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References

Secretion of Atrial Natriuretic Peptide and Digoxin-like Immunoreactive Substance during Pregnancy

P. Shrivastav, D. S. Gill, V. D'Souza, P. M. S. O'Brien,1 and P. Dandona2

We investigated the secretion of atrial natriuretic peptide (ANP) and digoxin-like immunoreactive substance (DLIS) during pregnancy, labor, and the puerperium, as measured in maternal and umbilical cord plasma. There were no significant changes in maternal concentrations of ANP during all three periods, and the concentrations were similar to those found in nonpregnant controls and in umbilical cord plasma. Maternal concentrations of DLIS increased significantly in the second half of pregnancy, peaked during labor, and then decreased abruptly within 24 h of expulsion of the infant and placenta to values approaching the nonpregnant range. DLIS concentrations in umbilical cord plasma, however, were significantly higher than in maternal plasma during labor. The abrupt fall in DLIS in maternal plasma and the absence of a significant difference in DLIS concentrations between arterial and venous cord plasma suggest that, during pregnancy, the fetus, not the placenta, is the source of DLIS in maternal plasma.

Additional Keyphrases: umbilical cord blood · hypertension · newborns · radioimmunoassay · source of digoxin-like immunoreactive substance(s)

Normal pregnancy is associated with an increased intravascular volume to perfuse the expanded vascular bed. The increase is greatest late in the third trimester, and the puerperium is associated with substantial diuresis by the
third or fourth day postpartum (1). In healthy, nonpregnant subjects, increases in intravascular volume, salt intake, and blood pressure are associated with an increase in the concentrations of atrial natriuretic peptide (ANP) (2, 3) and digoxin-like immunoreactive substance(s) (DLIS) in plasma (4).

There have been conflicting reports regarding ANP secretion during pregnancy. Increased concentrations during pregnancy have been reported by Curson et al. (5), but a recent report by Rutherford et al. (1) contradicts this. Increased concentrations of ANP demonstrated during the puerperium have been hypothesized to be responsible for ridding the body of the excess sodium and water accumulated during pregnancy. Increased concentrations of DLIS have been reported in third-trimester maternal plasma, amniotic fluid, placental homogenates, and umbilical cord blood (6). DLIS concentrations in plasma of the neonate have also been found to be increased for a variable period after birth (7). Increased concentrations of both ANP and DLIS have been reported in pre-eclampsia (8, 9).

We conducted this study to investigate the concentrations of ANP and DLIS in plasma during normal pregnancy and early puerperium in the mother and in the cord blood of the newborn.

Materials and Methods

Atrial Natriuretic Peptide

This study was conducted in two parts, the first during pregnancy and the second during labor and the puerperium.

Pregnancy. We studied 29 women with uncomplicated pregnancies: 16 in the first half of pregnancy (less than 20 weeks of gestation) and 13 in the second half. After the woman had been semirecumbent for at least 30 min, 10 mL of blood was collected from an antecubital vein into tubes containing sodium EDTA (20 mg) and aprotonin (400 kallikrein units). Without delay, the blood was centrifuged at 4°C and the plasma removed and stored at −70°C until assay in batches. The control group comprised 12 age-matched nonpregnant healthy women. Informed consent was obtained from each woman.

Labor and puerperium. An additional eight women with normal full-term pregnancies and in the active stage of labor volunteered for the study. All had vaginal deliveries. At the end of the second stage of labor, maternal blood and cord-blood samples were collected and processed as above. A second sample of maternal blood was taken 24 (±6) h after the first. Two subsequent blood samples were taken at intervals of 24 h.

