Ontogenesis of the Renin-Angiotensin System in Spontaneously Hypertensive and Normal Wistar Rats

By Alan Sinaiko and Bernard L. Mirkin

ABSTRACT

Kidney renin activity was investigated in spontaneously hypertensive and normotensive control Wistar rats. Kidneys were obtained from fetal rats after 18 and 21 days of gestation and from neonatal rats 1, 7, 14, and 21 days old. Kidney renin activity was determined by a modification of the radioimmunoassay for angiotensin I developed in this laboratory, and tissue from each age group was stained for juxtaglomerular granules. Kidney renin activity progressively increased in both strains from day 18 of fetal life until day 1 of neonatal life when a plateau level developed. In 1-day-old neonatal rats, significant differences in the kidney renin activity of the spontaneously hypertensive and the normotensive strains were initially noted. Kidney renin activity was maintained at significantly higher levels in the spontaneously hypertensive strain under 21 days of age when, because of a rise in the kidney renin activity of the normotensive control strain and a fall in the kidney renin activity of the spontaneously hypertensive strain, the difference between the two strains became statistically insignificant. In both strains, juxtaglomerular granules were first noted in 1-day-old neonatal rats and in each age group thereafter.

KEY WORDS kidney renin activity radioimmunoassay essential hypertension developmental hypertension juxtaglomerular granules blood pressure vasoactivity

Although the precise nature of essential hypertension has not been established in man, genetically determined factors appear to be significant in this disease (1, 2) which can be identified early in childhood (3). The development of hypertension-prone and hypertension-resistant strains of Sprague-Dawley rats (4) and of two spontaneously hypertensive strains of Wistar rats (5, 6) has provided an experimental model which may closely resemble essential hypertension in man.

The etiology of the hypertension in the Sprague-Dawley and the spontaneously hypertensive Wistar rats is unknown. Evidence from the Sprague-Dawley model has indicated that the kidney is responsible for producing the hypertensive state (7) via the release of an unidentified humoral agent (8). However, recent investigations have established that Sprague-Dawley rats from the hypertension-prone strain have a plasma renin activity significantly lower than that in rats from the hypertension-resistant strain (9) even before hypertension is induced. In the spontaneously hypertensive Wistar rats, elevated plasma renin activity has been demonstrated in one model (10, 11), but reduced activity occurs in the other model (12). This phenomenon, however, has been investigated only in rats old enough to have documented hypertension; levels of plasma renin activity have not been studied during earlier developmental stages.

In this study the ontogenesis of the renin-angiotensin system in normotensive and spontaneously hypertensive Wistar rats was studied to determine whether differences in kidney renin activity exist between these two genetically different strains during fetal and neonatal development and prior to the occurrence of systemic hypertension.

Methods

The spontaneously hypertensive Wistar rats used in this study (Carworth Laboratory Animals) were direct descendants of the Wistar strain developed by Okamoto and Aoki (5); normal Wistar rats served as controls. All rats were fed regular rat chow containing 0.42% sodium. Blood pressure was measured directly from the abdominal aorta with a Beckman strain-gauge pressure transducer in 5- and 16-week-old rats under light ether anesthesia.

Kidneys were obtained from fetal rats after 18 and 21 days of gestation and from neonatal rats 1, 7, 14, and 21 days old. All rats were decapitated and their kidneys were immediately removed and placed on
The kidneys were weighed and frozen in distilled water (100 ml/g tissue) at −20°C. At the time of death, five separate pools of renal tissue were collected for each age group. The number of rats used for each pool was not uniform and decreased as the age and size of the rats increased. However, kidney tissue from any one rat was not placed into more than one pool except in the 18-day fetal rats when both kidneys from every fetus were placed in one pool to provide enough tissue for renin extraction. Renin was isolated from minced kidney tissue after freezing and thawing the tissue three times. The tissue was then homogenized, and the supernatant fluid containing the renin was separated and frozen at −20°C until the time of assay.

