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Postnatal Development of Renin-Angiotensin System in Rats

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The changes occurring in several components of the rat renin-angiotensin system (RAS) were studied for the brief postnatal period, between the fourth and tenth week of life. The parameters were: plasma renin activity (PRA), plasma renin concentration (PRC), plasma renin substrate (PRS) and the plasma angiotensin II concentration (AII). A gradual decrease in PRA with age was noticed. Between the fourth and the eighth weeks of life, this was attributed to a corresponding decline in both PRC and PRS. However, between the eighth and tenth weeks, no changes in PRA could be detected, but PRC and PRS increased, perhaps as a consequence of the changes in renal function and the AII increase observed. In this second period, simultaneously with the RAS changes described, there was reduced sodium chloride excretion as the glomerular filtration rate (GFR) stabilized. The data presented suggest that this postnatal period is critical, in rats, for the maturation of the RAS component control mechanisms; they appear to be closely related to the development of the renal function.

Key words: Postnatal development, Renal function, Renin-Angiotensin system.

Circadian rhythms in the plasma renin activity (PRA) have been recognized (14). However, these are not the only temporal changes shown by the renin-angiotensin system (RAS). Numerous studies of several animal species and of human subjects (25) have revealed a gradual decrease of PRA during their development. In the rat this decrease is more evident in the early postnatal periods (19). The reasons for this decrease are not completely understood. Some authors have suggested that the PRA decrease might result from variations in renal renin secretion by the juxtaglomerular cells, due to changes in renal perfusion pressure (15), or to renin inactivation in the liver (19).

On the other hand, postnatal increases in angiotensin converting enzyme activity (23, 24) and in the plasma angiotensin II concentrations (AII) (25, 26) have

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been described. It has been shown that the increase in angiotensin converting enzyme activity may result from an elevation of the tissue concentration of this enzyme and not from an increase in its affinity for the substrate (23). This increase, together with the increased pulmonary perfusion observed during development, could be the cause of the increase of AII (26).

In addition, it is well known that kidney maturation continues during development (20, 21), and possibly the RAS changes may be related to this process. This paper reports a study of the influences of the RAS components on PRA and their relation to changes in renal function occurring between the fourth and tenth week of life, a brief postnatal period, but which in the rat, covers the transition from renal immaturity to the beginning of adulthood.

Materials and Methods

Animals and sample collection. Male Wistar rats were used for this study. Starting at the end of the 4th week of life, groups of eight animals were sacrificed at two week intervals under anaesthesia with sodium pentobarbitone (Nembutal, Abbot Lab. Madrid, Spain). The rats thus represent respectively, the 4th, 6th, 8th and 10 th weeks of development. All sacrifices were carried out at 12 a.m. The animals were kept for the previous 24 hours in individual metabolic cages to permit collection of 24 h-urine. Blood samples taken from the aorta were divided into two aliquots. Both were centrifuged immediately at 2500 g for 15 min at 4°C. The plasma and serum samples were stores at —20°C for subsequent use.

Renin-angiotensin assay. Plasma renin concentration (PRC), plasma renin substrate (PRS) and PRA were measured by incubating the plasma samples for 1 h at 37 °C and pH 7.4, in the presence of 10 μl of an alcoholic solution 0.8 M dimercaptopropanol and 10 μl of an aqueous solution of 0.34 M 8-hydroxyquinoline sulphate as inhibitors of the converting enzyme and angiotensinases.

For PRS estimation, 0.2 ml of plasma diluted in 0.04 M phosphate buffer at pH 7.4, were incubated with 0.1 ml of a solution of semipurified rat renin (6).

PRC was determined by incubating 0.2 ml of diluted plasma with 0.4 ml of plasma from 24 h-binephrectomized rats. The amount of added renin substrate was considered to be sufficiently in excess to maintain zero order kinetics for the period of analysis. At the same time, for the PRS determination, the addition of renin ensured that the substrate was the limiting factor in the reaction.

