Factors influencing plasma renin and renin substrate in premature infants

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Abstract

Concentrations of plasma renin (PRC) and plasma renin substrate (PRS) were measured during the first week of life in 52 infants born at less than 37 weeks' gestation (mean (SEM) gestation 30 (0.4) weeks, mean (SEM) birth weight 1.35 (0.08) kg). Both PRC (median 35, interquartiles 16.3, 94.5 ng/ml/hour) and PRS (median 2.3, interquartiles 1.3, 5.0 µg/ml) were raised compared with adults. The proportional rise in PRC was much greater than that in PRS, suggesting that PRS may be rate limiting in the generation of angiotensin I. Log₁₀ PRC was inversely proportional to gestational age and a high urinary sodium loss was associated with a significantly raised log₁₀ PRC. In hypoxaemic infants, there was a strong correlation between log₁₀ PRS and haemoglobin concentration; this is a new observation in human infants but consistent with data available from other species.

The renin-angiotensin system in adult man is concerned with the maintenance of intravascular fluid volume, blood pressure, and sodium and potassium homeostasis. However, studies in a number of different species have suggested that the renin-angiotensin system may have a more important role early in phylogeny and ontogeny,^{1 2} periods during which the more sophisticated autonomic nervous system is less well developed.

A number of investigators have studied the renin-angiotensin system in the newborn human infant since it was first observed that umbilical venous renin concentration exceeded maternal venous renin concentration.³ However, these studies have reported plasma renin activity (PRA), rather than plasma renin concentration (PRC) and plasma renin substrate (PRS) independently.⁴⁻⁷

PRA is a measure of the rate of generation of angiotensin I by a plasma sample under specified conditions; PRA may thus be low because of either relatively low enzyme or relatively low substrate concentrations. The reaction between the enzyme renin and its substrate appears to follow first order kinetics in adult man, with the enzyme being rate limiting.⁸ However, in human pregnancy, even though PRS is raised, it is the substrate which is rate limiting in the mother.⁹

Very little is known in the human neonate about the relative contributions of PRC and PRS to the raised PRA observed in newborn infants^{5 7} and whether PRC or PRS is rate limiting. PRC and PRS are under different control mechanisms.^{10 11}

The purpose of this study was to measure simultaneously PRC and PRS in premature newborn infants during early postnatal life and to examine the relationship of PRC and PRS to blood pressure and concentrations of urea, electrolytes, and arterial blood gases. These variables are known to relate to the renin-angiotensin system from previous studies of PRA in animals,^{12 13} adult man,^{14 15} and the human fetus^{2 3 9} and newborn.^{4-7 10 11 16}

Subjects and methods

SUBJECTS AND SAMPLES

The protocol was approved by the Nottingham hospitals' committee of ethics.

All babies born at less than 37 weeks' gestation were deemed to be eligible for the study if arterial cannulation was required during the first week of life. This selection criterion was necessary because it was not considered ethical to obtain samples by direct venepuncture. Gestation was derived from the date of last menstrual period if known. Otherwise, gestation was assessed by ultrasound morphometry in early pregnancy or, failing this, postnatal Dubowitz scoring.¹⁷

Blood samples (maximum 2.5 ml) were collected from an infant at 9 am on the first morning on which an arterial catheter was in place. If the infant was male, a six hour urine collection was made by attaching a polythene bag over the genitalia, starting the collection from the time at which blood was taken.

If the arterial catheter remained in place 48 hours later, and if the infant was still within the first week of postnatal life, further blood and urine samples were obtained.

ANALYSIS OF SAMPLES

An aliquot of 0.125 ml of blood was taken into a heparinised, sealed syringe and arterial blood gases measured immediately (Corning 168 pH/Blood Gas system, Corning Medical and Scientific). Another aliquot of 1.0 ml of blood was taken into a tube containing lithium heparin for subsequent measurement of plasma sodium, potassium, urea and creatinine concentrations (Corning 902 analyser, Corning Medical and Scientific). Plasma osmolality was measured by the depression of freezing point method (Freezing point osmometers, Advanced Instruments Ltd). A further aliquot 0.3 ml of blood was taken into a tube containing potassium EDTA for subsequent measurement of haemoglobin concentration (Coulter S+4 cell counter, Coulter Electronics Ltd). A final aliquot of

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1.0 ml of blood was taken into potassium EDTA, the sample centrifuged, and the plasma separated immediately. The plasma was frozen for later measurement of PRC and PRS, at physiological temperature and pH, by established radioimmunoassay methods already published.¹⁸ The high PRC found in many samples necessitated the use of shorter incubation times than usual (0, 15, 30, and 45 minutes). The intra-assay coefficient of variation for PRC and PRS were 4.4 and 5.1% respectively; interassay coefficients of variation were 14.5 and 13.5%.

