Plasma atrial natriuretic peptide and spontaneous diuresis in sick neonates

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SUMMARY Plasma concentrations of immunoreactive human atrial natriuretic peptide (human ANP) were sequentially determined in 12 infants with respiratory distress syndrome (RDS) or meconium aspiration syndrome (MAS) during various phases of diuresis to elucidate the role of human ANP in the occurrence of spontaneous diuresis in the newborn. Plasma immunoreactive ANP concentrations during the diuretic as well as the maximum diuretic phase were significantly ($p < 0.001$) higher than during the prediuretic phase. A gradual decrease occurred during the post diuretic phase, returning to prediuretic values after one week of life. Significant natriuresis, increased glomerular filtration rate, mild hyponatremia, and decreased blood pressure were observed in the diuretic phase in all the cases studied. These results suggest that hypersecretion of human ANP may play an important part in initiating spontaneous diuresis in sick neonates.

Spontaneous diuresis is often delayed in ill neonates, particularly in those who have suffered birth asphyxia and those with respiratory distress syndrome (RDS).¹ Failure to initiate spontaneous diuresis is related to the degree of severity of RDS or meconium aspiration syndrome (MAS).² Despite extensive studies on postnatal renal adaptation, the physiological importance of the renal function responsible for this diuresis has not yet been fully understood.³ Recently, atrial natriuretic peptide (ANP), with potent natriuretic and diuretic activities, has been isolated from rat and human atrial tissues.⁴ It has been reported that an intravenous infusion of synthetic human ANP causes a dramatic increase in sodium excretion and urinary volume in animals as well as in man.⁵ The physiological role of endogenous human ANP in the newborn, however, remains unknown. The purpose of this study was to clarify the role of human ANP in the incidence of spontaneous diuresis in relation to the renal function in neonates with RDS or MAS.

Patients and methods

Twelve neonates (nine boys and three girls), who were transferred to the neonatal intensive care unit, Kansai Medical University Hospital, for treatment of RDS ($n=5$) or MAS ($n=7$), were studied during the first four days of life. Age at admission was 3.2

(SD 4.4) hours. Informed consent was obtained from each patient's parents. The mean gestational age and birth weight of the infants was 35.5 weeks (26–41 weeks) and 2208 g (1040–4030 g), respectively. Nine infants were ventilated with a Bourns BP 2001 respirator. The mean volume of fluid administered (ml/kg/day) was 61.8 (14.2) on day 1, 68.3 (17.6) on day 2, 84.5 (17.4) on day 3, and 104.3 (19.4) on day 4, respectively. The fluids consisted of 10% glucose supplemented with calcium (100 mg/kg/day) until the third day of life, after which sodium chloride (35 mmol/l) and potassium chloride (20 mmol/l) were added. All fluid intake was administered parenterally. The mean fluid intake over each eight hour period was calculated from a fluid balance chart monitored every hour. Urine was collected in a collecting bag for all male infants and one female infant so as to measure directly the volume. In two other female infants the urine volume was calculated by weighing nappies immediately after voiding. Urine was consecutively collected during the first four days of life: onset of diuresis was defined as urine output greater than 1 ml/kg/hour, and the maximum urine output as the greatest output during the first four days of life by checking the urine volume every hour. The diuretic phases were arbitrarily divided into four eight-hour periods: the first (prediuretic) phase was defined as the beginning of urine collection just after admission

at 3.0 (4.7) hours of age; the second (diuretic) phase as the onset of diuresis with urine volume greater than 1 ml/kg/hour occurring at 17.5 (11.2) hours of age; the third (maximum diuretic) phase as the maximal urine output following the diuretic phase at a mean age of 43.3 (18.3) hours; the fourth (post diuretic) phase as 24 hours after maximum diuretic phase at a mean age of 67.3 (18.3) hours. The arterial pressure was measured every 30 minutes in the right arm using the Doppler ultrasound technique throughout the study.

Blood samples were obtained at the mid point of urine collections to determine human ANP, creatinine, urea, nitrogen, glucose concentrations, and electrolytes. Urine collected from all male infants and one female infant were pooled for the determination of osmolality, urinary sodium, potassium, and creatinine concentrations to calculate fractional excretion of sodium (FENa) and urine:plasma osmolar ratio in each phase. Creatinine clearance (Ccr) FENa, osmolar clearance (Cosm), and free water clearance (CH₂O) were determined using standard formulas shown below:

$$\text{FENa (\%)} = \frac{\text{UNa} \times \text{SNa}}{\text{Ucr} \times \text{Scr}} \times 100, \text{ Ccr (ml/minute)} = \frac{\text{Ucr} \times \text{V}}{\text{Scr}}$$

$$\text{Cosm (ml/minute)} = \frac{\text{Uosm} \times \text{V}}{\text{Posm}}, \text{ CH}_2\text{O (ml/minute)} = \text{V} - \text{Cosm}$$

Where V indicates urine flow (ml/minute); SNa, serum sodium concentration (mEq/l); UNa, urinary sodium concentration (mEq/l); Scr, serum creatinine (mg/dl); Ucr, urinary creatinine (mg/dl); Posm, plasma osmolality (mOsm/l); and Uosm, urinary osmolality (mOsm/l).