Analysis of the basal and generated angiotensin after incubation in presence of renin substrate or renin excess, or in endogenous enzyme and substrate conditions, was by radioimmunoassay (7).

Angiotensin II measurement. AII was measured by radioimmunoassay (2), following an extraction method in which 0.3 ml of plasma were gently mixed with 3 ml of absolute ethanol at 4 °C for 24 h. After centrifuging, at 2000 g, the supernatant was dried in a current of air at 37 °C and the resulting pellet rediluted in 0.04 M phosphate buffer pH 7.4.

Renal function. Glomerular filtration rate (GFR) was calculated from creatine clearance data. Endogenous creatine, in serum and urine, was determined by the Jaffe reaction in a Beckman analyzer.

Chloride concentrations, in serum and urine, were determined by an argentocoulombometric method (Clorurómetro Bioquímico. OM). Serum and urine concentrations of sodium and potassium were determined by ion-selective electrodes (Microlyte. Kone). Fractional chloride,

sodium and potassium excretions (FE_{Cl}, FE_{Na} and FE_K) were calculated using conventional formula.

When data from more than two groups were compared, statistical analyses employed one-way and two-way analysis of variance (ANOVA), and regression analysis. Student's t-test was used when data from two individual groups were compared. The 0.05 level of probability was used as the significance criterion.

Results

Postnatal changes in PRS and PRC (figure 1). PRS was noticed to decline steadly between the 4th and the 8th week and then to increase sharply in the 10th week of development. At the same time, after a significant descent between the 4th and 6th week of life, PRC was stable until the 8th week, but a the 10th week it had increased sharply. No significant difference was observed in PRC between the 4th and 10th week of development.

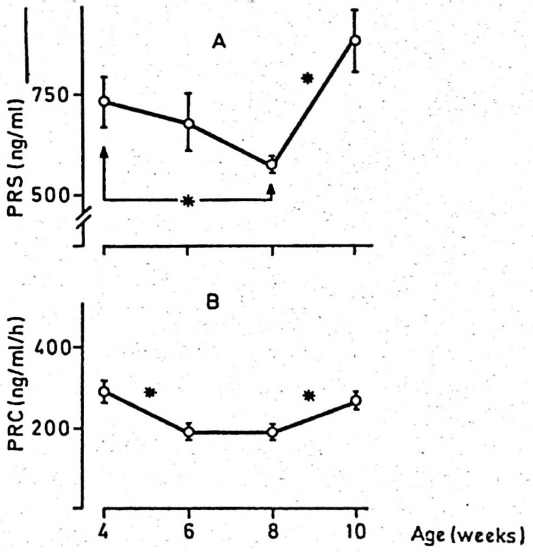

Fig. 1. *Plasma renin substrate concentration (A) and plasma renin concentration (B) in rats at various stages of development.*
Each point represents mean ± SEM of eight animals (* $p < 0.05$).

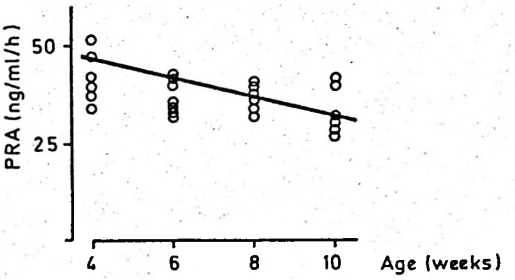

Fig. 2. *Plasma renin activity (PRA) during development of male rats between 4th and 10th week.*
Individual values are used in analysis of regression (L.R. = —0.51; $p < 0.05$).

Postnatal changes in PRA. Although no significant PRA changes were seen during the 4th, 6th, 8th and 10 th weeks of life, following a comparison of the means of the results in each group, a gradual descent of PRA with development was detected (fig. 2), except for two animals which showed a sharp increase in the plasma renin substrate levels at 10th weeks, and so their results were excluded from the simple regression analysis.

