Plasma renin activity as a function of age in two new strains of spontaneously hypertensive and normotensive rats

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Summary
1. Strains of spontaneously hypertensive and normotensive rats were selected by repeated in-breeding.
2. Brief ether anaesthesia was shown to produce a two- to three-fold increase in plasma renin activity in both strains.
3. Plasma renin activity was significantly higher in young spontaneously hypertensive than in normotensive rats of the same age (5-7 weeks). After the ninth week plasma renin activity decreased and, at week 45, became significantly lower in hypertensive than in normotensive rats.
4. When hypertension was established a significant inverse relationship was found between plasma renin activity and systolic blood pressure in spontaneously hypertensive and in normotensive rats.
5. It seems unlikely that the renin-angiotensin system plays a major role in the maintenance of the established spontaneous hypertension in this strain. However, renin hypersecretion may be important in the early pre-hypertensive stage of genetic hypertension in rats.

Key words: age, anaesthesia, genetically hypertensive rats, plasma renin activity.

Introduction
There have been several studies of the renin–angiotensin system in spontaneously hypertensive rats of the strains bred by Okamoto & Aoki (1963) and Phelan (1968). The results are often difficult to compare because of the widely varying protocols employed and, more important, the origin of the normotensive control rats used. We have isolated, by inbreeding, two strains of rats classified as hypertensive (SHR) and normotensive (NR) (Dupont, Dupont, Froment, Milon & Vincent, 1973). The NR strain can safely be used as control animals because they were selected from the same parent strain, over the same period of time and by the same procedure as the SHR strain.

To evaluate the role of the renin–angiotensin system in spontaneous hypertension we first studied the vascular reactivity to angiotensin II, which was found to be identical in the two strains of rats (Dupont & Sassard, 1974). In the present work we have followed the evolution of plasma renin activity as a function of age in the SHR and NR strains of the eighth generation.

Methods

Animals and procedures
Nine eighth-generation male NR and ten male SHR were used. These animals were originally of Sprague-Dawley stock and were maintained in constant conditions of temperature (22°C), lighting and humidity (50%). They received a standard diet (Iffa rat chow from Iffa Credo) and water ad libitum.

At the end of 5, 7, 9, 11, 13, 14, 15, 21 and 45 weeks of age, the following values were measured in
each of the nineteen rats. Systolic blood pressure (mmHg) was taken by an indirect oscillometric method without anaesthesia, after slight warming at 38°C for 10 min. Body weight (g) was measured the same day as systolic blood pressure. Plasma renin activity (pmol of angiotensin I h⁻¹ ml⁻¹) was evaluated by a previously described radioimmunoassay of angiotensin I (Vincent, Sassard & Cier, 1972), which was modified for use with rat plasma.

Optimum pH for renin activity was found to be at 6.5. To 400 μl of rat plasma, 100 μl of a solution of angiotensinase and converting enzyme inhibitor (EDTA, 14 mmol/l, and 8-hydroxyquinoline sulphate, 6.8 mmol/l) and 1.5 ml of phosphate-buffered (0.1 mol/l) sodium chloride solution (150 mmol/l, pH 6.9) were added. The final pH of this incubation mixture was 6.5 ± 0.1.

For each plasma sample, endogenous angiotensin I was measured in an aliquot maintained at +4°C and angiotensin I production was measured after 15, 30 and 120 min incubation at 37°C. In each experiment, an aliquot of standard rat plasma was introduced to detect variation in the assay. When the standard’s plasma renin activity was found to differ more than 2 SD from the mean value, that experiment was rejected.

Blood sampling

After ether anaesthesia of the rats, a small cutaneous incision was made and 1 ml of blood was drawn by a direct puncture of the jugular vein with a siliconized syringe containing 1 mg of disodium EDTA. The operation lasted less than 30 s. The incision was closed with a surgical clamp. By this technique, blood samples could be drawn frequently from the same animal.

Control study

To evaluate the influence of anaesthesia on plasma renin activity, a control study was conducted in eleven additional 10-week-old male rats (six NR strain and five SHR strain) belonging to the eighth generation.

Two blood samples were drawn: one without anaesthesia, through a polyethylene catheter (Becton-Dickinson PX 014) inserted in the right atrium 3 days before, and the other, 24 h later, by the previously described method after anaesthesia.

Results for each variable were expressed as the mean value ± SEM and further statistical analysis was carried out by Student's t-test and the paired t-test.

Results

Kinetics of angiotensin I formation in rat plasma

Angiotensin I formation was found to be a linear function of time during 30 min both in the NR strain plasma and SHR strain plasma (Fig. 1). Only such linear results were used for this study.

