI AND WILLIAM A. PETTINGER
Department of Pharmacology, Hoffmann-La Roche Inc., Nutley, New Jersey 07110

Czyzewski, Leo B., and William A. Pettinger. Failure of feedback suppression of renin release in the spontaneously hypertensive rat. Am. J. Physiol. 225(1): 234-239. 1973.-A model to test for the failure of feedback suppression of renin release was developed in normal rats and applied to spontaneously hypertensive (SH) and two strains of normotensive rats at various ages. The test system involved the subcutaneous administration of deoxycorticosterone acetate (DOCA) and the oral ingestion of sodium chloride. This test system suppressed serum renin activity in male and female normal Wistar and Charles River rats at 4, 8, 16, and 40 weeks of age. Normal suppression of renin release occurred at 4, 8, and 16 weeks of age in the SH rat. However, in both male and female SH rats, there was complete failure of suppression of renin release at 40 weeks of age, an abnormality similar to the one occurring in human malignant hypertension. Thus, in the context of renin release studied here, the SH rat appears to be a model resembling human essential hypertension that progresses to the malignant phase.

serum renin activity; angiotensin I; deoxycorticosterone acetate; hypertension

FAILURE OF FEEDBACK SUPPRESSION of renin release has been demonstrated in patients with malignant hypertension (4, 13), renal artery stenosis, and renal disease (30) and has been suggested to be a mechanism in women who become hypertensive while using antiovulatory agents (26, 28).

Failure of suppression of renin release in various forms of hypertension may result in a continuous release of renin from the kidneys and could be analogous to a continuous angiotensin infusion. This could be comparable to the phenomenon of autopotentiation, which has been reported in each of the following species: dog (14), rabbit (7), and man (11). The phenomenon of autopotentiation results from the continuous infusion of angiotensin at rates that initially have no effect on blood pressure, but which after one or more days of continuous intravenous infusion results in progressively increasing blood pressure. Angiotensin autopotentiation thus results in elevation of blood pressure, even though plasma levels of angiotensin II may be within the normal range. For this reason, normal or average levels of serum renin activity or angiotensin II may be meaningless in relating the pathogenic role(s) of this system to elevated blood pressure.

It has been suggested that the development of high blood pressure in the spontaneously hypertensive rat resembles, in some ways, the progressive increase in blood pressure found in the patient with essential hypertension (13, 21, 23). Thus, it was decided to study the spontaneously hypertensive rat for possible abnormalities of the renin-angiotensin system and to relate the findings to the human condition.

The model system that was used consisted of DOCA-induced sodium retention in the normal rat. This test system caused feedback suppression of renin release analogous to the system used in man (14, 19, 30). The test system was applied to spontaneously hypertensive rats and two strains of normotensive rats. The two major objectives of this study arc: 1) to establish if, at various ages and stages of hypertension, the serum renin activity in spontaneously hypertensive rats is different from that of two normotensive rat strains, and 2) to establish if failure of normal feedback suppression of renin release is a possible contributing mechanism of hypertension in spontaneously hypertensive rats.

METHODS

Spontaneously hypertensive (SH) rats were obtained by inbreeding of rats from the Okamoto and Aoki strain (21) at appropriate intervals to provide sufficient animals at 4, 8, and 16, and 40 weeks of age. Normotensive Charles River rats purchased from Charles River Breeding Laboratories, Wilmington, Mass., and normotensive Royal Hart Wistar rats from Royal Hart Laboratories, New Hampton, N.Y.

All rats were housed in individual cages (7 x 7 x 9.5 inches) for at least 3 days prior to the experiment day. “Newly arrived” normotensive animals were permitted to acclimate for 1 week prior to the test day. All rats were kept at 74 F in specially ventilated animal rooms and were exposed to a 12-hr light cycle from 6:00 AM to 6:00 PM by means of an automated control system. Unless specified otherwise, all experimental rats were permitted free access to tap water and diet. The diet consisted of Purina Laboratory rat chow.

