

NOTE

Changes in Plasma Atrial Natriuretic Peptide during Hemodialysis: Mechanism of Elevated Levels in Patients with Chronic Renal Failure

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Abstract. An elevated plasma atrial natriuretic peptide (ANP) concentration was observed in patients with chronic renal failure, and it was significantly ($P<0.01$) decreased by hemodialysis with or without fluid removal. The ANP concentration was decreased by dialysis without fluid removal and the decrease was significantly ($P<0.01$) correlated with the pre-dialysis value. The decrease in this peptide during fluid removal without diffusion was significantly ($P<0.05$) correlated with the decrease in the circulating plasma volume measured by dye dilution. The decrease in the ANP level was therefore considered to be related to a fall in right atrial pressure caused by the decline in the circulating plasma volume. The circulating plasma volume, osmotic pressure, and blood pressure were not changed by hemodialysis without fluid removal. Moreover, the filtrate concentration of ANP (mean: 5.5 pg/ml) during fluid removal without dialysis was only about 8% of the plasma level, so filtration of ANP from the dialyzer was negligible. The decrease in ANP during hemodialysis without fluid removal may therefore have been caused by the removal of or a change in the level of substances, for example electrolytes, epinephrine, and uremic toxins. In addition to volume expansion, such a substance(s) might influence plasma ANP levels in patients with chronic renal failure.

Key words: Atrial natriuretic peptide, Chronic renal failure, Hemodialysis, Circulating plasma volume.
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THE PLASMA concentration of atrial natriuretic peptide (ANP) is reported to be increased in patients with chronic renal failure (CRF) [1, 2]. ANP secretion is stimulated by stretching [3] of the atrial muscle or an increase in the right atrial pressure [3, 4]. In addition, ANP secretion is stimulated by vasopressin, angiotensin II and phenylephrine in rats [5, 6], and steroid and thyroid hormones have also been found to stimulate ANP secretion in some *in vitro* experiments [7, 8]. Moreover, Gibbs *et al.* [9] have reported that extracellular osmolality was one of the factors modulating ANP secretion. In patients with CRF,

however, many factors other than an increase in plasma volume may be involved and the principal determinants of the ANP level are still unknown.

To investigate the factors regulating ANP secretion in CRF in addition to the change in right atrial pressure, we therefore compared the changes in plasma ANP induced by hemodialysis (HD) with or without fluid removal and fluid removal without HD.

Materials and Methods

Subjects

Twenty CRF patients on HD, 12 males and 8 females aged 34–84 yr (mean \pm SEM: 58 \pm 8 yr), were studied. The cause of CRF was diabetic

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nephropathy in 8 patients, chronic glomerulonephritis in 11 patients, and primary nephrotic syndrome in 1 patient. None of them had severe congestive heart failure, valvular heart disease, arrhythmia, liver disease, or endocrine disease. The patients had been on dialysis between 1 and 20 months (mean \pm SEM: 3.9 ± 3.1 months) and received regular HD for 4 h 2–3 days per week. Sodium intake was restricted to about 7 g/day.

Hemodialysis procedure

HD was carried out with TAF-08 or TAF-10 hollow fiber dialyzers (Teijin, Japan). The membrane was made of saponified cellulose and the total membrane area of these dialyzers was 0.8 m² and 1.0 m², respectively. After 20 min of bedrest, the patients received HD without fluid removal for 1 h and then received fluid removal without diffusion for the following 1 h. Standard HD with removal of 0.7–1.6 kg of fluid was then performed for 2 h. During HD, blood flow was kept at 200 ml/min and the dialysate flow rate was set at 500 ml/min. HD without fluid removal was controlled with an automatic bedside console (DCS-22, Nikkiso, Japan), and fluid removal without diffusion was done by performing extracorporeal ultrafiltration during which the dialysate was not drained into the dialyzer. The dialysate contained 135 mEq/l Na⁺, 2.5 mEq/l K⁺, 107 mEq/l Cl⁻, and 30 mEq/l HCO₃⁻ without glucose.