The volumes of the six hour urine collections were recorded and the samples assayed the same day for sodium, potassium, urea, and creatinine concentrations (Corning 902 analyser, Corning Medical and Scientific). Osmolality was measured by depression of freezing point.

Systolic and diastolic blood pressure were measured immediately before blood sampling using either the oscillometric technique (44 babies; Dynamap Neonatal Vital Signs Monitor, Critikon Inc) or the direct intra-arterial measurement (eight babies; pressure transducer at heart level coupled to S and W monitor, S and W Medico Teknik A/S).

ANALYSIS OF DATA

Creatinine clearance and fractional excretions of sodium and potassium were calculated from the data obtained in this study. A correction was applied to the small number of blood pressures measured intra-arterially to allow comparison with the larger number of measurements made oscillometrically.¹⁹

Analyses were performed using the SPSSX statistical package. Where appropriate, data were transformed logarithmically before analysis to achieve a more normal distribution. This is a standard statistical tool which is frequently necessary with skewed biological data. If this failed to normalise the data, non-parametric analyses were used. Arithmetic or geometric means were calculated. Arithmetic means (SEM) are quoted. Medians and interquartiles are quoted for non-normally distributed data. Comparisons were made using paired or unpaired *t* tests as appropriate and correlation coefficients calculated by method of Pearson. The straight line equations shown in figs 1–3 were generated by linear regression analysis.

Results

Blood samples were obtained from 52 infants, 28 boys and 24 girls, mean (SEM) gestation 30.0 (0.4) weeks, mean (SEM) birth weight 1.35 (0.08) kg. Ten infants had birth weights below the third centile for gestation age,²⁰ of whom only one was born at less than 28 weeks' gestation. The mean (SEM) postnatal age was 2.4 (0.2) days. A second blood sample was obtained 48 hours later from 31 babies; these paired data are considered separately below. Urine collections were made from 25 boys but data were incomplete for four of these. A further urine sample was obtained 48 hours later from 12 of these infants (see methods). As

would be expected, both systolic and diastolic blood pressures rose significantly ($p < 0.0001$, $p < 0.02$) with increasing gestational age at delivery. The serum osmolality fell appreciably with increasing gestational age ($p < 0.0001$).

ENZYME

The median PRC at the time of first sampling was 35.0 (16.3, 94.5) ng/ml/hour, significantly higher than in a group of 26 healthy young adults (2.4 (1.7, 3.3) ng/ml/hour; $p < 0.0001$). (We have no data on PRC and PRS concentrations, using the current assays, in normal term infants.) $\log_{10}$ PRC fell significantly with increasing gestation age ($p < 0.02$; fig 1). However, there was no independent association between $\log_{10}$ PRC and body weight ($p = 0.682$). There was also a significant positive correlation between $\log_{10}$ PRC and $\log_{10}$ fractional sodium excretion ($\log_{10}$ FeNa; fig 2, $p < 0.05$). There were no apparent associations between $\log_{10}$ PRC and either systolic or diastolic blood pressure, pH, blood gas tensions, or glomerular filtration rate.

Figure 1 Association between $\log_{10}$ PRC and gestation showing significant inverse correlation over the range studied in this paper ($r = 0.33$; $p < 0.02$). $\log_{10}$ PRC (ng/ml/hour) = $3.25 - 0.06$ gestational age (weeks).

Figure 2 Association between $\log_{10}$ PRC and $\log_{10}$ fractional excretion of sodium (FeNa) determined in 21 of the boys at a mean (SEM) postnatal age of 2.4 (0.2) days. The $\log_{10}$ FeNa was positively correlated with $\log_{10}$ PRC ($r = 0.47$; $p < 0.05$). $\log_{10}$ PRC (ng/ml/hour) = $1.63 + 0.47 \log_{10}$ FeNa (%).

Figure 3 Association between $\log_{10}$ PRS and base excess showing a significant correlation between these two variables, ($r=0.32$; $p<0.02$). $\log_{10}$ PRS ($\mu\text{g/ml}$) = $0.59 + 0.04$ base excess (mmol/l).