Fig. 1. Kinetics of angiotensin I production in (a) normotensive and (b) spontaneously hypertensive rat plasma. Results are expressed as pmol of angiotensin I (mean ± SEM) in plasma incubated at 37°C after subtraction of the endogenous angiotensin I concentration (n = number of measurements).
Influence of ether anaesthesia on plasma renin activity

A sharp increase of plasma renin activity was found both in NR and SHR strains after anaesthesia (Fig. 2); the increase in the SHR strain appeared to be of the same magnitude as that of the NR strain but far less reproducible. There was no significant difference between the responses of the SHR and NR strains ($P > 0.30$).

Influence of ageing on plasma renin activity, systolic blood pressure and body weight in rats

Plasma renin activity was seen to be higher in SHR strain than in NR strain until 14 weeks of age, but this difference was significant only at 5 ($P < 0.002$) and 7 weeks ($P < 0.05$; Fig. 3). After 9 weeks, the activity decreased and at 45 weeks was significantly lower in the SHR strain than in NR strain ($P < 0.01$).

Small, but sometimes significant, differences were found in body weight, which was usually greater in the SHR strain than in NR strain.

Relations between plasma renin activity and systolic blood pressure

There appeared to be a positive but non-significant correlation between these in the SHR and NR strains in rats aged from 5 to 9 weeks ($P > 0.05$). Conversely, a significant negative correlation was found between these two variables in rats of both strains, aged from 11 to 45 weeks (Fig. 4). This correlation was greater in the NR strain than in the SHR strain.

Discussion

Most studies of plasma renin activity or renal renin content have been carried out in several groups of different rats killed at different ages. Observations with the SHR strain have been compared with those obtained in control rats of different origin. Normal
rats belonging to different strains can exhibit large variations in plasma renin activity and renal renin content, as well as in renin responses to different stimuli (Conradi, Jelinek & Gross, 1969; Czyzewski & Pettinger, 1973; Forman & Mulrow, 1974). Thus meaningful comparisons are difficult.

Throughout this study, males originating from two strains of SHR and NR, selected by the same procedure, were used. Moreover, all the experimental variables were followed, at different ages, in the same rat. These procedures were essential for valid comparison of results.

Body weight was measured as in some studies it has been used as a standard of comparison rather than age. In comparison with the New Zealand strain (Lee, 1973) at each age, our SHR were not smaller than their control animals.

A few minutes after the start of ether anaesthesia plasma renin activity increased more than twofold. This is in agreement with observations from other workers (Leenen, De Jong & De Wied, 1973; Carvalho, Shapiro, Hopper & Page, 1975. As no significant differences could be demonstrated between the responses of SHR and NR strains, anaesthesia did not modify the interpretation of our data.

In the present experiments the only significant increase of plasma renin activity was found in 5- and 7-week-old SHR. This is consistent with the results of Sen, Smeby & Bumpus (1972) and of Sinaiko & Mirkin (1974).

Plasma renin activity decreased after 9 weeks; thus our strain shows similarities with both the Okamoto (De Jong, Lovenberg & Sjoerdsma, 1972; Sen et al., 1972) and New Zealand (McKenzie & Phelan, 1969) strains.

In contrast, Lee (1973) found a progressive decrease of plasma renin activity in the New Zealand strain, and Shiono & Sokabe (1973), Czyzewski & Pettinger (1973) and Forman & Mulrow (1974) reported no change in plasma renin activity in the Okamoto strain. Plasma renin activity was much more stable in our NR strain than in our SHR strain. Similar absence of change in plasma renin activity with age has been reached in normotensive Wistar rats (De Jong et al., 1972).

After 11 weeks, plasma renin activity decreased more strikingly in the SHR strain than in the NR strain. As a consequence, significantly lower plasma renin activities were found in SHR after 45 weeks of age. In SHR of Okamoto and Phelan strains, such a low plasma renin activity or renal renin content has been reported after 6 weeks (Lee, 1973), 12 weeks (Saito, Mukaino & Ogino, 1974), 16 weeks (McKenzie & Phelan, 1969), 24 weeks (Gresson, 1972) and 28 weeks (Koletsky, Smook & Rivera-Velez, 1970). Differences in substrate concentration could still account for some of the observed differences in plasma renin activity, although this seems unlikely in these studies. Such a decrease of plasma renin activity exhibited by SHR might reflect renal

FIG. 4. Correlation between plasma renin activity and systolic blood pressure in (a) normotensive rats and (b) spontaneously hypertensive rats aged from 11 to 45 weeks.
sodium retention or a normal baroceptor response of the juxtaglomerular apparatus to an extra-renally elevated blood pressure. This last explanation is supported by the demonstration of an inverse relationship between plasma renin activity and systolic blood pressure in the SHR and NR strains from 11 to 45 weeks of age.

In conclusion, results reported here make it unlikely that the renin–angiotensin system plays a major role in the maintenance of an established spontaneous hypertension. Thus, at this stage, the SHR strain exhibited a lower plasma renin activity than the SHR strain, and in a previous study we have shown that these rats do not show an increased vascular response to angiotensin (Dupont & Sassard, 1974).

However, renin hypersecretion may play a role in the early pre-hypertensive stage.

References