Measurements

Blood samples were taken from the arterial blood access site of the 13 patients just before HD as well as 60, 120, and 240 min after the initiation of dialysis. Twenty milliliters of filtrate was collected during fluid removal without diffusion. Plasma samples were taken not only at the arterial but also venous blood access sites 120 min after the start of HD in the other 7 patients.

Body weight was measured with a scale bed. Plasma and filtrate concentrations of sodium, potassium, chloride, urea nitrogen and creatinine were measured with an autoanalyser. Hematocrit was determined by the centrifugation method and the plasma and filtrate osmotic pressures were measured by the freezing point method. Plasma ANP concentrations were measured by a previously described radioimmunoassay [10]. ANP levels in

the filtrate were measured as follows. After washing a Sep-pak C-18 cartridge (Waters Associates, Milford, MA) with 0.1% acetic acid in ethylhydroxide and water, the filtrate was passed through the column and 2 ml of 0.1% acetic acid in ethylhydroxide was used for elution. The eluate was evaporated, reconstituted with 0.6 ml of the assay buffer, and measured by the same radioimmunoassay as was used for plasma [10].

In 8 out of the 13 patients, the circulating plasma volume (CPV) was measured by the dye dilution method [11, 12]. Five milligrams of indocyanine green was dissolved in 5 ml of distilled water and rapidly injected into a peripheral vein. The dye dilution curve was then obtained by means of the earpiece densitometer [11, 12] and a cardiac output computer (MLC-4100, Nihon Denken, Japan). The end-tail density was read from the curve and CPV was calculated with the following formulae:

$$1) \text{TBV} = 5 / \text{De}$$

$$2) \text{CPV} = \text{TBV} \times (100 - \text{Ht}) / 100$$

Where TBV is total blood volume (L), De is end-tail density (mg/dl), CPV is circulating plasma volume (L), and Ht is hematocrit (%).

Statistical analysis

The differences in the changes in the mean values for the two parameters were analyzed by Student's *t*-test for paired data. Results are expressed as the mean \pm SEM.

Results

Body weight was found to not change for the first 60 min of HD without fluid removal (Table). However, it was significantly ($P < 0.01$) decreased by fluid removal without diffusion, and was further decreased by HD with fluid removal ($P < 0.01$, Table). Systolic and diastolic blood pressure decreased, but the change was not significant (Table).

The urea nitrogen, creatinine, and potassium concentrations were significantly ($P < 0.01$) decreased by HD with or without fluid removal, but they were not significantly changed by fluid removal without diffusion (Table). Plasma sodium was significantly increased by HD without fluid removal, but it was not changed thereafter by fluid removal without diffusion and HD with fluid

Table. Changes in various parameters during hemodialysis

	Blood				Filtrate
	Before	60 min	120 min	240 min	
Body weight (kg)	47.3±2.5	47.3±2.5	46.7±2.5**	46.4±2.0**	
Systolic BP (mmHg)	151±4	146±5	148±6	141±6	
Diastolic BP (mmHg)	77±3	72±5	76±3	74±3	
Urea nitrogen (mg/dl)	62.6±5.9	47.0±4.5**	47.8±4.5	32.4±3.7**	46.1±4.9
Creatinine (mg/dl)	8.7±0.9	6.9±0.8**	7.0±0.8	4.8±0.6**	6.7±0.7
Na (mEq/l)	135±1	137±1*	137±1	138±1	137±1
K (mEq/l)	4.7±0.2	4.3±0.1**	4.3±0.1	3.7±0.1**	4.2±0.3
Cl (mEq/l)	101±2	102±1	102±1	102±1	101±1
Hematocrit (%)	20.8±0.7	20.8±0.7	21.2±0.8	21.5±0.9	
Osmotic pressure (mOsm/l)	303±2	304±2	299±3	293±3*	300±3
CPV (ml)	4230±400	4120±410	3350±320**	2470±100**	
ANP (pg/ml)	109±15	79±11**	56±7**	41±6**	5.5±1.0**

BP, blood pressure; CPV, circulating plasma volume; ANP, atrial natriuretic peptide. Hemodialysis (HD) without fluid removal was performed for the first 60 min. Fluid removal without diffusion and standard HD with fluid removal were then performed for the subsequent 60 and 120 min, respectively (see text). The values were compared with those obtained just before each period. Filtrate concentrations were compared with the plasma levels 60 min after the start of HD. Values are presented as the mean±SEM (*, $P<0.05$; **, $P<0.01$).

removal. Plasma chloride and hematocrit levels did not change significantly, and plasma osmotic pressure was significantly decreased only at the end of normal HD (Table). CPV was fairly constant during HD without fluid removal, but then it showed a gradual decrease (Table).