ANP estimation. We measured ANP by a radioimmunoassay adapted from that used by Sagnella et al. (10). To briefly describe the procedure: we extracted ANP from 5 mL of plasma on Sep-Pak C18 cartridges (Waters Associates, Milford, MA) that had previously been activated with 5 mL of methanol followed by 5 mL of distilled water. After washing the resin with 5 mL of distilled water, we eluted the adsorbed ANP with 4.5 mL of an 800 g/L solution of ethanol containing 40 g of glacial acetic acid per liter into glass tubes containing 100 μL of a 10 g/L solution of bovine serum albumin. The extracts were evaporated at 50°C under a stream of nitrogen and the dry residues were redissolved in 250 μL of phosphate/Triton buffer (per liter, 50 mmol of sodium phosphate, 10 mmol of sodium EDTA, 2 g of bovine serum albumin, and 1 g of Triton X-100, pH 7.4). Plasma ANP extraction efficiency was determined by using plasma supplemented with known amounts of 125I-labeled ANP and extracted as described above. Analytical recovery was 95% (SD 5%).

125I-Labeled human α-ANP (Amersham International, Bucks, U.K.) was dissolved in 1 mL of the assay buffer (phosphate/Triton; pH 7.4) and stored in aliquots at −20°C until assay. ANP antiserum raised in rabbits against synthetic human α-ANP (Amersham International) was also diluted in 12.5 mL of assay buffer. We incubated, overnight, 50-μL aliquots of extracted serum samples or standards at 4°C, in duplicate, with 50 μL of 125I-labeled ANP and 50 μL of the diluted antiserum solutions. The unbound 125I-labeled ANP was then separated by precipitation with dextran-coated charcoal. The supernate, containing the bound fraction, was then separated by centrifugation and counted for radioactivity (we used a "Selektronik" gamma counter). Standard curves were constructed from data on human α-ANP (Peninsular Laboratories, Merseyside, U.K.) dissolved in phosphate/Triton buffer to give concentrations of 1 to 600 pg per tube. The intra-assay CV for standards and samples was 9.6%, the interassay CV 12.5%.

Digoxin-like Immunoreactive Substance

This study was also conducted in two parts.

Pregnancy. Blood was sampled from an antecubital vein from another 14 women with uncomplicated pregnancies. Six were in the first half of pregnancy, eight in the latter half. Six age-matched, nonpregnant healthy women were the control group. The samples were collected and stored as described earlier for the ANP samples, and analyzed in batches for DLIS.

Labor and puerperium. Eight women with normal term pregnancies and in active labor were selected. Maternal blood and cord-blood samples were collected at the end of the second stage of labor and during the puerperium, as described for ANP. The plasma samples were stored and DLIS activity was estimated in batches.

Arterial and venous blood from umbilical cord. Because initial results showed that concentrations of DLIS in umbilical blood exceeded those in maternal blood, we considered the possibility that the fetus or the placenta, or both, might be responsible for the increased secretion of DLIS. Therefore, we collected arterial and venous cord-blood samples from an additional five newborns and analyzed the plasma for DLIS activity.

DLIS estimation. We measured DLIS by radioimmunoassay (RIAEN™ Digoxin; New England Nuclear, Dreieich, F.R.G.), using the procedure described by the manufacturer as modified by Wijdicks et al. (11). These modifications resulted in a lower limit of detection for DLIS: 0.03 μg/L.

Results

Atrial natriuretic peptide. Concentrations of ANP measured in 29 women with normal pregnancies (median: 2.5; range: 1.0–10.4 ng/L) did not differ significantly (by the Mann—Whitney two-tailed test) from those in the nonpregnant control group (n = 12; median: 3.2; range: 1.4–8.6 ng/L). Furthermore, ANP concentrations in the second half of pregnancy (n = 13; median: 2.4; range: 1.0–7.5 ng/L) did not differ significantly from those in the first half (n = 16; median: 3.0; range: 1.2–10.4 ng/L).

The concentrations of ANP in the mother's plasma at the end of the second stage of labor (n = 8; median: 1.9; range: 1.2–4.0 ng/L) were not significantly different from the concentrations during pregnancy. The concentration of ANP
in cord plasma (median: 2.5; range: 1.6–13.2 ng/L) was not significantly different from those in maternal blood at the end of the second stage. Similarly, there was no increase in ANP in the mothers during the postpartum period: day 1—median: 2.3; range: 1.3–5.6 ng/L; day 2—median: 2.0; range: 1.3–4.1 ng/L; and day 3—median: 2.8; range: 1.2–4.6 ng/L.