Postnatal changes in AII (fig. 3). The circulating levels of AII exhibited an exponential increase ($y = 0.52e^{0,29x}$; LR = = 0.64, $p < 0.05$) during development, showing a marked rise between the 8th and 10th week of life.

Table I shows the changes observed in some parameters of renal function. Diuresis increased significantly between the 4th and 6th week of life, and then was stable for the last four weeks. A gradual and significant rise in GFR was observed between the 4th and 8th week of life, followed by stabilised values in the 10th week of development.

FE_{Na} and FE_{Cl} showed no change from their initial values until the 8th

Table I. *Renal function in rats between the fourth and tenth weeks of life.* The results are the means ± SEM of 8 determinations. Unpaired t-test was used.

Week	Diuresis (µl/min)	GFR (µl/min)	FE_{Na} (%)	FE_{Cl} (%)	FE_{K} (%)
4th	1.7 ± 0.4	13.7 ± 1.1	14.0 ± 1.5	26.5 ± 2.9	463.5 ± 37.2
6th	6.4 ± 1.4*	26.6 ± 6.7*	10.9 ± 2.8	20.6 ± 2.4	539.8 ± 126.6
8th	9.4 ± 2.0	62.1 ± 3.8*	11.5 ± 1.4	21.0 ± 1.7	433.6 ± 32.6
10th	9.1 ± 1.8	72.8 ± 7.3	7.0 ± 1.3*	11.8 ± 2.9*	391.7 ± 85.1

* $p < 0.05$.

week of life; at this time both had decreased significantly. No changes were observed for FE_K in the period studied.

Figure 4 shows the relations between the RAS components and renal function for all the experimental situations. During postnatal development PRC and PRS accounted for 51 % and 21 % of the changes in AI and PRA, respectively. A relationship was also found between AI and PRA (determination coefficient, R = 0.35). No relation was observed between AI-AII.

Angiotensin II concentration accounted for 16 % of the GRF changes.

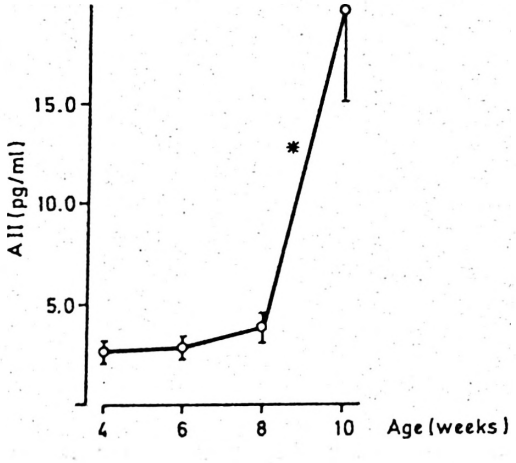

Fig. 3. *Plasma angiotensin II concentration (AII) in rats at different ages.* Each point represents mean ± SEM of eight animals (* $p < 0.05$).

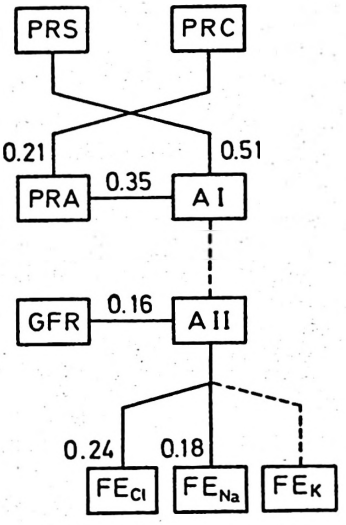

Fig. 4. *Relationship between kinetic parameters of renin-angiotensin system (RAS) and renal function during 4th and 10th week of life in rats.*

Discussion

A gradual decrease in PRA was observed in agreement with previous reports (19, 25). The decrease might result from the interplay of several factors affecting RAS components.