The mean plasma ANP concentration before HD (109 ± 15 pg/ml) was significantly ($P<0.01$) higher than that in normal controls (39 ± 9 pg/ml) [13]. There was no significant correlation of ANP levels with age, sodium or potassium concentration, systolic and diastolic blood pressure, or the disease underlying CRF. The ANP level was significantly reduced by HD without fluid removal to 79 ± 11 pg/ml ($P<0.01$, Table), and was further decreased to 56 ± 7 pg/ml by fluid removal without diffusion. ANP levels at the end of the study period were within the normal range in 8 out of 13 patients and the mean value (41 ± 6 pg/ml) was significantly lower than at any other time ($P<0.01$, Table).

The decrease in ANP during HD without fluid removal was not correlated with the changes in plasma sodium and creatinine levels or plasma osmotic pressure. The magnitude of the decrease in the ANP concentration during HD without fluid removal showed a significant correlation with the pre-HD value (Fig.). On the other hand, the decrease in ANP during fluid removal without

diffusion was significantly correlated with the decrease in CPV (Fig.). There were no significant differences between the plasma ANP concentrations in arterial (96 ± 30 pg/ml) and venous (94 ± 30 pg/ml) blood access sites.

Mean filtrate concentrations of sodium, potassium, chloride, urea nitrogen, and creatinine did not differ significantly from those in plasma. Filtrate osmotic pressure also did not differ significantly from that of plasma. The filtrate ANP concentration ranged from 1.7 to 9.9 pg/ml (mean: 5.5 ± 1.0 pg/ml) (Table). The mean filtrate / plasma ANP concentration ratio was 0.08 ± 0.02 , which was far lower than the ratios for urea nitrogen and creatinine.

Discussion

The present study showed that the plasma ANP concentration is high in CRF patients receiving HD, a finding which agrees with previous reports [1, 2, 13]. This increase in ANP in CRF patients has been considered to be due to an increase in right atrial pressure [1, 2] consequent on volume expansion. In addition, we [13] have previously reported that the metabolism of plasma ANP is slower in CRF patients. On the other hand, Marumo *et al.* [14] reported that renal failure per

