ATRIAL NATRIURETIC FACTOR CONCENTRATIONS DURING PREGNANCY AND IN THE POSTPARTUM PERIOD

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ABSTRACT

Atrial natriuretic factor (ANF) is a hormone that regulates fluid and electrolyte homeostasis. Increased intra-atrial pressure or atrial distention, which might occur secondary to intravascular volume expansion, stimulate the secretion of ANF by human atrial myocytes. During normal human pregnancy, there is a progressive increase in total intravascular fluid volume. Thus, we asked the following question: Does this physiologic adaptation to pregnancy result in an increase in ANF concentrations? Concentrations of α-human ANF (α-hANF) were measured by a specific radioimmunoassay in venous blood samples obtained longitudinally in the first, second, and third trimesters of pregnancy, during the intrapartum period, in the early postpartum period, and 6 to 8 weeks postpartum from 11 normal women who had no antepartum, intrapartum, or postpartum complications. Maternal circulating α-hANF levels were not different from those seen in the nonpregnant state. However, higher α-hANF concentrations were noted in the early postpartum period. Although the hypervolemia of normal pregnancy is not associated with higher α-hANF concentrations, other possibilities (such as increased ANF clearance, dilutional effects) need to be investigated. Finally, the etiology for the transient increase in α-hANF levels in the early postpartum period remains to be elucidated.

During normal human pregnancy, there is a progressive increase in both plasma and total extracellular fluid volume.[1] The mechanism that regulates this physiologic adaptation to pregnancy remains poorly understood. It is not known whether primary renal sodium and fluid retention results in an increase in plasma and total extracellular fluid volume or whether primary expansion of the capacity of the vascular compartment leads to renal sodium and water retention.

Atrial natriuretic factor (ANF) designates a family of peptide hormones secreted by specialized cells in atrial myocytes in a variety of species.[2] These peptides have been shown to be potent natriuretic, diuretic, and vasodilatory agents.[3] ANF levels are elevated in states of central hypervolemia and appear to be directly related to right atrial pressure.[4] We therefore asked whether maternal circulating ANF concentrations are increased during pregnancy compared with the nonpregnant, postpartum state (6 to 8 weeks). ANF concentrations were determined longitudinally in 11 normal pregnant women throughout their pregnancies and in the postpartum period. The results of this study are the subject of this report.

MATERIALS AND METHODS

The study was approved by the Clinical Research Practices Committee at the Bowman Gray School of Medicine. Eleven pregnant women were recruited to participate in this study. Maternal venous plasma was obtained at 10 to 12 weeks, 24 to 28 weeks, 34 to 38 weeks, during the intrapartum period, 2 to 3 days postpartum, and 6 to 8 weeks after delivery. These patients had no previous history of hypertension or renal disease and did not develop hypertension during their pregnancy. They were taking no

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medications except iron or prenatal vitamins. There were no antepartum, intrapartum, or postpartum complications. Selected demographic and laboratory parameters for these 11 patients are reported.

Blood Sampling

Blood samples were obtained between 9:00 AM and 1 PM. Intrapartum blood samples were obtained in the latent phase, between contractions, and prior to placement of conduction anesthesia. After at least 15 minutes rest in the left lateral decubitus position, approximately 8 ml of venous blood was obtained from a peripheral vein and was placed into chilled glass tubes containing disodium ethylene diaminetetraacetic acid (EDTA). The specimens were immediately centrifuged at 4°C and each plasma sample was divided into 1 ml aliquots that were kept frozen at −40°C. Under these conditions no significant loss of ANF was noted over a 6-month period. A few maternal venous samples were not obtained due to logistic problems.

Atrial Natriuretic Peptide Assay

ANF in the plasma samples was extracted by a procedure that was described by Brier et al[5] and validated in our laboratory.[6] One ml of plasma and 1.5 ml of 0.1 M acetic acid were mixed. Methanol (2.5 ml) was then added to the extraction tube. The latter was mixed for 10 minutes. The tubes were then centrifuged at 6000 rpm, at 4°C for 20 minutes. The supernatant was poured into a new tube containing 2.5 ml of chloroform and mixed with total inversion for 10 minutes. The tubes were again centrifuged as before. The top (aqueous) layer was removed to a new tube and lyophilized. The extracted samples were stored in −40°C until assayed. They were then allowed to thaw and reconstituted before the assay with 1 ml of 80 mM phosphate buffer (pH 7.4). Using this procedure recovery of unlabeled ANF was 50.9 ± 3.0%. Recovery of routinely monitored in every assay and was not dependent on the timing of the sampling.