A single dose of deoxycorticosterone acetate (DOCA) (Mann Research Laboratories) in 0.1 ml of olive oil was administered subcutaneously to each rat at the times indicated in the results or, for the standard test procedure, 48 hr prior to decapitation. Placebo-treated animals received 0.1 ml of olive oil alone. The DOCA-treated rats were only permitted free access to 0.9% NaCl solution, whereas the placebo group was limited to tap water ad libitum. DOCA, 40 mg/kg, and ingestion of 0.9% NaCl (DOCA Na) for 2 days gave optimal depression of serum renin activity and was used as the test model.
A modification of the procedures of Williams et al. (31) was used to record systolic blood pressure by cyclical occlusion of the tail artery pulse (cuff and pressure system from E & M Instrument Co., Houston, Texas). Blood pressures were measured in unanesthetized rats previously warmed for 5–10 min in an animal chamber (13 inches height × 12 inches diam) that was maintained at 35°C by a PDU-50 power dial reostat lamp (Lutron Electronics Co. Inc., Emmons, Pa.). The animals were placed into a rat holder and both a sensing and occluding cuff were placed on the tail. The pneumatic sensing cuff which was attached to a pneumatic pulse transducer (catalogue no. 92-600-70, E & M Instrument Co. Inc.) sensed the pulse return. The pulse return was recorded on a two-channel polygraph (Hewlett Packard, model no. 7702B, Sanborn Division, Waltham, Mass.). The average of five systolic blood pressure readings, determined in succession on 1 day in each rat during the week prior to application of the test system, was used in calculating the mean and standard error for each group of rats. Correlations were determined for the mean systolic blood pressure and the serum renin activity.

The rats were sacrificed between 9 and 11 AM, and a 4-sec blood sample was collected from the trunk following decapitation. The blood sample was collected into siliconized plastic tubes (13 X 100 mm) immersed in ice, permitted to clot, and centrifuged at 10,000 × g for 20 min at 4°C. The serum was frozen in 1 ml aliquots from each animal. The remaining serum was pooled, frozen, and later used for standard angiotensin binding curves in the assay. The techniques of incubations and radioimmunoassay of angiotensin I were previously published (25) and used with the following modifications. The pH of the serum sample was adjusted to 5.9 with 1/10 volume of 1.0 M Na citrate-2H₂O/HCl, pH 5.0, and EDTA was added to give a final concentration of 3 × 10⁻³ M. The serum obtained from both placebo and DOCA-Na-treated rats was incubated for 24 hr at 37°C. The average of quadruplicate results from each serum sample was used to calculate the mean and standard error of each group of rats and was expressed as nanograms of angiotensin I accumulated per 100 ml/hr. Angiotensin I-¹²⁵I was purchased at monthly intervals from Schwarz-Mann, Orangeburg, N.Y.

RESULTS

Deoxycorticosterone acetate (DOCA) in oil, 40 mg/kg sc, when combined with 0.9% NaCl ingestion (DOCA-Na), induced maximum suppression of serum renin activity within 2–4 days (Fig. 1) in normal rats. A 1- to 2-week interval was required after DOCA, 40 mg/kg, for return of serum renin activity to control levels (Fig. 1). This prolonged effect indicates sustained release of DOCA from the injection site.

Dose response relationships of DOCA suppression of serum renin activity are given in Fig. 2. DOCA was administered 2 days prior to decapitation. The serum renin activity following DOCA administration at 4 mg/kg was significantly depressed whereas a marked suppression followed the 40 mg/kg dose. This latter dose combined with 0.9% NaCl as drinking water was used as the model to test for failure of feedback suppression of renin release in both SH and normotensive rats.

The serum renin activity of placebo and DOCA-Na-treated male and female Charles River, Wistar, and SH rats at ages 1, 2, 4, and 10 months of age are given in Figs. 3–6.