SUBSTRATE

The median PRS at the time of first sampling was 2.3 ($1.3, 5.0$) $\mu\text{g/ml}$, significantly higher than that in healthy young adults (1.1 ($0.8, 1.2$) $\mu\text{g/ml}$; $p<0.001$). $\log_{10}$ PRS did not alter significantly with gestational age, indices of renal function, or blood pressure. However, a significant positive correlation was observed between $\log_{10}$ PRS and base excess ($p<0.02$; fig 3), although there was no correlation with pH or blood gas tensions. Nineteen of the premature infants who were severely hypoxaemic at the time of study (arterial oxygen tension (PaO_2) <7.2 kPa) were found to show a significant positive correlation between $\log_{10}$ PRS and haemoglobin ($p=0.001$) and packed cell volume ($p=0.003$). This association was not seen in 16 premature but normoxaemic infants of similar gestation ($\text{PaO}_2 >9.0$ kPa).

PAIRED DATA

Paired data on PRC and PRS were available from 31 infants. Median PRC fell between the first sample and the second sample by 6.0 ($-36.0, +12.0$) ng/ml/hour but this fall was not significant. Median PRS also fell slightly, by 0.3 ($-0.8, +1.5$) $\mu\text{g/ml}$, but this change was also not significant. There were no significant changes over the period studied in arterial blood pressure, blood gas tensions, or packed cell volume.

Discussion

LIMITATIONS OF THE STUDY

Because of the ethical constraints of investigating only those infants with an indwelling arterial catheter, the sample studied was not representative of the population of all infants born prematurely, although the more immature the infant, the more representative the sample becomes. All umbilical arterial catheters were sited with the tip above the level of the diaphragm but we cannot exclude the possibility that the presence of an umbilical arterial catheter may alter renal blood flow. Ideally, serial daily samples would have been obtained, but again this could not be justified ethically, and 24 hour

urine collections would have been obtained from all infants enrolled in the study. However, it was felt that the technique of bag urine collection was only reliable in boys. A six hour period was considered the most feasible duration for which the bag could be kept attached and the urine uncontaminated. As fluid compartments in the neonate correlate closely with body weight, all parameters are expressed per unit body weight.²¹

ENZYME AND SUBSTRATE

The measurement of PRA gives an idea of the angiotensin I generating capacity of a plasma sample. Assuming that angiotensin converting enzyme activity is not rate limiting, this thus gives information about the angiotensin II generating capacity, which is, of course, of direct biological interest in the absence of a direct measurement of angiotensin II. In the non-pregnant adult PRC appears to be rate limiting in the enzyme:substrate reaction and changes in PRA thus follow closely those of PRC. In pregnancy and the neonate, however, it appears to be PRS which is rate limiting⁹ so that interpretation of underlying changes in enzyme and/or substrate synthesis or release becomes impossible. Enzyme and substrate are regulated by different mechanisms, and it was thus of interest to determine the individual plasma concentrations of PRC and PRS, and to attempt to correlate them with potential stimuli for release.

In the human neonate, it has been shown that PRA is high at birth⁵ and higher the earlier the gestation.⁷ PRA falls with increasing postnatal age^{5,6} and this trend continues throughout infancy and childhood. In the newborn puppy²² and rat,²³ however, there is a further postnatal increase in PRC before the decline to adult values and this delayed increase in renin concentration may reflect the relative immaturity of these mammals at birth. PRA has been shown to be inversely correlated with sodium intake and directly correlated with urine osmolality over the first week of postnatal life in term infants.¹⁶ PRA also appears to increase in response to hypovolaemia, at least after 31 weeks' gestation.²⁴ These studies therefore show that, at least by the last trimester, the renin-angiotensin system can be activated by similar stimuli to the adult.