Kidney renin activity was determined by a modification of the radioimmunoassay for angiotensin I (13) developed in this laboratory. This procedure was based on the reaction between renin extracted from rat kidney and renin substrate present in plasma obtained from sheep nephrectomized 48–72 hours before bleeding. Prior to each assay, the supernatant fluid from the homogenized renal tissue was diluted with distilled water to yield 400 μg tissue/ml. All procedures other than incubation were carried out at 4°C. The following incubation mixture was used: 2 ml of sheep plasma, 0.5 ml of the supernatant fluid from homogenized renal tissue (equivalent of 200 μg of kidney tissue), 6.5 ml of phosphate buffer (pH 6.5), 0.2 ml of ethylenediaminetetraacetic acid (EDTA) (2 mg), 0.002 ml of 2,3-dimercapto-1-propanol (BAL) (0.2 mg), and 0.01 ml of 8-OH-quinalone (0.66 mg). This mixture was continuously agitated during incubation for 3 hours in a 37°C water bath; then the flasks were removed and brought to 4°C. The radioimmunoassay was immediately performed using 0.05-ml samples of the incubated material. An angiotensin I radioimmunoassay kit for renin activity (Schwarz-Mann) was used. Kidney renin activity was expressed as nanograms of angiotensin I (ng AI/ml incubate 3 hour)−1. Separately incubated samples of sheep plasma and sheep renal tissue supernatant fluid generated negligible amounts of angiotensin I. Student's t-test was used for the analyses of statistical significance.

Rat kidney tissue from each age group was placed in 10% phosphate-buffered Formalin and then embedded in paraffin. This tissue was stained for juxtaglomerular granules using a crystal violet technique (14).

Results

**Blood Pressures of Spontaneously Hypertensive and Control Wistar Rats**

Intra-aortic blood pressures in both adult and 5-week-old spontaneously hypertensive Wistar rats were significantly elevated compared with those in the normotensive controls. The mean blood pressure in the adult spontaneously hypertensive Wistar rats was 158/100 mm Hg and that in the adult controls was 106/75 mm Hg (P < 0.005). The mean blood pressure in the 5-week-old spontaneously hypertensive Wistar rats was 137/95 mm Hg and that in their respective controls was 108/75 mm Hg (P < 0.005).

**Kidney Renin Activity of Spontaneously Hypertensive and Normotensive Control Wistar Rats**

Intrastrain Kidney Renin Activity.—The kidney renin activity of both spontaneously hypertensive and normotensive control Wistar rats increased progressively from day 18 of fetal life until day 1 of neonatal life when a plateau level was reached (Fig. 1). The level of kidney renin activity in the 21-day-old fetal rats and the 1-day-old neonatal rats was significantly different (P < 0.0025) in both strains. In contrast, no significant differences in kidney renin activity were noted in either strain between the 1- and the 7-day-old neonates or the 7- and the 14-day-old neonates. Although insignificant changes in kidney renin activity occurred between the 14-
and the 21-day-old neonatal spontaneously hypertensive Wistar rats, the kidney renin activity in the 21-day-old neonatal normotensive rats was significantly higher than that in the 14-day-old neonatal normotensive controls ($P < 0.005$).

**Interstrain Kidney Renin Activity.**—Significant differences between the kidney renin activity of the spontaneously hypertensive and the normotensive strains were initially noted in the 1-day-old neonatal rats (Fig. 1). Statistically significant data were not obtained for the 18-day-old fetuses, since replicate assays were not performed. In the 21-day-old fetuses, the mean kidney renin activity was $1.38 \text{ ng AI}$ in the spontaneously hypertensive rats and $1.49 \text{ ng AI}$ in the normotensive control group ($P > 0.05$).

In the 1-day-old neonates, the mean kidney renin activity in the spontaneously hypertensive rats ($4.65 \text{ ng AI}$) was significantly greater ($P < 0.025$) than that in the normotensive controls ($2.69 \text{ ng AI}$). A similar difference was observed in the 7-day-old neonates; spontaneously hypertensive rats had a kidney renin activity of $5.22 \text{ ng AI}$ and normotensive rats had an activity of $2.36 \text{ ng AI}$ ($P < 0.01$). Likewise, in the 14-day-old neonates, there was a significant difference in kidney renin activity between the spontaneously hypertensive and the normotensive Wistar rats ($5.30 \text{ ng AI}$ and $2.07 \text{ ng AI}$, respectively, $P < 0.01$). In contrast, in the 21-day-old neonates, the mean kidney renin activity in spontaneously hypertensive Wistar rats ($3.85 \text{ ng AI}$) and in controls ($4.18 \text{ ng AI}$) did not differ significantly.