A comparison of serum renin activity of normotensive male Charles River and Wistar rats showed that of the 4-week-old male Charles River (163.6 ± 9.7) to be greater (P < 0.01) than that of the 4-week-old male Wistar (129.0 ± 5.1) (Fig. 3), and the 40-week-old male Charles River (195.8 ± 21.1) to be greater (P < 0.01) than the 40-week-old Western Kentucky University, 1975. 473-474.

![Graph](http://ajplegacy.physiology.org/Downloadedfromhttp://ajplegacy.physiology.org/Downloadedfromhttp://ajplegacy.physiology.org/Downloadedfromhttp://ajplegacy.physiology.org/)
old male Wistar (112.8 ± 16.8) (Fig. 6). The serum renin activity (186.4 ± 12.2) from normotensive 4-week female Charles River rats was significantly higher (P < 0.001) than 128.2 ± 2.7 obtained from female Wistar rats of the same age.

A comparison of serum renin activity within each sex of both normotensive strains showed significant differences only in female Charles River rats. When 4-week-old Charles River female rats were compared to 8-, 16-, and 40-week-old Charles River females, the following differences were found: 4-week 186.4 ± 12.2 vs. 8-week 98.4 ± 20.9 (P < 0.01); vs. 16-week 69.5 ± 14.3 (P < 0.001); vs. 40-week 130.4 ± 17.7 (P < 0.05) (Figs. 3–6).

The serum renin activity within the spontaneously hypertensive rats at 4, 8, 16, or 40 weeks of age was not significantly different when compared either within each sex or between male and female rats (Fig. 7).

A comparison of the serum renin activity between spontaneously hypertensive rats and the two normotensive strains showed the following significant differences: 4-week male SH 134.4 ± 4.2 vs. Charles River male 163.6 ± 9.7 (P < 0.05) (Fig. 3); 40-week male SH 124.2 ± 13.9 vs. Charles River male 195.8 ± 21.1 (P < 0.05) (Fig. 6); 4-week female SH 137.6 ± 17.7 vs. Charles River females 186.4 ± 12.2 (P < 0.05) (Fig. 3); 16-week female SH 113.2 ± 12.0 vs. Charles River female 69.5 ± 14.3 (P < 0.05) (Fig. 5); 8-week female SH 87.6 ± 19.2 vs. Wistar females 155.2 ± 20.5 < 1. (P < 0.05) (Fig. 4).

DyNa suppressed serum renin activity in both normotensive strains, males and females, at 1, 2, 4, and 10 months of age (Figs. 3–5). In both the male and female SH rat, DOCA-Na also suppressed serum renin activity at 4, 8, and 16 weeks of age (Figs. 3–5), but failed to suppress serum renin activity in either the male or female SH rat at 40 weeks of age (Fig. 6).

Systolic blood pressures were already higher (P < 0.01) in female SH rats at 1 month of age than in Wistar or Charles River normotensive females (Fig. 8). This elevation in systolic pressure progressed until 40 weeks of age and averaged 190 ± 5 mm Hg. Similar patterns occurred in male SH rats with systolic pressure reaching 196 ± 11 mm Hg at 40 weeks of age. There were no significant differences in blood pressure between SH and Charles River rats at month of age.

A comparison of blood pressures between male and female normotensive strains showed the following significant differences. The pressure of 123 ± 3 obtained from 4-week-old Charles River male rats was significantly greater (P < 0.001) than 106 ± 2 obtained from Wistar rats of the same age (Fig. 9). The pressure of 133 ± 2 of 40 week old Char
RENIN RELEASE IN THE SH RAT 237

FIG. 7. Serum renin activity (SRA) in spontaneously hypertensive male and female rats.

FIG. 8. Systolic blood pressure of Charles River, Wistar, and SH female rats at 4, 8, 16, and 40 weeks of age. Each value is mean ± SE. Number of animals is given in parentheses. *** P < 0.001 indicates significance of difference from mean of comparably aged SH rats. Normotensive controls, Charles River (●), Royal Hart Wistar (○), and spontaneously hypertensive (⊗).