**Digoxin-like immunoreactive substance.** DLIS was undetectable in nonpregnant women and in pregnant women during the first half of pregnancy. Median DLIS concentrations in plasma in the second half of pregnancy were 0.13 μg/L (n = 8; range: 0.05–0.17 μg/L), a significant (P < 0.001) increase. DLIS concentrations during labor (n = 8; median: 0.18; range: 0.13–0.23 μg/L) were significantly (P < 0.01) higher than those in the second half of pregnancy.

The puerperium was associated with an abrupt decline in DLIS in the mothers’ blood. Within 24 h of delivery, plasma DLIS concentrations had dropped significantly (P < 0.001) to a median of 0.05 μg/L (range 0.03–0.06 μg/L) and remained at these concentrations during the following two days (Figure 1).

**Concentrations of DLIS in arterial and venous cord plasma.** DLIS concentrations in umbilical cord arterial plasma (n = 5; median: 0.45; range: 0.32–0.50 μg/L) did not differ significantly from those in cord venous plasma (n = 5; median 0.40; range: 0.37–0.57 μg/L). Both significantly (P < 0.002) exceeded those in maternal plasma.

**Discussion**

Evidently, during pregnancy the concentrations of ANP do not differ significantly from those in the nonpregnant controls. Pregnancy is associated with an increased intravascular volume, presumably to adequately perfuse the expanded intravascular compartment and the placental bed. Increased ANP secretion as a result of this increased intravascular volume would tend to negate this rise by causing natriuresis and diuresis. Possibly, the altered hormonal/metabolic milieu during pregnancy prevents ANP secretion in response to the volume expansion.

Acute stress has been shown to increase secretion of ANP in experimental animals (3). However, the stress of labor was not associated with an increase in ANP secretion, because the concentration of ANP in maternal plasma at the end of the second stage of labor was similar to that in pregnancy. ANP concentrations in cord plasma were not significantly different from those in maternal plasma.

ANP may have a diuretic role in the puerperium (1), but we did not observe an increase in the ANP concentrations during serial blood sampling over the first 72 h in the postpartum period, by which time the diuresis of the puerperium should have been well established. The role, if any, of ANP during normal pregnancy, labor, and the puerperium remains unexplained. Increased concentrations of ANP have been observed in pre-eclampsia (6), a condition associated with vasospasm and consequently a diminished intravascular volume. The significance of this observation remains unexplained.

Cusson et al. (5), using a radioimmunoassay with no extraction phase, demonstrated an increase in ANP concentrations during pregnancy. Their normal mean plasma ANP concentrations were 80 (SD 20) ng/L. Possibly they measured a peptide distinct from that measured by us, Sagnella et al. (10), and Rutherford et al. (1), because all of us found markedly lower normal concentrations of ANP in plasma.

Plasma DLIS concentrations appear to increase throughout pregnancy, peaking at the time of labor. However, within 24 h of delivery DLIS values for maternal plasma approach those seen in nonpregnant women. Cord plasma concentrations of DLIS exceeded those in maternal plasma. The abrupt decline in DLIS concentrations in maternal plasma shortly after delivery would indicate that the infant or the placenta, or both, are the source of this substance, and that it has a short biological half-life. The absence of any arteriovenous difference in the DLIS concentrations of umbilical cord plasma would exclude the placenta as a possible source of secretion. It is possible that the fetus is the source of this substance, which then spills over into the maternal circulation. DLIS has been demonstrated in placental homogenates (12). In the absence of any arteriovenous difference in its concentrations in umbilical cord plasma, it is possible that this reflects the DLIS content of the fetal and maternal blood contained within the placenta. Among other substances, progesterone and dehydroepiandrosterone sulfate, both abundantly present in cord blood, have been known to cross-react with digoxin-specific antibodies (13). However, the concentrations of these steroids required to interfere in the digoxin assay are several-fold those in cord serum (6).

