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Atrial Natriuretic Factor During the Perinatal Period: Equal Depletion in Both Atria[1]

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ABSTRACT. Data in the literature concerning atrial natriuretic factor (ANF) physiology in the fetus and newborn, although limited, suggest significant activity during the perinatal period. To characterize further ANF physiology during this time, we documented immunoreactive ANF (IR-ANF) concentrations in the right and left atria before and immediately after birth as well as in the hearts of immature and adult rats. There was a significant decrease in the concentration of IR-ANF in both right and left atria on the d before birth that persisted for the first 48 h of life [d 20 fetal right 570 (106, 90), left 580 (86, 75); d 21 fetal right 270 (70, 55), left 214 (117, 75); 1 d right 206 (39, 33), left 229 (41, 35); 2-d right 352 (35, 32), left 237 (26, 23) { geometric mean (upper SE, lower SE) in ng ANF/mg protein }]. Repletion of ANF stores began in the right atrium on d 2 of life and in the left atrium between d 2 and 5. The highest levels of IR-ANF were observed at d 15 [d 5 right 1439 (53, 51), left 1547 (83, 79); d 15 right 2034 (90, 86), left 1943 (108, 102); adult right 1380 (119, 109), left 963 (118, 105)]. In contrast to normal adult animals, factors mediating the observed change affect both atria equally during the perinatal period. The concentration of IR-ANF in the right and left atrium of the fetal, newborn, and immature animals was equal. These data document significant alterations in intraatrial IR-ANF concentrations in the perinatal period. The changes in tissue concentration reflect an alteration in the synthesis/release relationship that may be either a response to or evidence of involvement in the modulation of intravascular vol at the initiation of extrauterine life. (*Pediatr Res* 25:339–341, 1989)

Abbreviations

ANF, atrial natriuretic factor
IR-ANF, immunoreactive atrial natriuretic factor

ANF, a peptide synthesized and secreted by atrial myocytes, has potent natriuretic, diuretic, vasodilatory, and antimineralocorticoid properties (1–7). These characteristics suggest that ANF may play a significant role in salt and water homeostasis and in blood pressure control (1, 8).

Data in the literature concerning ANF physiology in the fetus and newborn, although limited, suggest significant activity during the perinatal period. IR-ANF is present in the fetal rat heart shortly after formation of the four chamber heart, and at 20 d of gestation, levels of IR-ANF in plasma are markedly higher than in the adult rat (9, 10). During late gestation of the fetal sheep, intravascular expansion causes an increased concentration of plasma IR-ANF (11). In human newborns, arterial cord plasma levels and venous plasma concentrations obtained during the first 4 d of extrauterine life are reported to be greater than plasma levels in adults (12–14). Unexpectedly, IR-ANF has also been found in high concentrations in the 20-d fetal rat ventricle, with a marked decrease occurring in the first few days of life (10, 15). The significance of ventricular ANF remains unclear.

To define more clearly ANF physiology during the perinatal period, we measured IR-ANF concentrations in the right and left atria before and immediately after birth as well as in the hearts of immature and adult rats. We then compared ANF levels between right and left atria and among age groups.

MATERIALS AND METHODS

Tissue harvest. Pregnant Sprague-Dawley rats at 20 and 21 d of gestation (term = 21–22 d), pups at 1, 2, 5, and 15 d of age, and adult nonpregnant female animals (Harlan, Indianapolis, IN) were killed by stunning and decapitation. After the hearts of the fetuses, pups, and adult animals were removed, individual atria were dissected free, frozen in liquid nitrogen, and stored at −70°C until extraction. Tissue from fetuses and pups was harvested from two litters at each age.

Extraction and recovery of ANF. Frozen tissue samples were partially thawed on ice then individually processed. We added 2 mL of hot (100°C) 1.0 M acetic acid to each specimen. The samples were homogenized for 60 s (Tissumizer, Tekmar, Cincinnati, OH), placed in boiling water for 10 min, cooled on ice, and centrifuged at 30 000 × *g* for 30 min at 4°C. An aliquot of the supernatant was lyophilized for subsequent RIA and the remainder stored at −70°C for protein determination and recovery. Protein concentration was measured by the method of Lowry *et al.* using BSA as the standard (Sigma Chemical Co., St. Louis, MO) (16).

Recovery of IR-ANF from the acid extraction procedure was quantified using extracts of both right and left atria from each age group. Selected samples were separated into two equal vol. A known amount of unlabeled ANF (two to three times greater than concentration present in the extract) was added to one portion and to an equal vol of RIA buffer. All extracts were processed as previously described, and the amount of ANF in the extracts and buffer samples was determined by RIA. Recovery was calculated as the difference between samples with and without added ANF, divided by the concentration of ANF in the buffer samples.