River male rats was significantly less (P < 0.001) than 151 ± 3 obtained from Wistar rats of the same age and sex (Fig. 9). Forty-week-old Charles River females showed a pressure of 127 ± 2, which was significantly less (P < 0.001) than 139 ± 3 obtained from Wistar rats (Fig. 8).

A comparison was made between the mean systolic blood pressures of Charles River male and female rats and showed that pressures in male rats at 4, 8, and 16 weeks of age (141 ± 5, 138 ± 2, and 133 ± 2, respectively) were significantly greater (P < 0.001, P < 0.05, and P < 0.001, respectively) than comparable female pressures (102 ± 2, 131 ± 3, 121 ± 3) at the same age (Figs. 8 and 9).

A comparison of the mean blood pressures of Wistar male and female rats was performed and showed that of the 40-week-old male rats (151 ± 3) to be significantly greater (P < 0.01) than 139 ± 3 obtained from female rats of the same age (Figs. 8 and 9).

An analysis of correlation between blood pressure and serum renin activity in rats receiving either placebo (0.1 ml olive oil) or DOCA (40 mg/kg sc in 0.1 ml olive oil) was performed. The analyses were performed on each placebo and DOCA test group (serum renin activity) as presented in Figs. 3–9, which list the blood pressures and corresponding serum renin activities. Four of the groups tested showed a negative correlation (blood pressure increasing and serum renin activity decreasing) between blood pressure and serum renin activity (P < 0.05). These were the Charles River female rats receiving the placebo at 40 weeks (Figs. 6 and 8) and DOCA at 16 weeks (Figs. 5 and 8), the Royal Hart (Wistar) female rats receiving the placebo at 16 weeks (Figs. 5 and 8), and the spontaneously hypertensive female rats receiving DOCA at 40 weeks (Figs. 6 and 8).

DISCUSSION

The induction of a positive sodium balance in man with several types of hypertension fails to suppress serum renin activity to the extent that it occurs in the normotensive population (14, 30). Because of angiotensin's autopotentiating capacity, the apparent failure of feedback suppression of renin release could contribute to elevation of blood pressure (7, 12, 15). The SH rat (21, 23) served as a model for these studies, since it has biochemical (20) and pharmacologic (1, 8, 10, 16, 17, 19, 22) abnormalities similar to those in human subjects with essential hypertension (24, 29). In order to test for failure of feedback suppression of renin release in the SH rat, various combinations of DOCA and sodium chloride were used as the test system for the suppression of serum renin activity in both normal and SH rats.

The DOCA-Na test model suppressed serum renin activity in both normotensive strains at 4, 8, 16, and 40 weeks of age and in the SH rats at 4, 8, and 16 weeks of age. However, a failure of feedback suppression of renin release was evidenced in both male and female SH rats at 40 weeks of age. The failure of the negative feedback system in the 40-week-old SH rat could have become evident at once, or subtle changes could have been occurring at an earlier age, but were not readily detectable by the test system used. This failure which could precede the onset of the hypertensive phase could result in a continuous renin release and conse-
quent angiotensin formation. Such a continuous formation of small amounts of angiotensin II for long periods of time could result in a progressive rise in blood pressure and explain the onset of the hypertensive phase in the young SH rat. These findings (failure of feedback suppression of renin), together with no significant differences in serum renin activity in the SH rat (of either sex) at 4, 8, 16, or 40 weeks of age, could be explainable in terms of the autopotentiation phenomenon of angiotensin II.

The continuous infusion of angiotensin at rates that initially have no effect on blood pressure will result in progressively increasing blood pressure after one or more days of continuous infusion. The increase in blood pressure resulting from such an infusion of angiotensin is said to be a result of the autopotentiation phenomenon of angiotensin II. The elevation of blood pressure will return to control levels at the termination of infusion, the rate of return being dependent on the length of time angiotensin II was infused (3, 7, 11, 14).