Umbilical cord serum containing DLIS has been shown to inhibit the leukocyte sodium pump as measured by the ouabain-sensitive sodium efflux rate constant (14). Whether DLIS, like digoxin, has a positive inotropic effect on myocardial cells, and whether this contributes to the enhanced maternal cardiac output that normally occurs during pregnancy, remains to be established.

**Fig. 1.** DLIS concentrations in cord plasma (1) and maternal plasma during labor (2) and the puerperium

3 is day 1, 4 is day 2, and 5 is day 3 of the puerperium. By Mann-Whitney two-tailed test, the following differences are significant: for 1 vs 2, P < 0.002; for 2 vs 3, 4, or 5, P > 0.01
In conclusion: ANP concentrations do not increase in pregnancy, and there is no significant increase in ANP concentrations during the puerperium to account for the marked postpartum diuresis. dlIS, on the other hand, increases during pregnancy but abruptly declines postpartum. Umbilical cord plasma has high dlIS concentrations, and it is therefore possible that maternal dlIS is derived from the fetus.

References

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Development and Evaluation of an Automated Dye-Binding Assay for Protein in Cerebrospinal Fluid
C. M. Huang

Addition of sodium dodecyl sulfate (SDS) to Coomassie Brilliant Blue reagent equalizes the binding variability of the dye to various proteins and markedly improves the accuracy of quantification of protein in cerebrospinal fluid. In the presence of SDS, the absorption spectrum and the absorption maximum are affected by reaction time and temperature, age of the dye preparation, and protein constituent. I automated this procedure, to optimize precision, enable use of a smaller sample, decrease hands-on time, maintain consistency in the time of reading, and avoid carryover. The results (y) compared well with those of the curve (x), with a Deming de-biased regression equation of \( y = 0.991x + 14.1 \text{ mg/L} \), \( S_{xy} = 33.4 \text{ mg/L} \). The within-run and between-run precision (CV) was 2.5% and 4.5%, respectively. Commonly used antibiotics, fucosamine, or amphotericin B do not interfere. This automated procedure is fast and accurate and requires only 10 \( \mu \text{L} \) of sample.

Additional Keyphrases: centrifugal analyzer · turbidimetry compared

Analysis of cerebrospinal fluid (CSF) for protein is an important tool for the laboratory diagnosis of potentially serious disorders. At present, the most commonly used methods are turbidimetric techniques (1), in which sulfosalicylic acid, trichloroacetic acid, or alkalinized benzethonium chloride is used to produce turbidity. All of these procedures require large quantities of CSF and respond differently to different proteins (2). Among those attempting to find better techniques or to improve the efficacy for measuring the total protein concentration, McIntosh (3) applied the Bradford method of the Coomassie Brilliant Blue (CBB) binding (4) for quantifying protein concentrations in CSF. Results correlated rather poorly with those of the turbidimetric method (r = 0.65). Because the CBB dye showed wide variability in sensitivity to various proteins (5, 6), Johnson and Lott (7) recommended use of a reference standard in which 70% of the protein content was albumin and 30% was globulin; they used a bichromatic approach to extend the linearity of the method.

Macart and Gerbaut (8) substantially improved the dye-binding method for determinations of protein in CSF by adding sodium dodecyl sulfate (SDS) to the CBB reagent. The SDS equalizes the binding sensitivity of the dye to albumin, transferrin, and IgG. However, the absorption spectrum of the protein–dye complex may be unstable, owing to the dissociation properties of this detergent. Furthermore, interference by antibiotics is not well studied.

I optimized the CBB binding method and automated the measurement of total protein in CSF, using a centrifugal analyzer to eliminate carryover in the dye-binding method of analysis.

Clinical Pathology Department, Clinical Center, National Institutes of Health, Bethesda, MD 20892.
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