Factors affecting angiotensin II concentrations in the human infant at birth

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Summary

1. A radioimmunoassay for the measurement of angiotensin II in 1 ml of plasma has been developed and used to measure angiotensin II in maternal peripheral, cord venous and cord arterial blood in 45 patients at delivery.

2. In babies delivered vaginally, cord venous and cord arterial concentrations of angiotensin II were significantly higher than maternal venous blood concentrations. There was a significant relationship between both cord venous and cord arterial concentrations and maternal concentrations of angiotensin II.

3. Cord venous concentrations of angiotensin II were significantly greater than those in cord arterial blood in babies delivered vaginally but not in those delivered by lower-segment Caesarean section. This suggests the possibility that, during labour, the placenta may contribute to foetal concentrations of angiotensin II.

4. Maternal and cord venous concentrations of angiotensin II were significantly higher in patients with hypertensive disease of pregnancy than in those who had remained normotensive throughout pregnancy.

5. Cord venous concentrations of angiotensin II increased significantly with increasing duration of the second stage of labour.

Key words: angiotensin II, Caesarean section, newborn infant, placenta.

Introduction

The kidneys of immature mammals have long been known to contain proportionately more renin than do those of adults (Grossman & Williams, 1938). Investigations using bioassay techniques have shown that newborn rabbits and lambs not only have higher circulating concentrations of angiotensin II than those in adults but also produce higher concentrations of angiotensin II in response to a standard haemorrhage (Broughton Pipkin, Mott & Roberton, 1971; Broughton Pipkin, Kirkpatrick, Lumbers & Mott, 1974a). Both plasma renin activity and plasma renin concentration are higher in the human newborn than in the mother (Mott, 1975) but there is little information on concentrations of angiotensin II at this time. In view of the marked changes in angiotensin II which occur in the perinatal period in other animals, an investigation into the concentrations in humans at birth and those factors which affected them has been undertaken.

Methods

Patients

Blood samples were obtained at delivery from 95 babies. Because of the length of time needed to extract angiotensin II from the plasma (~2½ h) only those patients were sampled whose expected time of delivery was convenient for
the laboratory. Within this limitation, samples were obtained from consecutive vaginal deliveries and deliveries by elective lower-segment Caesarean section.

All mothers were taking an unrestricted diet and were not known to have pre-existing renal or metabolic disease. Fifty-two of the mothers were primigravidae. Age ranged from (mean 23.0) years in the 81 who delivered vaginally and from 17 to 45 (mean 30.0) in the 14 delivered by elective Caesarean section.

Blood pressures were measured at each attendance at the ante-natal clinic by trained midwives using Accoson sphygmomanometers (A. C. Cossor and Son Ltd, London, N.4). A cuff size of 25 cm × 14 cm was used throughout and the abrupt change of sound was taken as the diastolic blood pressure. If there was any uncertainty, the blood pressure was taken again by a second member of staff. Patients were classified into three groups on the basis of their recorded blood pressures. Those who remained normotensive throughout (blood pressure less than 140/90 mmHg) were classified as group 1. Those who were normotensive until the onset of labour but during labour had blood pressures equal to, or in excess of, 140/90 mmHg on at least two occasions were put into group 2 and group 3 was those mothers who were normotensive up to the twentieth week of pregnancy but thereafter had blood pressures equal to or in excess of 140/90 mmHg on at least two visits.

No patient had received diuretics, antibiotics or anti-hypertensive agents during the week before labour. Maternal hypertension was treated with bed rest in hospital. Mothers who delivered vaginally without epidural anaesthesia were given 100 mg of pethidine and 25 mg of promazine hydrochloride intramuscularly on admission to the labour suite. Forty patients were infused with oxytocin (Syntocinon: Sandoz) during labour and eight were given oral prostaglandin E₂ (Prostin E₂: Upjohn). Ten mothers were delivered under epidural anaesthesia and, in four of these, forceps were used.