The failure of feedback suppression in the SH rat could result in an angiotensin formation at a rate that initially causes no increase in blood pressure but which, after a period of time, does initiate a progressive blood pressure increase as a result of the autopotentiation effect. Thus, a continued low rate of angiotensin formation would not be readily detectable in the SH rat and, hence, would be in agreement with the normal values of serum renin activity in the SH rat reported in this study. The low rate of angiotensin formation could, however, result in increased blood pressure, depending on the initial onset of the lesion and the length of time an inappropriate renin release was in progress. The failure to detect differences in the serum renin activity of the SH rat at all ages studied does not necessarily negate the renin-angiotensin system from a role in the development of the hypertensive phase in this species, but must be considered with regard to the autopotentiation phenomenon of angiotensin II (7, 11, 14). In certain individuals with mild essential hypertension, the angiotensin II levels have been reported to be both below and above the normal value, as well as showing no variation (4, 14). Whether failure of feedback suppression of renin release is responsible for the varied levels of angiotensin found in certain individuals is unknown. However, angiotensin's known pharmacologic activities, coupled with inappropriate renin release, suggest a potential mechanism for rapid acceleration of pathologic lesions in malignant hypertension.

In the SH rat, the onset of this abnormality (failure of feedback suppression of renin release) correlates with both the biochemical (18) and pharmacologic abnormalities (1, 8, 10, 16, 17, 27) that are similar to human subjects with essential hypertension (27). These observations appear to be in accord with the failure of feedback suppression of renin release that occurs in several forms of hypertensive disease. This failure has been demonstrated in hypertensive patients with renal artery stenosis, renal disease, and malignant hypertension (13), and in patients with essential hypertension who develop high angiotensin levels with no apparent deficiency of extracellular fluid volume (4).

An interesting question relating to the normal levels of serum renin activity in SH rats is whether these normal values may, in fact, represent two- or threefold relative elevations of serum renin activity for this model. For example, there is a well-known reciprocal relationship between effects of angiotensin and renin release. Effects of angiotensin are: aldosterone release with sodium retention (2, 5, 6, 9), increased sodium content of vascular smooth muscle (29), supersensitivity to vasoconstrictor substances (20), and elevation of blood pressure, the latter three effects having been reported to occur in the SH rat (8, 10, 15, 18).

Thus, according to observed and reported effects and the reciprocal relationships of renin release to these effects, the serum renin activity should be significantly lower in the SH rat and patients with essential hypertension if the feedback suppression loop were functional. It must be considered that normal levels of serum renin activity in the SH rat and in human patients with essential hypertension may actually be the result of inappropriate renin release.

These results illustrate that the spontaneously hypertensive rat is an excellent model for the study of the changes that occur as essential hypertension progresses to malignant hypertension. The present observations suggest that one of the changes that takes place in the SH rat is a significant loss of responsiveness of the negative feedback system concerned with renin release. This effect becomes evident at 40 weeks of age. The failure of feedback suppression of renin release in the 4 week old SH rat may be analogous to an inappropriate renin release and may be comparable to that in the malignant hypertensive individual where it has been shown that a failure of feedback suppression does occur and can result in high levels of angiotensin II (13).

The authors thank Dr. M. J. Kopac of the Department of Biology, New York University, for his assistance and guidance, Dr. W. D. Horst for his helpful discussions of this problem, and Mr. G. Bautz for the preparation of the antibodies used in the radioimmunoassay.

The work presented here is in partial fulfillment of the requirements for the degree of Doctor of Philosophy, Department of Biology, New York University.

A preliminary report of these investigations was presented at the joint meeting of the American Society for Pharmacology and Experimental Therapeutics Inc. and the Division of Medicinal Chemistry of the American Chemical Society, Burlington, Vermont, August 1971 (Clin. Res. 20: 26, 1972).

Present address of W. A. Pettinger: Departments of Pharmacology and Medicine (Clinical Pharmacology), University of Texas, Northwestern Medical School, Dallas, Texas.

Received for publication 13 November 1972.

REFERENCES

RENIN RELEASE IN THE SH RAT