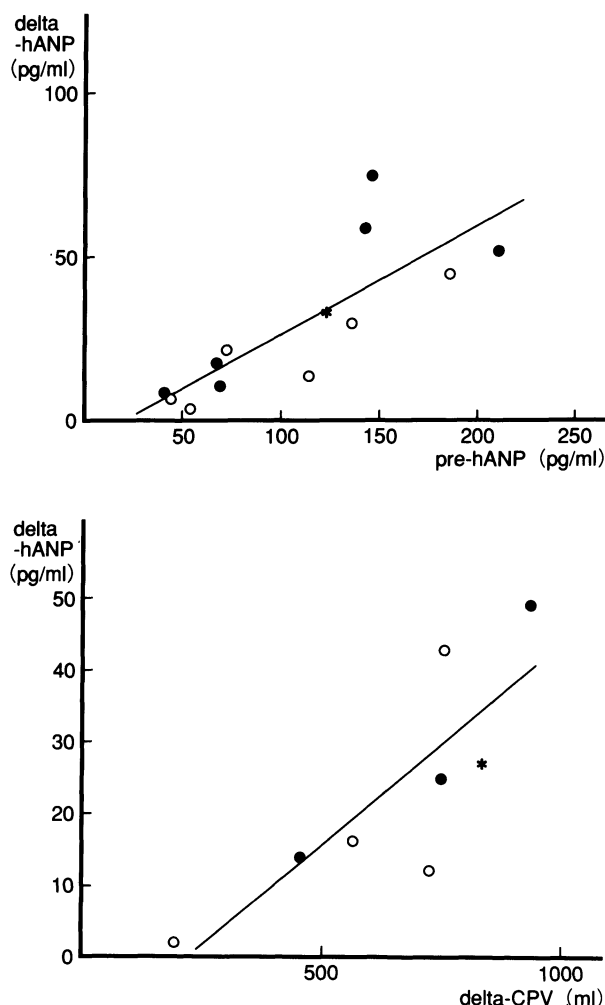


Fig. Correlations between ANP concentration before HD (pre-ANP) and the magnitude of the decrease in ANP (delta ANP) during HD without fluid removal ($r=0.80$, $P<0.01$) (upper panel), and between the change in circulating plasma volume (delta CPV) and delta ANP during fluid removal without HD ($r=0.83$, $P<0.05$) (lower panel). ●, diabetic nephropathy; ○, chronic glomerulonephritis; *, primary nephrotic syndrome.

se did not influence the plasma ANP level. The mechanism(s) underlying the increase in ANP in CRF patients is (are) therefore still unclear.

We therefore investigated the changes in ANP during dialysis when diffusion and ultrafiltration were performed separately. The plasma ANP level decreased when CPV was decreased by ultrafiltration without diffusion, and the decrease in the ANP concentration showed a significant correlation with the decrease in CPV. This decrease in ANP was therefore probably related to the fall in right atrial pressure due to the decrease in CPV.

However, when HD was performed without

fluid removal so that the body weight showed no significant change, the plasma ANP concentration also decreased significantly. Another study failed to show a significant change in ANP under similar conditions [15], where the CPV was not measured during HD. The reason for the discrepancy is unknown, however, it may depend on the difference in the study subjects or the dialysis prescription. As the CPV, osmotic pressure, and blood pressure were not changed by HD without fluid removal in the present study, the decrease in plasma ANP cannot be explained by a mechanism involving any of these parameters. However, the following possible explanations for this decrease in ANP may be considered: 1) ANP was filtered by the dialyzer through diffusion, 2) removal of humoral factors or uremic toxins by the dialyzer through diffusion caused a secondary decrease in ANP, 3) secretion of ANP was modulated through the nervous or endocrine system in response to the stress of the HD and 4) the ANP level was influenced by the changes in plasma electrolytes.

The filtrate concentration of ANP (mean: 5.5 pg/ml) was only about 8% of the plasma level, and there were no significant differences between the ANP concentrations in the blood samples taken from the arterial and venous access sites. The immunoreactive ANP detected in the present study mainly represents a C loop portion of ANP. It is therefore possible that the ANP measured in the filtrate may have included inactive fragments, and that we may have overestimated the extent of filtration of the whole ANP molecule. Filtration of ANP by the dialyzer would therefore make a negligible contribution to the decrease in the plasma level if any at all.

Secretion of cortisol and catecholamines is the most representative response to stress, but cortisol increases, rather than decreases, the plasma concentration of ANP [7]. A stress-induced cortisol response would therefore be unlikely to cause a decrease in ANP during HD without fluid removal. However, the stress-induced increase in epinephrine may have influenced the plasma ANP level, because epinephrine is reported to decrease ANP [16]. However, the effect of catecholamines on ANP secretion is still controversial and it has also been reported that ANP levels are raised by catecholamines [17].

The plasma concentration of sodium was significantly increased and that of potassium was

significantly decreased during HD without fluid removal, but the osmotic pressure did not change. Changes in these electrolytes may therefore influence the plasma ANP concentration, although no supportive experimental data are available to confirm this hypothesis. As the magnitude of the decrease in ANP correlated with the pre-HD value, some dialysable substances may influence ANP concentrations.

The decrease in ANP during HD without fluid removal may therefore have been caused by the removal of or a change in the level of some

substance(s), including electrolytes, epinephrine, and uremic toxins, and this substance(s) might have a direct or indirect effect on ANP levels in CRF patients. Eskay *et al.* [18] reported that neuronal influences were important in promoting the release of ANP by a volume load in experiments with denervated heart preparations, and suggested that there was an indirect effect of some substance on ANP secretion. It is necessary to further investigate the other mechanisms controlling ANP secretion in CRF in addition to volume expansion.

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