To determine the sodium balance for each phase sodium intake was calculated from the sodium content of fluids, medicine, and blood products administered. Sodium output was measured in a pooled urine sample for each phase.

Determinations of creatinine, urea, nitrogen, and glucose concentrations, and electrolytes in serum and urine were performed using an autoanalyser (Beckman Astra-8), and urine osmolality was measured by freezing point depression (Fiske OR osmometer).

Radioimmunoassay of human ANP. Plasma (0.5 ml) was extracted by ODS cartridges (Sep-Pak, Waters Associates, Milford, Minnesota), as reported.⁶ Radioimmunoassay of human ANP was performed by using an antibody generated in rabbits immunised with human α -ANP¹⁻²⁸ (Peptide Institute, Osaka, Japan) coupled to bovine thyroglobulin by carbodi-imide. The antibody cross reacted fully with α -human ANP¹⁻²⁸, rat (r) ANP¹⁻²⁸ (40%), but less

with α -human ANP⁷⁻²⁸ (2-5%), rANP⁵⁻²⁸ (atrio-peptin III), rANP⁵⁻²⁷ (atrio-peptin II), and rANP⁵⁻²⁵ (atrio-peptin I), and not at all with angiotensin II, arginine vasopressin, nor bradykinin. The assay buffer was 50 mM phosphate buffered saline (pH 7.4), containing 0.1% bovine serum albumin, 1 mM edetic acid, 0.1% triton X-100, and 500 U/ml aprotinin. The incubation mixture, which consisted of 0.1 ml standard human α -ANP or plasma extract and 0.1 ml antibody (final dilution—1/10 000), was preincubated at 4°C for 24 hours, followed by the addition of 0.1 ml ¹²⁵I-labelled α -human ANP (Amersham International, Tokyo, Japan). After additional incubation at 4°C for 24 hours, bound ligands were separated from free ligands by the double antibody method. The lowest concentration of human ANP yielding a binding significantly different from that found in the absence of standard α -human ANP at the 95% confidence interval was 3 pg/tube, and the 50% intercept was 20 pg/tube. The minimum detectable concentration of immuno-reactive human ANP in plasma was 30 pg/ml. The interassay and intraassay coefficients of variations were less than 10%. The recovery of immunoreactive human ANP applied to Sep-Pak cartridge was 50-60%. All values were corrected for the recovery during the extraction procedure.

Statistical analysis was performed by Student's *t* test or Welch's *t* test following the F-test. Data were presented as mean ($\pm$ SD).

Results

The Figure shows that the mean plasma concentration of immunoreactive human ANP was significantly ($p < 0.001$) higher during the diuretic phase (305.4

Figure Sequential changes of plasma human ANP concentrations and urine volume in newborn infants with RDS or MAS. Open circles indicate mean plasma concentrations of immunoreactive human ANP and closed circles indicate mean urinary volume during various diuretic phases; bars show standard deviation.

(208.7) pg/ml) and the maximum diuretic phase (314.0 (107.2) pg/ml), than during the prediuretic phase (80.6 (40.2) pg/ml), and that it gradually decreased during the post diuretic phase (168.4 (120.9) pg/ml), returning to prediuretic concentrations after one week of age (62.7 (27.4) pg/ml). Plasma concentrations of immunoreactive human ANP during the prediuretic phase and after one week of age were comparable with those of normal cord blood (61.3 (22.5) pg/ml; $n=7$) and those of healthy adults (40 (20) pg/ml; $n=20$). There were significant ($p<0.01$) inverse correlations between immunoreactive human ANP concentrations and plasma osmolality ($r=-0.62$), or mean arterial blood pressure ($r=-0.58$), in the prediuretic and diuretic phases, while plasma human ANP concentrations did not correlate with body weight nor serum sodium concentrations.

Tables 1 and 2 show the studies of renal function during each phase. The increased urine output during

the diuretic phase was associated with a significant increase of FENa ($p<0.01$). There were also significant increases in creatinine clearance ($p<0.05$) and free water clearance ($p<0.01$) during the maximum diuretic phase. The serum sodium concentrations decreased slightly during the diuretic phase. Output: intake ratio was significantly decreased during the prediuretic phase ($p<0.01$). Systolic and diastolic blood pressure were slightly decreased during the diuretic phase, but returned to the basal values during the maximum diuretic and post diuretic phases. Pulse rate was unchanged throughout the study.