The indications for elective lower-segment Caesarean section were: breech presentation (seven patients); major degree of placenta praevia (two); cephalopelvic disproportion (two); previous poor obstetric history (three). Patients were premedicated with atropine (0.6-1.2 mg intramuscularly) and magnesium trisilicate solution (15–30 ml) by mouth. They were given 100% O₂ to breathe before induction of anaesthesia with thiopentone (225–300 mg intravenously), alphadolone acetate and alphaxalone (3–4.5 ml intravenously; Althesin, Glaxo) or methohexitone (70–100 mg intravenously; Brietal, Lilley). Anaesthesia was maintained with nitrous oxide and oxygen.

### Sampling and assay

One of us (F.B.P.) collected all the foetal samples. Maternal samples were obtained only when an extra member of staff was available to take the blood without interfering with labour ward routine.

The blood samples were put immediately into chilled polyethylene tubes containing a mixture of o-phenanthroline (25 mmol/l) and EDTA (125 mmol/l) (Düsterdieck & McElwee, 1971). The tubes were replaced in ice and taken immediately to the laboratory where they were centrifuged at 4°C and 2000 rev./min. Angiotensin II was assayed immediately from the plasma by the method of Düsterdieck & McElwee (1971) and the tubes containing the residue were stored at −18°C. Angiotensin II was assayed by a scaled-down version of the method of Düsterdieck & McElwee (1971), in 1 ml portions of plasma. An antiserum of high avidity was raised in a New Zealand White rabbit against angiotensin II amide (Hypertensin, Ciba Ltd, Horsham, Surrey, U.K.) coupled to bovine serum albumin (Sigma Chemical Co., St Louis, Mo., U.S.A.); the cross-reactivity characteristics of this antiserum are summarized in Table 1. The limit of detectability of the assay was taken to be the smallest amount of hormone that differed from the percentage bound in the absence of added angiotensin (zero binding) by at least 3 SD of replicate variation. The SD of replicates from ten standard curves for zero binding was 0.38%; thus on our standard curve 5 pmol/l was taken as the limit of detection. Over the range 7–28 pmol/l of plasma the mean coefficient of variation of 25 samples assayed in duplicate was 13-2 ±1.9%. The corresponding figure for 25 samples in the range 100–300 pmol/l was 7.74 ±1.3%. One sample from a pool was put through seven assays over a period of 6 weeks;
Angiotensin II in babies

TABLE 1. Percentage cross-reaction of angiotensin II antiserum (1:1200) on a molar basis with other components of the renin-angiotensin system and oxytocin at 50% of initial zero standard bound on the standard curve

<table>
<thead>
<tr>
<th>Cross-reaction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asn1-Val2-angiotensin II (Hypertensin, Ciba)</td>
</tr>
<tr>
<td>Asp1-Ileu1-angiotensin II (Medical Research Council)</td>
</tr>
<tr>
<td>Val1-heptapeptide (Schwarz-Mann)</td>
</tr>
<tr>
<td>Val1-hexapeptide (Schwarz-Mann)</td>
</tr>
<tr>
<td>Oxytocin (Syntocinon, Parke Davis: up to 100 μunits/ml)</td>
</tr>
</tbody>
</table>

the mean angiotensin II value was 155·0±5·8 pmol/l with a coefficient of variation of 9·9%.

Statistical methods

Results are expressed as mean values ± 1 SEM. Student's t-test was used to assess the statistical significance of the difference between two means where appropriate.

For further analysis, the angiotensin II values were transformed logarithmically to normalize the variances. Linear models were fitted to these data for the analysis of covariance using the General Linear Interactive Modelling package of the Royal Statistical Society's Working Party on Statistical Computing (Nelder, 1974).

Results

Vaginal delivery

Paired samples. In 31 instances, blood was obtained from the cord artery, the cord vein and a maternal antecubital vein. Paired cord venous/maternal venous samples were obtained from another 20 cases and since an F-test showed no significant difference between the variances (P>0·10) these results have been pooled. Cord arterial angiotensin II concentrations were higher than in maternal blood in 24 of the 31 instances; a paired t-test showed this difference to be highly significant (P<0·005). Cord arterial and maternal venous concentrations were significantly related to each other (r = 0·37; P<0·05). Angiotensin II was higher in cord venous than in maternal blood in all except three instances, and this difference was also highly significant (P<0·001) as was the relationship between maternal venous and cord venous concentrations (r = 0·69; P<0·001).