RIA. Lyophilized tissue extracts were reconstituted in RIA

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buffer (0.1 M sodium phosphate, 0.05 M NaCl, 0.1% BSA, 0.1% Triton X-100, 0.01% sodium azide, pH 7.4). RIA was performed using the double antibody method (17). Rabbit anti-hANF, ^{125}I-hANF, and α-hANF standard were obtained from Peninsula Laboratories (Belmont, CA). Goat antirabbit γ-globin was purchased from Antibodies Inc. (Davis, CA). The cross-reactivity of rabbit anti-hANF is 100% with rat ANF (Ile^{12} α-hANF), 100% with rat atriopeptin III, 5% with rat atriopeptin II, and 0% with rat atriopeptin I. The final dilution of ANF antibody was 1:120 000. The sensitivity of the assay in our laboratory is 2.6 pg/tube. The intraassay and interassay coefficients of variation are 7% ($n = 9$) and 11% ($n = 34$), respectively.

Analysis. To normalize the distribution of the data, a log transformation was performed before the analysis. To test for differences in IR-ANF concentrations at various ages and between the right and left atria, an ANOVA was performed using the Student-Newman-Keuls test for *a posteriori* comparisons. A paired t test was used to test for right-left differences at each age. The SAS (SAS Inc., Cary, NC) computer program package was used for data management and analysis. A value of $p < 0.05$ was considered significant.

RESULTS

The recovery of ANF in the acid extracts of the right and left atrial samples was determined (see Materials and Methods) in three samples from each atria at each time period. The range of the recoveries was 71.3% to 104.2%; the mean was 88.4 ± 2.3% ($\bar{X}$ ± SE). Reported concentrations of IR-ANF were corrected for mean recovery of α-hANF from the right and left atria at each age. Samples from both atria at each age diluted in parallel to the standard curve of the RIA as previously reported (9). Samples from either atria which did not produce values on the acceptable portion of the standard curve for either the protein determination or ANF RIA were excluded. The concentrations of IR-ANF [geometric mean (upper, lower)] in the right and left atria at each age are displayed in Figure 1.

Levels of IR-ANF decreased in both the right and left atria from d 20 to d 21 of gestation and remained lower than d 20 through the first 2 d of life (*). In the right atrium, the concentration rose between d 1 and d 2 (**), but there was no difference between the same ages in the left atrium. After d 2, the amount of IR-ANF increased in both. Levels in the right and left atria on d 5, the right and left atria on d 15, and the right atria of adults were higher than concentrations during the perinatal period (▲). In the adult left atria, however, the amount of IR-ANF was lower than the left atrial concentrations on d 15 (●). In addition, the IR-ANF level in the left atria of adults was not different from that of the 20-d fetus.

There was no difference in the concentration of IR-ANF between the right and left atria at the same age except on d 2 of life (■). In the adult animals, right atrial concentration was greater than left atrial concentration, but this difference did not reach statistical significance ($p < 0.0559$).

DISCUSSION

This study documents a significant decrease in the concentration of IR-ANF in both right and left atria on the d before birth that persists for the first 48 h of life. Repletion of ANF stores begins in the right atrium by d 2 and in the left atrium between d 2 and 5. The highest observed concentration of IR-ANF occurs on d 15. In contrast to normal adult animals, factors mediating the observed changes effect both atria equally during the perinatal period. The concentration of IR-ANF in the right and left atrium of the fetal, newborn, and immature animals is equal.

The decrease in the concentration of intraatrial IR-ANF on d 21 of gestation suggests that factors modulating the concentration of ANF are active before extrauterine life. The stimuli responsible for the change in ANF concentration in the fetal rat are unknown; however, data from the sheep and humans suggest that the recognized stimuli of ANF secretion in adults (elevated