Studies carried out on animals and human subjects show that during development a reduction in PRC occurs (9, 25). It has been suggested that the descent in PRC might be caused by a decrease in the rate of renin release from the jux-

taglomerular cells (19), or by an elevation of plasma volume (4), or by an increased hepatic breakdown of renin and its subsequent metabolic clearance (19). Although this decrease between the fourth and the eighth weeks of life is confirmed by the present work, at the tenth week a slight increase in PRC had occurred. This increase in PRC could be associated with the fact that the kidney was now completely mature (21). In this sense, the GFR, used as an index of evaluation of the renal function (10), revealed a stabilisation between the eighth and tenth week of life.

At the eighth week, a sharp rise in AII concentration was observed. It is well known that this hormone redistributes renal plasma flow (RPF) from the cortical to the juxtamedullary nephrons (27), where the renin content and single nephron glomerular filtration rate (SNGFR) are lower (17, 22). The redistribution of RPF induced by AII might stimulate renin release from cortical nephrons and the subsequent increase in PRC. On the other hand, its antinatriuretic activity and RPF redistribution, could be the cause of decrease of the sodium and chloride fractional excretion. The lack of changes in the excretion of the potassium fraction in the period studied, could be explained by the fact that distal nephron matures earlier; around the 24th day of life (13), and so AII could not influence potassium transport (1).

The existence of a direct relationship between the PRA and PRC changes found in other experimental situations, supports the hypothesis that the PRA changes may well be caused by modifications of PRC. However previous studies have revealed that PRA changes are also dependent on PRS (11). In this way, although PRS values of 1 day-old rats showed no significant difference from those found in adult animals (15), fluctuations during development were found between the fourth and eighth week of life when there was a sharp fall in PRS. This decrease cannot be attributed to an increase in renin concentration (12); it might result from a decrease in hepatic synthesis, but this was not studied.

After the eighth week, a sharp increase in PRS occurs, coinciding with two important situations: firstly, with an increase in plasma AII, this being a positive feed-back stimulus of further hepatic synthesis (18); secondly, it coincides with the onset of rat puberty (16, 29), a period of significant changes in the levels of many enzymes and hormones, some of which (i.e. adrenal steroids) control the production of renin substrate in the liver (5, 28).

The observation of the PRS and PRC increases in the tenth week of life, suggests that an increase in PRA might occur because, in the rat, the endogenous renin substrate is the limiting factor of reaction velocity (3). However, no change in PRA was detected. This could imply an age-related change in the catalytic activities of renin, or changes in the structure of the angiotensinogen molecule involving the catalytic site(s) (25); or both of these factors. It might also reflect the appearance of an inhibitor to the renin reaction (8).

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Resumen

Se estudia en ratas, entre la 4.ª y 10.ª semana de vida, los cambios en varios componentes del sistema renina-angiotensina (RAS): actividad renina plasmática (PRA), concentración de renina en plasma (PRC), concentración de sustrato de renina en plasma (PRS) y concentración de angiotensina II (AII). Durante este periodo tiene lugar un paulatino descenso en la PRA. Este descenso, entre la cuarta y

octava semana de vida se produce como consecuencia de la disminución de PRC, así como de PRS. Sin embargo, entre la octava y décima semana, se observa un aumento en PRC y PRS, presumiblemente debido a los cambios en la función renal y aumento en la concentración de AII, que no afecta a la PRA. En este último periodo, simultáneamente con los cambios descritos en los componentes del RAS, se produce una reducida excreción de cloruro sódico y una estabilización en la tasa de filtración glomerular (GFR). Los datos presentados muestran que en la rata el periodo de vida estudiado es crítico para los mecanismos de control de los componentes del SRA, apareciendo una relación de los mismos con el proceso de maduración renal.