Maternal angiotensin II concentrations were higher in the hypertensive than in the normotensive patients (Table 2) and the difference between mean values in group 1 and group 3 was significant (P<0·02). Cord arterial values

<table>
<thead>
<tr>
<th>Angiotensin II (pmol/l of plasma)</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal venous blood</td>
<td>59·2±11·6</td>
<td>104·9±33·9</td>
<td>132·3±9·7</td>
</tr>
<tr>
<td>Cord venous blood</td>
<td>210·2±34·0</td>
<td>144·6±21·0</td>
<td>337·6±45·6</td>
</tr>
<tr>
<td>Cord arterial blood</td>
<td>183·1±48·8</td>
<td>145·5±31·2</td>
<td>219·5±54·3</td>
</tr>
</tbody>
</table>
did not differ significantly in the three groups. Cord venous angiotensin II was, however, significantly raised in group 3 by comparison with both group 1 \((P<0.05)\) and group 2 \((P<0.01)\).

A total of 51 paired cord venous and cord arterial samples was obtained. The mean concentrations were respectively 226.2 ± 20.1 and 169.0 ± 20.0 pmol of angiotensin II/l. There was a very highly significant relationship between these paired measurements \((r = 0.65; P<0.001)\). Cord venous concentrations of angiotensin II were higher than cord arterial values in 41 instances and a paired t-test over all 51 samples showed this difference to be highly significant \((P<0.001)\).

Factors affecting angiotensin II in cord venous blood. A total of 81 cord venous samples were obtained at vaginal delivery. Those which were not paired with either maternal or cord arterial samples as previously detailed did not have significantly different variances from them \((P>0.10)\) and all results for cord venous blood were therefore pooled. There was a wide scatter of data, with cord venous angiotensin II ranging from 33 to 1453 pmol/l. Information was available concerning maternal blood pressure, length of gestation, body weight of the baby, whether or not oxytocin or prostaglandins had been given during labour and the duration of the second stage of labour. The data were therefore subjected to an analysis of covariance, taking this information into account.

Analysis of covariance revealed that there was no significant difference in angiotensin II concentrations between cord venous blood in the babies of group 1 and group 2 (mean values 240.8 ± 36.0 and 184.3 ± 31.7 pmol/l). Babies born to mothers with hypertensive disease of pregnancy did, however, have significantly higher concentrations than did the babies of either of the other groups (326.2 ± 32.8 pmol/l; Table 3).

Cord venous concentrations of angiotensin II were also significantly related to the duration of the second stage of labour \((P<0.05\); Table 3). Increasing length of the second stage of labour was associated with raised concentrations: for example, a baby born after a second stage of 55 min had 799 pmol of angiotensin II/l in cord venous blood and another born after a second stage of 130 min had 1453 pmol/l.

In addition, there was a difference in the effect of the duration of the second stage of labour on concentrations of angiotensin II according to the mother’s blood pressure group \((P<0.05\); Table 3). Thus although the mean duration of the second stage of labour was similar in the babies of group 1 and group 3 (31.3 ± 3.3 min compared with 37.1 ± 4.7 min; \(P>0.1\)), there was a greater increase in circulating angiotensin II in the babies of the hypertensive mothers in response to increasing length of the second stage of labour (Table 3). None of the other factors studied reached significance for their effect on cord venous blood concentrations.

Caesarean section

Paired maternal venous, cord venous and cord arterial blood samples were obtained from 14 mothers and babies delivered by elective lower-segment Caesarean section at term.

| Table 3. Analysis of variance of cord venous blood angiotensin II concentrations |
|---------------------------------|---------------------------------|----------------|--------|
| Model                          | Degrees of freedom | F ratio | \(P\)   |
| Fitted term                    | Current model       | 81      |        |
| Second stage                   | Overall mean        | 80      | 5.425  | <0.05 |
| Maternal blood pressure group  | Overall mean        | 79      | 3.908  | <0.05 |
| MBPG+SS                        | SS                  | 78      | 3.573  | <0.05 |
| MBPG+SS                        | MBPG                | 78      | 4.733  | <0.05 |