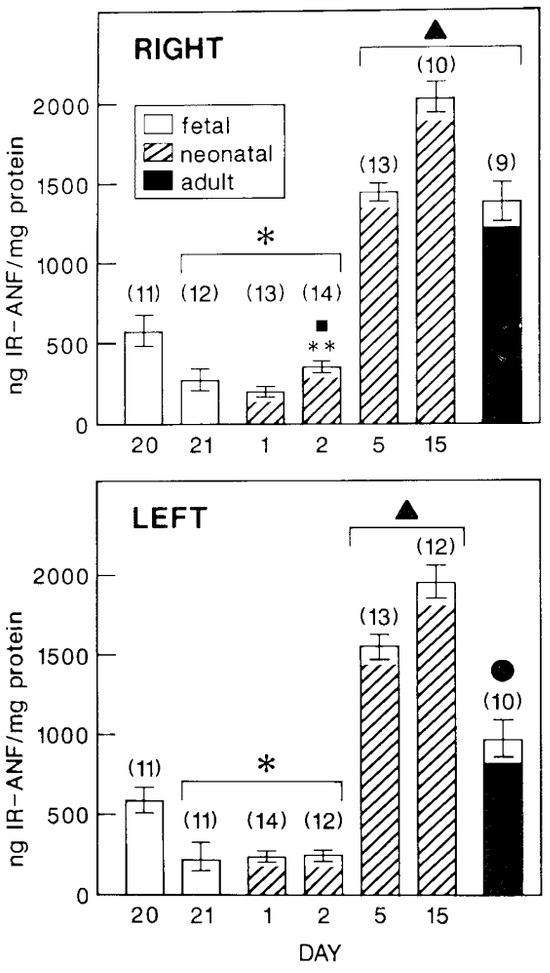

Fig. 1. Immunoreactive atrial natriuretic factor concentration in acid extracts from right and left atria at various ages. Geometric mean (upper SE, lower SE). SE are uneven due to log transformation. () = n; * = different than Day 20 to gestation; ** = different than day 1 of life; ▲ = different than day 20 fetal through day 2; ● = different than day 15; ■ = different than left atria of same age ($p < 0.05$).

intraatrial pressure or vol and increased heart rate) may be active in late gestation and the perinatal period (1). Exogenous vol expansion in late gestation of the fetal sheep stimulates ANF release (11). Despite the fact that physiologic intravascular expansion has not been shown to mediate a similar response, reports suggest that events before birth may be associated with net fluid shift into the intravascular space. Specifically, decreased lung fluid production has been documented in the lamb 2 d before delivery (18). In addition, increased intrauterine hydrostatic pressure associated with labor could expand the intravascular vol and increase ANF secretion as observed with water immersion therapy (19, 20). At birth, hemodynamic changes result in a rise in intraatrial pressure (20). Finally, marked alterations in heart rate are associated with uterine contractions during labor (20). Thus, the known stimuli of ANF release may influence intraatrial ANF concentration in the perinatal period.

Normal adult animals respond to physiologic and pharmacologic stimuli by the release of ANF from the right atrium (21–24). A similar conclusion was reached concerning the fetal rat. Investigators administered indomethacin to rat dams on d 20 of gestation and subsequently documented a decrease in the concentration of ANF in right fetal atrium but no change in the left fetal atrium (10). The response was presumed secondary to vol and pressure changes in the right atrium associated with closure of the ductus arteriosus (10). In the present study, the equal response of both atria suggests that the aforementioned changes

in blood flow and pressure are not the sole mediators of depletion of intraatrial ANF in the perinatal period. Either the fetus and newborn respond differently than adults to the known physiologic stimuli of ANF release or other, as yet unrecognized, factors are operative only during the perinatal period that result in an equal response of both atria.

Repletion of ANF stores began in the right atrium on d 2 of life and in the left atrium between d 2 and 5. This suggests that the factors stimulating the depletion of ANF in both atria are less effective or less active on d 2 in the right atrium or that the synthetic capability of the right atrium is greater than the left atrium. Others have reported repletion of atrial ANF by d 3 of life (10, 25). Although these reports did not assess the concentration in the right and left atria separately, all of the data support a rise in atrial ANF concentration between d 3 and 5 of life to a level greater than in the 20-d fetus.

The highest observed concentration of intraatrial ANF was observed on d 15. We have previously reported an identical pattern in both the Dahl salt-sensitive and salt-resistant animal at the same age (26). The significance of this pattern is not understood.

A number of investigators have reported a greater concentration of ANF in the right than in the left atrium in adult animals (22–24). Studies of the 20-d fetal rat and the 3-wk-old spontaneously hypertensive rat, however, found no difference in the concentration of ANF between the atria (10, 23). Although analysis of the present data in adult animals generated a p value that did not quite reach statistical significance, this study supports and extends the previous findings of equal ANF concentrations in both atria in fetal, newborn, and immature animals.

The concentration in the 20-d fetal rats is three times greater than we have previously reported (9). The only known differences between the experiments are that the measurements in this study were obtained in each atrium from individual animals *versus* the atria taken as a whole in pooled litter mates.

Due to technical difficulties in harvesting blood from the fetal and newborn animals, we are unable to report simultaneous plasma ANF concentrations. Others, however, have recently reported an identical pattern of change in atrial ANF concentration in the fetal and newborn rat associated with a significant increase in plasma ANF concentration on d 21 of gestation that was maintained for the first 3 d of life (27). This report is consistent with our data and suggests that the decrease in ANF concentration is a result of greater release than synthesis in the perinatal period.

To conclude, this study documents a significant nadir in the concentration of IR-ANF in the right and left atria during the perinatal period with the highest value observed in immature rats. In contrast to normal adult animals, factors mediating the observed change affect both atria equally. The data support a potential role for ANF in the modulation of intravascular vol at the initiation of extrauterine life. Additional studies are required to explore the relative contribution of changes in ANF synthesis and release during this period and why the perinatal atria respond in concert.

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