Discussion

This study clearly shows that an increase of plasma immunoreactive human ANP concentrations coincided with the occurrence of spontaneous diuresis in neonates with RDS or MAS. Plasma immuno-

Table 1 Mean (SD) changes in body weight, plasma atrial natriuretic peptide (ANP), serum sodium, blood pressure, and pulse rate during various phases of diuresis in newborn infants with RDS or MAS

Variable	Phase			
	Prediuretic	Diuretic	Maximum diuretic	Post diuretic
Age (hours)	3.0 (4.7)	17.5 (11.2)	43.3 (18.3)	67.3 (18.3)
Bodyweight (gm)	2145 (930)	2223 (901)	2143 (887)	2089 (883)
Plasma human ANP (pg/ml)	80.6 (40.2)	305.4 (208.7)*	314.0 (107.2)†	168.4 (120.9)
Serum sodium (mmol/l)	139 (3)	136 (4)	137 (3)	138 (3)
Arterial pressure (mm Hg)				
Systolic	59 (6)	55 (4)	60 (6)	64 (7)‡
Mean	47 (5)	45 (4)	49 (6)	53 (7)
Diastolic	36 (4)	34 (2)	38 (5)¶	40 (5)‡
Pulse rate (beat/minute)	153 (17)	149 (18)	143 (10)	139 (6)

† $p<0.001$, * $p<0.01$ v prediuretic phase, respectively.

‡ $p<0.01$, ¶ $p<0.05$ v diuretic phase, respectively.

Table 2 Mean (SD) changes in renal function tests and fluid and sodium balance during various phases of diuresis in newborn infants with RDS or MAS

Variable	Phase			
	Prediuretic	Diuretic	Maximum diuretic	Post diuretic
Creatinine clearance (ml/minute)	0.97 (0.64)	1.68 (1.40)	2.29 (1.5)‡	2.40 (1.70)‡
Fractional excretion of sodium (%)	0.45 (0.45)	7.1 (6.8)*	5.6 (4.5)*	4.3 (4.7)‡
Osmolality (mOsm/kg)				
Plasma	288 (6)	287 (6)	288 (10)	289 (7)
Urine	520 (285)	225 (87)*	187 (92)*	248 (125)*
Free water clearance (ml/minute)	-7.4 (1.4)	2.2 (3.9)†	143 (119)**¶	7.3 (5.1)*
Fluid balance (ml/kg/hour)				
Intake	2.6 (1.0)	2.6 (0.9)	3.6 (1.3)§	4.1 (1.2)*
Output	0.3 (0.2)	1.8 (0.7)†	5.6 (1.3)**¶	3.2 (1.4)†
Output/intake (%)	13 (8)	81 (43)†	172 (67)†¶	94 (38)†
Sodium balance (mmol/kg/hour) ($\times 10^3$)				
Intake	0	70 (140)	800 (800)	1200 (1200)
Output	70 (80)	1400 (1100)*	2660 (1440)†	3250 (1700)†

† $p<0.001$, * $p<0.01$, ‡ $p<0.05$ v prediuretic phase, respectively.

¶ $p<0.01$, § $p<0.05$ v diuretic phase, respectively.

reactive human ANP concentrations increased during the diuretic and maximum diuretic phases, followed by a gradual decrease during the post diuretic phase, and returned to prediuretic values after one week of age. These changes were observed not only in mature infants, but also in premature infants. Plasma immunoreactive human ANP concentrations were almost the same in mature and premature sick neonates. These results suggest that human ANP is normally secreted in neonates, irrespective of maturity.

The major renal effect of human ANP in adults is an increase in glomerular filtration rate which does not affect renal blood flow but shifts the glomerular balance toward natriuresis.⁷ In fact, a noticeable natriuresis and an increase in glomerular filtration rate were observed during spontaneous diuresis in all cases studied. It has recently been reported that secretion of endogenous human ANP stimulated by the extracellular volume expansion is responsible for the observed natriuresis and diuresis.⁸⁻¹⁰ The mechanism by which human ANP is secreted in the newborn has not yet been established. As fluid restriction is normally used to treat patients with respiratory problems volume expansion has been considered to be less common in the newborn period. Costarino *et al.*, however, showed that the diuresis in patients with RDS is a water diuresis resulting from an endogenous water overload, and that spontaneous diuresis after birth represents the infant's physiological response to extracellular water load.³ In this study positive water balance was observed in the prediuretic phase, and negative correlation between plasma human ANP concentrations and plasma osmolality was also observed. Therefore, extracellular volume expansion induced by the relative overhydration and the endogenous water overload may lead to the release of human ANP in the newborn period. Furthermore, it has been shown that plasma renin activity and angiotensin II concentrations are increased during the newborn period,¹¹ causing an increased extracellular volume in the neonatal period. Taken together, it seems possible to speculate that the extracellular volume expansion in the early neonatal period may

trigger release of human ANP through the distension of atrial wall or the increase of intra-atrial pressure or both. We speculate that endogenous human ANP, possibly stimulated by an extracellular volume overload, is one humoral factor responsible for the spontaneous diuresis in sick neonates.

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