The total residual mean square was taken as the ‘overall’ variance. The reduction in this variance, achieved by successively fitting individual factors, is used to demonstrate significance of the effect of the various factors. SS = duration of the second stage of labour. MBPG = maternal blood pressure groups 1, 2 and 3.
mean maternal blood angiotensin II was $66.1 \pm 9.1$ pmol/l of plasma; mean cord venous and cord arterial blood values were $76.8 \pm 14.5$ and $81.6 \pm 13.7$ pmol/l respectively. No statistically significant relationship could be demonstrated between maternal venous and either cord venous or cord arterial angiotensin II concentrations. However, a highly significant relationship was found between the cord venous and cord arterial concentrations ($P < 0.001$).

Cord venous blood angiotensin II was also measured in an additional 13 babies of comparable gestation age at elective Caesarean section. There was a significant overall inverse relationship between body weight and angiotensin II in these infants ($r = 0.441$; $P < 0.05$; Fig. 1).

**Discussion**

There is general agreement that cord plasma renin levels are markedly higher than those in the mother after vaginal delivery (Brown, Davies, Doak, Lever, Robertson & Tree, 1964; Geelhoed & Vander, 1968; Wernze & Seki, 1972; Hayduk, Krause, Huenges & Unbehaun, 1972; Soveri, Fyhrquist & Widholm, 1975). Plasma renin substrate concentrations appear to be lower in cord blood and blood taken 1–12 h after delivery than in maternal blood (Wernze & Seki, 1972; Kotchen, Strickland, Rice & Walters, 1972).

This paper shows that plasma of the human newborn, in common with all other species so far studied, has high concentrations of angiotensin II at birth. Similar results have recently been found by C. Godard, U. Hafschmid, R. Gaillard & M. Vallotton (personal communication). Cord venous and cord arterial blood concentrations are almost identical in babies born after elective Caesarean section or vaginal deliveries in which the second stage of labour was short and are somewhat higher than the maternal concentrations. This suggests that although there is limited blood flow to the lungs in utero (Rudolph & Heymann, 1970), converting enzyme is present before birth. This may be in contrast to the chronically cannulated foetal lamb, in which concentrations of angiotensin II are similar in mother and foetus, whereas renin values are up to tenfold those of the mother (Broughton Pipkin, Lumbers & Mott, 1974b; Smith, Lupu, Barajas, Bauer & Bashore, 1974).

The differences in angiotensin II in the babies of group 1 and group 2 were small. There was, however, a considerable increase in concentration in both cord arterial and cord venous blood in those babies born to mothers with established hypertensive disease of pregnancy (group 3). This is, to our knowledge, the first time that the renin–angiotensin system has been studied in infants with reference to maternal blood pressure. The existence of a statistically significant relationship between maternal diastolic blood pressure and concentration of angiotensin II measured simultaneously in a group of primigravid women at term (Symonds, Broughton Pipkin & Craven, 1975) suggests that there may be alterations in the renin–angiotensin system of both mother and baby in hypertensive disease of pregnancy. It is not yet known whether these changes are a response to diminished utero-placental blood flow or antedate it.

It seems possible that the placenta itself may possess considerable converting enzyme activity, since in some cases the blood angiotensin II in cord venous was substantially higher than in the cord arterial blood. This was especially noticeable where there had been a prolonged second stage of labour, and may reflect increasing hypoxia. There is some evidence to suggest that in both the newborn human and the newborn mouse, alveolar hypoxia is associated
with significantly raised angiotensin converting enzyme activity (Mattioli, Zakheim, Mullis & Molteni, 1975) but conditions affecting such activity in the placenta are not known.

The high angiotensin II concentrations found in the cord venous blood of babies born to hypertensive mothers may also be related to altered placental metabolism of angiotensin I or II. It would seem to be very unlikely that maternal angiotensin II could contribute to foetal values, since there is a considerable foetal/maternal concentration gradient, and its transplacental passage has not, to our knowledge, been satisfactorily demonstrated.