**Juxtaglomerular Granules**

Juxtaglomerular granules were not identified in fetal kidney tissue. They were first noted in the 1-day-old rats of both the spontaneously hypertensive and the control strain and in each age group thereafter. The granules were always found adjacent to formed glomeruli and never in areas of early glomerular development, i.e., S-shaped or metanephric vesicles.

**Discussion**

The presence of kidney renin activity was detected by radioimmunoassay in the fetuses of both spontaneously hypertensive and normal Wistar rats as early as the eighteenth day of gestation. An increase in fetal kidney renin activity was noted between 18 and 21 days of gestation; however, no statistically significant differences in kidney renin activity between strains was observed until 1 day after birth. Kidney renin activity was significantly greater in the spontaneously hypertensive strain until 21 days; kidney renin activity of the normotensive controls then increased and that of the spontaneously hypertensive rats decreased so that the differences between the two strains were statistically not significant.

The lack of correlation between morphologically demonstrable juxtaglomerular granules and the presence of kidney renin activity in the rat has been reported previously (15). However, the histological technique used in this study was qualitative in nature, and granules which were not identified in other tissue sections may have been present.

No comparable studies contrasting the kidney renin activity of spontaneously hypertensive and normotensive strains of rats during fetal or early neonatal periods of development could be found. However, data are available from the three strains of genetically hypertensive rats for a comparison between rats 4 weeks of age and older. Sen et al. (10) compared the kidney renin activity of spontaneously hypertensive Wistar rats and normotensive controls using a bioassay technique. They divided their rats into groups according to body weight and found significantly increased amounts of kidney renin activity in spontaneously hypertensive rats weighing 100–150 g. This weight should correspond to rats approximately 7–8 weeks old. In rats weighing more than 150 g, the normotensive strain had significantly greater levels of kidney renin activity. These findings are in slight conflict with the data presented in this study which indicated that the difference in kidney renin activity between the spontaneously hypertensive and the normotensive strains was no longer significant at 21 days of age. Their results also differed from those of an earlier study (16) which showed no statistically significant difference in the kidney renin activity in 5-week-old spontaneously hypertensive Wistar and Donryn rats. When adult rats are compared, kidney renin activity of Donryn rats is similar to that of normal Wistar-Kyoto rats. The investigations of de Jong et al. (11) have also confirmed the absence of any significant difference in kidney renin activity between spontaneously hyper-

**Circulation Research, Vol. XXXIV, May 1974**
tensive and normotensive control Wistar rats at 8, 12, 16, and 20 weeks of age.

In the New Zealand strain of spontaneously hypertensive Wistar rats (6), kidney renin activity has been compared with that of normal control rats of 60, 90, and 120 days of age (12). The data from this study indicate no significant difference in kidney renin activity between the 60-day-old rats but show significantly reduced levels of kidney renin activity in spontaneously hypertensive rats at both 90 and 120 days of age. Data about kidney renin activity in the Sprague-Dawley strain of hypertension-prone rats are similar to those for the New Zealand Wistar model. Kidney renin activity in the hypertension-prone strain is significantly decreased compared with that in the hypertension-resistant strain in rats 5–12 weeks of age (9) and during the time before hypertension is induced.

The etiology of the hypertension which develops in the Sprague-Dawley and Wistar rats is unknown. In the Wistar rat, conflicting evidence exists regarding the renin-angiotensin system. The present study and others (10, 11) suggest that the renin-angiotensin system is important in the development of spontaneous hypertension. However, the fact that kidney renin activity in the hypertensive rats reverts to normal at approximately the time when hypertension develops and that kidney renin activity is significantly lower in the hypertensive rats than it is in control rats when older animals are compared (12, 17) conflicts with this hypothesis. Furthermore, available evidence from the Sprague-Dawley model indicates that kidney renin activity is significantly reduced in the hypertensive strain after 5 weeks of age. Perhaps, increased kidney renin activity is only required in the early developmental stages to establish a pattern which is maintained throughout later life.

Evidence obtained from man as well as from rats suggests that genetic factors influence the development of essential or spontaneous hypertension (1, 2). If evidence from studies on rats proves relevant to man, additional studies in the developing rat to define the events occurring in early development which may exert long-term influences on the renocardiovascular system are warranted.

References

Ontogenesis of the Renin-Angiotensin System in Spontaneously Hypertensive and Normal Wistar Rats

ALAN SINAIKO and BERNARD L. MIRKIN

Circ Res. 1974;34:693-696
doi: 10.1161/01.RES.34.5.693

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1974 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/34/5/693