References

1. Borrensen, H. C., Rorvik, S., Guldvog, I. and Aakvaag, A.: *Scand. J. Clin. Lab. Invest.*, **42**, 87-92, 1982.
2. Cain, M. D., Catt, K. J., Coghlan, J. P. and Blair-West, J. R.: *Endocrinology*, **86**, 955-964, 1970.
3. Carretero, O. and Gross, F.: *Am. J. Physiol.*, **213**, 695-700, 1967.
4. Davis, J. O.: *Circ. Res.*, **28**, 301-306, 1971.
5. Dzau, V. J. and Herrmann, H. C.: *Life Sci.*, **30**, 577-584, 1982.
6. Haas, E., Goldblatt, H., Gipson, E. C. and Lewis, L.: *Circ. Res.*, **19**, 739-749, 1966.
7. Haber, E., Koerner, T., Page, L. B. Kliman, B. and Purnode, A.: *J. Clin. Endocrinol.*, **29**, 1349-1355, 1969.
8. Hauger-Klevene, J. H. and Levin, G. M.: *Medicina (Buenos Aires)*, **36**, 219-222, 1976.
9. Hayduk, K., Krause, D. K., Huenges, R. and Umbehaum, V.: *Experientia*, **28**, 1489-1490, 1972.
10. Ichikawa, I.: In «The kidney during development: morphology and function» (Spitzer, A., ed.), Masson, New York, 1982, pp. 119-125.
11. Jiménez, E., Montiel, M., Narváez, J. A., Miranda, M. T., Parras, L. and Morell, M.: *Horm. Metabol. Res.*, **16**, 315-318, 1984.
12. Jiménez, E., Montiel, M., Narváez, J. A. and Morell, M.: *Acta Endocrinol (Kbh)*, **105**, 505-510, 1984.
13. Larsson, I. and Maunsbach, A. B.: In «The kidney during development: morphology and function» (Spitzer, A., ed.), Masson, New York, 1982, pp. 115-117.
14. Molteni, A., Warpeha, R. L., Brizio-Molteni, L,, Albertson, D. F. and Kaurs, R.: *Ann. Clin. Lab. Sci.*, **9**, 518-523, 1979.
15. Pohlová, I. and Jelinek, J.: *Pflügers Arch.*, **351**, 259-270, 1974.
16. Ramírez, V. D.: In «Handbook of Physiology. Endocrinology» (Greep, R. O. and Astwood, E. E., eds.), American Physiological Society, Washington, 1973, volume II, pp. 1-28.
17. Rouffignac, C., Bonvalet, J. A. and Menard, J.: *Am. J. Physiol.*, **226**, 150-154, 1974.
18. Schambelan, M. and Stockigt, J. R.: In «Pathophysiology of the renin-angiotensin system» (Brenner, B. M. and Stein, J. H., eds.), Churchill Livingstone, New York, 1979, pp. 1-39.
19. Solomon, S., Iaina, A., Eliahou, H. and Serban, I.: *Biol. Neonate*, **32**, 237-242, 1977.
20. Solomon, S.: *J. Physiol.*, **272**, 573-589, 1977.
21. Solomon, S.: In «The kidney during development: morphology and function» (Spitzer, A., ed.), Masson, New York, 1982, pp. 283-289.
22. Spitzer, A.: In «The kidsey during development: morphology and function» (Spitzer, A., ed.), Masson, New York, 1982, pp. 127-132.
23. Wallace, K. B., Bailie, M. D. asd Hook, J. B.: *Am. J. Physiol.*, **234**, R141-R145, 1978.
24. Wallace, K. B., Bailie, M. D. and Hook, J. B.: *Am. J. Physiol.*, **236**, R57-R60, 1979.
25. Wallace, K. B., Hook, J. B. and Bailie, M. D.: *Am. J. Physiol.*, **238**, R432-R437, 1980.
26. Wallace, K. B., Roth, R. A., Hook, J. B. and Bailie, M. D.: *Am. J. Physiol.*, **238**, R395-R399, 1980.
27. Wilkinson, R.: *Drugs*, **23**, 195-206, 1982.
28. Weigand, K., Wernze, H. and Falge, C.: *Bioch. Biophys. Res. Comm.*, **75**, 102-109, 1977.
29. Wong, C., Döhler, K., Geerlings, H. and Mühlen, A.: *Horm. Res.*, **17**, 202-215, 1983.