Samples and standards were incubated for 24 hours at 4°C with 100 µl of anti α-hANF antibody (obtained from Dr. Kozo Hashimoto) in 80 mM phosphate buffer (pH 7.4). ^{125}I-α-hANF (5000 to 10,000 cpm/tube) was then added and the tubes were incubated for an additional 24 hours. The antigen-antibody complex was than precipitated using 100 µl of goat antirabbit immunoglobulin G (IgG) and 100 µl of normal rabbit serum. These tubes were then incubated for 2 hours at room temperature. Phosphate buffer (100 µl) was added and the tubes were centrifuged for 20 minutes at 1500 × g. The supernatant was decanted and the dried pellets were counted for 5 minutes in a gamma counter. Synthetic α-hANF (Peninsula Laboratories) (3 to 1000 pg/tube) was used to construct the standard curve. All samples were assayed in duplicate and the standards were assayed in triplicate. The interassay coefficient of variation ranged from 10 to 14% and the intra-assay variation was between 6 and 8%. Results were analyzed by utilizing a four parameter logistic equation.[7] The IC_{50} ranged from 40 to 50 pg/tube. The results are expressed as pg/ml and have been corrected for percent recovery; although the conclusions drawn from these results are unaffected by this correction.

Results are presented as the mean ± SEM, unless noted otherwise. Statistical analysis was carried out by analysis of variance with repeated measures test. Multiple comparisons were evaluated by Newmann-Keuls test. A $p < 0.05$ was considered significant.

RESULTS

Tables 1 and 2 show selected clinical and demographic parameters for the study population. All patients were delivered at term of appropriate for gestational age infants. As noted earlier, the patients' pregnancies were uncomplicated. None of these patients was hypertensive nor did they develop hypertension during pregnancy or in the postpartum period. Mean arterial pressure (MAP) in the second trimester was lower than in the early postpartum period. They gained weight at a normal rate. Their weight returned to prepregnant levels by 6 to 8 weeks postpartum. Finally, there was a transient decrease in hemoglobin and hematocrit (Hb/Hct) levels in the early postpartum period.

Figure 1 shows maternal plasma α-hANF concentrations during the antepartum, intrapartum, and postpartum periods. Mode of delivery did not affect α-hANF levels. There were no statistically significant differences between the values at various points in gestation and the nonpregnant state (6 to 8 weeks postpartum). However, α-hANF levels in the early postpartum period were higher than those observed in the first trimester. There were no correlations between α-hANF levels and MAP, maternal weight gain, or Hb/Hct levels.

DISCUSSION

In human pregnancy, one of the major physiologic adaptations is the expansion of intravascular and extracellular fluid volume. The underlying mechanisms responsible for this remarkable change are not clearly defined. Whether this physiologic hypervolemia is the result of primary renal sodium and water retention or whether it is seen in response to maternal vasodilation and increased intravascular capacitance is unclear. This study was undertaken to evaluate a volume-sensitive hormonal system relative to the marked changes in volume status that occur during pregnancy and in the puerperium. We measured maternal venous levels of α-hANF during pregnancy and

Table 1. Patients' Clinical Parameters

Number	11
Age (yr)	30.36 ± 1.26
Estimated gestational age (weeks)	40.41 ± 0.42
Gravidity	
1	5
≥2	6
Parity	
0	5
≥1	6
Mode of delivery	
Vaginal	9
Cesarean section	2
Anesthesia	
Conduction	5
Local/pudendal	4
General	1
None	1
Neonatal birthweight (gm)	3632.34 ± 219.03

Table 2. Maternal Weight, Mean Arterial Pressure (MAP), Hemoglobin, and Hematocrit

Time	*1st Trimester (A)*	*2nd Trimester (B)*	*Intrapartum (C)*	*Postpartum (2–3 days) (D)*	*Postpartum (6 weeks) (E)*
Weight (kg)*	141.4 ± 10.3	154.9 ± 9.6	169.6 ± 9.9		146.6 ± 9.7
MAP (torr)†	78.3 ± 2.4	72.6 ± 2.9	79.8 ± 3.6	86.0 ± 1.9	83.2 ± 2.7
Hemoglobin (gm/dl)‡	13.0 ± 0.3	11.7 ± 0.3	12.6 ± 0.2	11.1 ± 0.4	
Hematocrit (%)§	37.7 ± 0.8	34.6 ± 0.8	36.5 ± 0.4	31.4 ± 1.2	

*p = 0.0000 by ANOVA repeated measured test across time.
†p = 0.026 by ANOVA repeated measured test for E>B.
‡p = 0.002 by ANOVA repeated measured test across time and for A>B, A>D, C>B and C>D
§p = 0.0000 by ANOVA repeated measured test across time and for A>B, A>D, B>D and C>D

in the postpartum period, since ANF levels have been shown to reflect the volume status of the organism as perceived by atrial volume sensors.[3]

We did not measure intravascular volume nor did we measure intra-atrial pressures in our subjects. However, our assumption about the increase in intravascular volume in these normal pregnant women is well supported in the literature.[8] Longitudinal measurements of central hemodynamic data in normal pregnancy are not justified. Given these potential limitations, our results indicate that maternal plasma ANF concentrations did not significantly change during pregnancy; actually, values in the first trimester were significantly lower than those measured 2 to 3 days postpartum. However, pregnant α-hANF levels were not different from those observed in the nonpregnant state (6 to 8 weeks postpartum). Thus, no increase in the maternal plasma ANF concentrations accompanied the presumed increment in plasma and blood volume. It should be noted that these observations are in agreement with published cross-sectional studies in pregnant humans[9] and rats.[10] However, other studies in humans[11] have suggested an increase in circulating ANF concentrations at term. The reason for these conflicting observations is not known.