Preliminary work in our laboratory with pregnant sheep and guinea pigs indicates that angiotensin II does not cross from the foetal to the maternal circulation intact but as one of the larger peptide fragments (F. Broughton Pipkin, N. Benjamin & C. Macallan, unpublished work).

The possibility must be considered that the placenta may itself be capable of synthesizing angiotensin II. Renin has been found in the placenta of various species (Stakemann, 1960; Gross, Schaechtelin, Ziegler & Berger, 1964; Bing & Faarup, 1966; Skinner, Lumbers & Symonds, 1968) and its synthesis has been shown in cultures of chorion, placenta and myometrium (Symonds, Stanley & Skinner, 1968). Uterine venous renin has also been shown to be higher than uterine arterial renin in women with toxaemia in two studies (Smith, Selinger & Stevenson, 1969; Kokot & Cekanski, 1972). Whether the placenta can also produce angiotensin II remains to be demonstrated.

A further possibility must also be considered. The antisera used in the radioimmunoassay of angiotensin II all cross-react to a considerable extent with the larger known angiotensin fragments (Table 1). Thus it is quite possible that the raised angiotensin II observed after a prolonged second stage of labour are in fact raised concentrations of one or more of the fragments. It has recently been shown that at least one of these fragments, the des-Asp'-angiotensin II, has considerable biological activity, especially at the adrenal cortex (Goodfriend & Peach, 1975; Freeman, Davis, Lohmeier & Spielman, 1976; Steele, Neusy & Lowenstein, 1976). It is fascinating to hypothesize that the placenta itself might be producing, and be a site for activity of, another such fragment.

The increased concentrations of angiotensin II found in babies born after a prolonged second stage of labour may be compared with the low values seen after elective lower-segment Caesarean section (Fig. 2). Similar marked differences in concentrations were found by Broughton Pipkin et al. (1974a) in lambs delivered vaginally and by Caesarean section. The mean cord venous concentration found after Caesarean section is still significantly higher than that found in non-pregnant adults (71.0 ± 8.8 pmol/l compared with 24.6 ± 4.6; \( P < 0.001 \)) but is very similar to the mean value of 58.4 ± 8.9 pmol/l found in 25 infants in the first week of life (Broughton Pipkin & Smales, 1975). It thus seems likely that some factor such as haemorrhage, which is known to stimulate the system in other species (Broughton Pipkin et al., 1974a, b), or hypoxia (Mattioli et al., 1975) or the other hormonal changes occurring during labour may be evoking an increase in circulating angiotensin II both from the foetus itself, since the cord arterial blood concentrations are high, and possibly from, or mediated by, the placenta. It is possible also that the rapidly changing placental blood flow
during delivery may result in changing concentrations of foetal angiotensin II.

Krauss (1970) found the blood pressure of 52 babies delivered by Caesarean section to be consistently lower than that of 125 babies delivered vaginally throughout the first 5 days of life. The immature young of some species appear to depend, at least in part, on the renin–angiotensin system for the maintenance of their arterial blood pressure (Broughton Pipkin, 1973) and it is tempting to postulate that this lower blood pressure may be associated with low concentrations of angiotensin II.

A decrease in angiotensin II and plasma renin activity with increasing body weight has previously been noted in other species (Broughton Pipkin et al., 1974a, b; Granger, Rojo-Ortega, Casado Perez, Boucher & Genest, 1971). In the human neonate plasma renin activity falls with increasing age (Mott, 1975; Dillon & Ryness, 1975). Whether this decline is in fact linked to increasing body size or, as is more likely, to maturation of the kidney and the mechanisms for sodium and water handling, remains unclear. Kidney function at birth differs markedly from that in the adult (Edelmann, 1967) and sodium conservation is relatively inefficient.

The renin–angiotensin system is intimately involved with sodium and water homeostasis in the adult (Guyton, Cowley & Coleman, 1972) and might well be stimulated to considerable activity by the immature functioning of the neonatal kidney. The high concentrations of angiotensin II observed after normal delivery thus appear to be functions both of naturally high 'basal' concentrations and increases, sometimes very large, superimposed during labour.

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References