Our data have now shown that PRC can be extremely high in premature infants in the first few days of postnatal life, considerably higher than in term infants.²⁵ Symonds *et al* measured PRC in samples obtained at cordocentesis between weeks 16 and 24 and also found PRC to be high (approximately 26 ng/ml/hour).²⁶

It appears from our data that although PRS is somewhat higher in premature infants than at term²⁷ or in later life, PRS is nevertheless probably rate limiting in the generation of angiotensin I in vivo at this gestation, given the proportionately much higher concentrations of PRC. The large volumes of blood which would be needed for formal kinetic studies in the infant make such studies impossible at present. Investigation of the postnatal development of

the renin-angiotensin system in the rat also suggested that plasma renin substrate concentration may be rate limiting in the newborn rat but not in the adult.²³ Symonds *et al* reported PRS concentrations within the normal adult range in the samples obtained at cordocentesis.²⁶ It is not possible to determine from our data whether the higher PRS which we found at a later gestation (measured in the same assay system) is a consequence of increasing gestation or of premature delivery. PRS is an α_2 globulin synthesised by the liver and the rise in PRS concentration with gestation may simply reflect a maturation of hepatic globulin production. Alternatively, less substrate may be consumed as PRC falls during the last trimester.

CHANGES IN RELATION TO RENAL FUNCTION

The relationship demonstrated in this study between PRC and gestation shows an inversality common to all large mammalian species studied in late gestation,² and this may be related to maturing nephrogenesis, which is complete by 36 weeks' gestation in the human. Urinary aldosterone excretion increases significantly between 30 and 41 weeks' gestation,⁷ and the urine potassium to sodium ratio, an index of aldosterone dependent distal tubular activity, is positively correlated with conceptional age.²⁸ Therefore, as gestation proceeds, glomerulotubular balance improves, renal sodium reabsorption increases, and renin concentrations fall.

This concept of end organ insensitivity driving the renin-angiotensin system in the more premature infants is supported by the data in fig 2. When the fraction of filtered sodium appearing in the urine was high, PRC was also high. Previous work has shown that premature infants can increase PRA in response to a negative sodium balance,^{7, 29} but the high renal sodium losses of immature infants are due in part to a blunting of aldosterone synthesis or release and in part of the limited responsiveness of the distal tubule to aldosterone stimulation.³⁰⁻³²

OTHER FACTORS WHICH MAY ACTIVATE THE RENIN-ANGIOTENSIN SYSTEM

The renin-angiotensin system is known to be activated via β adrenergic stimulation in the adult.^{32, 33} It has also been observed that angiotensin II concentrations, which parallel PRA in infancy,³⁴ are significantly raised in infants with severe respiratory distress syndrome,³⁵ and the severity of respiratory distress syndrome increases with decreasing gestation. This study did not show any significant association between PRC and blood pressure or arterial blood gas tensions and in a previous study of 11 term infants with congestive cardiac failure and raised values of PRA, no relationship was found between PRA and systolic or diastolic blood pressure.³⁶

ENZYME, SUBSTRATE, AND HEMOGLOBIN

We report for the first time a correlation between log₁₀ PRS and both packed cell volume

and haemoglobin concentrations in hypoxaemic premature infants. We have recently made a similar observation in prematurely delivered pony foals (F Broughton Pipkin, J Ousey, and P Rosedale, work in progress). PRS and erythropoietin are both α_2 globulins, both are manufactured in the liver and show apparent close structural homology,³⁷ both are activated by a renal factor, and both are higher in the newborn than in later life. Circumstantial evidence for a relationship between oxygen delivery and the renin-angiotensin system comes from data from chronically hypoxic rats in which PRS concentrations were raised and correlated closely with erythropoietin concentrations.³⁸ Similarly, the renin-angiotensin system can be activated in adult man by chronic altitude exposure.³⁹

CLINICAL RELEVANCE

We have confirmed that the renin-angiotensin system is very active in premature human infants. What remains unclear is whether this is desirable. It is possible that an activated renin-angiotensin system is an appropriate response to the low blood pressure,³⁵ low glomerular filtration rate,⁴⁰ and glomerulotubular imbalance⁴¹ of early postnatal life. However, angiotensin II is the most potent circulating vasoconstrictor known, and acts as a pulmonary vasoconstrictor in adult man⁴² and in fetal lambs⁴³ and the high concentrations of angiotensin II associated with respiratory distress syndrome³⁵ may exacerbate pulmonary hypertension and salt and water retention.⁴⁴ The consequences of an activated renin-angiotensin system for the premature cerebral circulation are unclear. Leslie *et al* have recently reported high PRA in 10 very low birthweight infants who had suffered severe intraventricular haemorrhage during the first two days of postnatal life.⁴⁵ Eight of the infants we studied had also suffered moderate to severe intraventricular haemorrhage during the first week. Six of these had PRS above the group median and four had grossly raised PRC (>120 ng/ml/hour).

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