The results of this study demonstrate that circulating ANF concentrations do not rise during normal pregnancy. Perhaps the chronic nature of the extracellular volume expansion that occurs during pregnancy does not result in elevated levels of ANF. This is in contrast to the situations of acute volume expansion in nonpregnant women and sheep during which ANF levels increase significantly.[12,13] A dilutional effect on ANF levels from the physiologic volume expansion during pregnancy may be implicated. This could have masked a possible increased production of ANF. A third possibility is that the increased intravascular volume is not perceived by atrial volume sensors because the capacity of the maternal vascular compartment is similarly increased. Chronic volume expansion in this setting may not result in an appreciable response with regard to α-hANF levels.[13] Finally, an increased rate of clearance of ANF in pregnancy may be counterbalanced by the increased synthesis resulting in unchanged ANF concentrations. There is no practical or ethical means of studying ANF clearance in human pregnancies. Thus, further experiments in nonhuman models will be necessary to distinguish between these possibilities.

Interestingly, at 2 to 3 days postpartum, circulating ANF concentrations are somewhat higher than those seen during the first trimester of human pregnancy. The latter increase expands the observations in the rat pregnancy in which there is an immediate postpartum rise in ANF levels.[10] One possible explanation for this rise in ANF levels

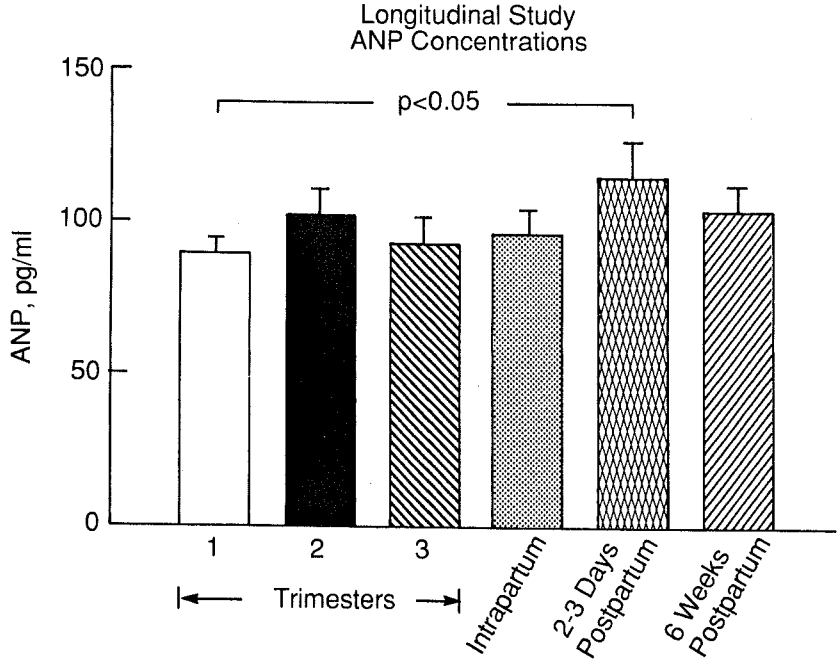

Figure 1. Maternal venous plasma atrial natriuretic factor (ANF) concentrations during the first (n = 11), second (n = 11), and third (n = 9) trimester of pregnancy, in the intrapartum period, and 2 to 3 days postpartum (n = 10) and 6 to 8 weeks postpartum (n = 9).

is that, in the immediate postpartum period, there is an acute increase in intravascular volume due to "autotransfusion."[8] This may be reflected in the decrease of Hb/Hct seen in our patients. In our study, we did not measure ANF concentrations in the immediate postpartum period. Autotransfusion is thought to be secondary to closure of the uteroplacental circulation. The latter, combined with the possible reversal of systemic vasodilation, may result in a functional decrease in the size of the maternal vascular compartment with secondary increase of circulating ANF levels. It is plausible that this sequence of events results in the facilitation of diuresis and natriuresis in the immediate postpartum period. Recent echocardiographic studies in postpartum women have shown a significant increase in atrial dimensions at 48 hours after delivery.[14] These observations could explain the transient rise in ANF levels in the early postpartum period.

We conclude that α-hANF concentrations during pregnancy are not different from those seen in the nonpregnant state. We would like to emphasize that this study is unique in that we report on α-hANF concentrations obtained longitudinally in the same group of normal pregnant women. This is a preferable and more sensitive experimental design than the cross-sectional studies that have been reported to date.

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