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Significance of adrenomedullin under cardiopulmonary bypass in children during surgery for congenital heart disease.*

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Abstract

To elucidate the effect of adrenomedullin (AM) on fluid homeostasis under cardiopulmonary bypass (CPB), we investigated the serial changes in plasma AM and other parameters related to fluid homeostasis in 13 children (average age, 28.2 months) with congenital heart disease during cardiac surgery under CPB. Arterial blood and urine samples were collected just after initiation of anesthesia, just before commencement of CPB, 10 min before the end of CPB, 60 min after CPB, and 24 h after operation. Plasma AM levels increased significantly 10 min before the end of CPB and decreased 24 h after operation. Urine volume increased transiently during CPB, which paralleled changes in AM. Simple regression analysis showed that plasma AM level correlated significantly with urinary vasopressin, urine volume, urinary sodium excretion, and plasma osmolarity. Stepwise regression analysis indicated that urine volume was the most significant determinant of plasma AM levels. Percent rise in AM during CPB relative to control period correlated with that of plasma brain natriuretic peptide ($r = 0.57$, $P < 0.01$). Our results suggest that AM plays an important role in fluid homeostasis under CPB in cooperation with other hormones involved in fluid homeostasis.

KEYWORDS: adrenomedullin, cardiopulmonary bypass, vasopressin, pediatric cardiac surgery, brain natriuretic peptide

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*Original Article***Significance of Adrenomedullin under Cardiopulmonary Bypass in Children during Surgery for Congenital Heart Disease**Mamoru Takeuchi*, Kiyoshi Morita, Tatsuo Iwasaki, Yuichiro Toda,
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To elucidate the effect of adrenomedullin (AM) on fluid homeostasis under cardiopulmonary bypass (CPB), we investigated the serial changes in plasma AM and other parameters related to fluid homeostasis in 13 children (average age, 28.2 months) with congenital heart disease during cardiac surgery under CPB. Arterial blood and urine samples were collected just after initiation of anesthesia, just before commencement of CPB, 10 min before the end of CPB, 60 min after CPB, and 24 h after operation. Plasma AM levels increased significantly 10 min before the end of CPB and decreased 24 h after operation. Urine volume increased transiently during CPB, which paralleled changes in AM. Simple regression analysis showed that plasma AM level correlated significantly with urinary vasopressin, urine volume, urinary sodium excretion, and plasma osmolarity. Stepwise regression analysis indicated that urine volume was the most significant determinant of plasma AM levels. Percent rise in AM during CPB relative to control period correlated with that of plasma brain natriuretic peptide ($r = 0.57$, $P < 0.01$). Our results suggest that AM plays an important role in fluid homeostasis under CPB in cooperation with other hormones involved in fluid homeostasis.

Key words: adrenomedullin, cardiopulmonary bypass, vasopressin, pediatric cardiac surgery, brain natriuretic peptide

A drenomedullin (AM) was originally isolated from human pheochromocytoma and normal adrenal medulla as a potent hypotensive peptide [1]. AM is localized in renal glomeruli, cortical distal tubules, and medullary collecting duct cells [2], and intrarenal infusion of AM increases glomerular filtration rate and fractional sodium excretion [2, 3]. Recent studies also demonstrated that exogenously infused AM results in significant increases in urine volume in rats [4] and humans [5]. These reports suggest that AM is considered to be physiologically important as a natriuretic

peptide.

Operation under general anesthesia represents an endocrinologically specific condition characterized by marked elevation of plasma arginine vasopressin (VP) independent of changes in plasma osmolarity [6, 7]. High levels of VP are also detected during cardiac surgery in patients undergoing cardiopulmonary bypass (CPB) [8, 9]. Under CPB, which induces marked hemodynamic changes, it is thought that AM plays a significant role in diuresis, in cooperation with other hormones involved in fluid homeostasis. However, to our knowledge, no previous studies have examined the dynamics of AM in children with congenital heart disease under CPB during cardiac surgery.

In the present study, we investigated the serial

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changes in plasma AM and other parameters related to fluid homeostasis in children with various congenital heart diseases who underwent cardiac surgery under CPB. The aim of our study was to elucidate the role of AM in fluid homeostasis, and especially its relationship with other hormones such as VP, atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), and aldosterone (ALD). These substances are reportedly involved in regulation of water and arterial pressure balance.

Materials and Methods

We selected a total of 13 children who underwent cardiac surgery under CPB at our hospital. Table 1 shows the clinical background of each child. Subjects included 3 boys and 10 girls, with an average age of 28.2 ± 7.3 months (mean \pm SEM; range, 7 months to 6 years and 4 months) with an average body weight (BW) of 10.8 ± 1.5 kg (range, 4.4–22.5 kg). Preoperative diagnoses included various congenital heart diseases, as shown in Table 1. The Qp and Qs ratio (Qp/Qs) and cardiothoracic ratio (CTR) of our cases were 2.1 ± 0.3 (range, 1.0–4.2) and $56.7 \pm 0.8\%$ (range, 52.0–60.0%), respectively. None of the patients had clinically evident heart failure or cyanosis. No patient had a history of liver or renal disease. The study protocol was approved by the Human Ethics Review Committee of Okayama University Medical School, and informed consent was obtained from the parents/guardian of each patient.

All patients were premedicated orally with midazolam 0.2–0.3 mg/kg approximately 30 min before the scheduled operation. General anesthesia was induced with fentanyl 10 μ g/kg, and maintained with 0.5–1.5% isoflurane, 50–60% nitrous oxide in oxygen, and intermittent bolus of fentanyl 5–10 μ g/kg. Just after induction of anesthesia, an i.v. line was established in an upper extremity. The radial artery, a central vein, and the urinary bladder were also catheterized in each patient. All patients were mechanically ventilated during surgery and closely monitored. CPB commenced approximately 60 to 90 min after induction of anesthesia. The bypass circuit, involving a membrane oxygenator (Dideco LilliPUT 902), was primed with a mixture of lactated Ringer's solution. Heparin 3 mg/kg was injected to maintain the activated clotting time > 400 s. Blood flow was nonpulsatile and commenced at 2.4 l/min/m², and was titrated to maintain systemic venous oxygen saturation $> 70\%$. Methoxamine was used intermittently to maintain perfusion

pressure ≥ 40 mmHg. Mild hypothermia (30–33 °C rectal temperature) was induced in all patients. Furosemide 20 mg was used in all cases at the commencement of CPB, and plasma protein solution was additionally used in some cases. Mannitol 0.8 g/kg was given to all patients through the CPB circuit. Dopamine (5 μ g/kg/min) was used to maintain cardiac output from the end of CPB during surgery in all cases. Two or three doses of furosemide 1 mg/kg were given to all patients over the 24 h after operation. All patients were extubated in the intensive care unit within 6 h after operation.

Arterial blood and urine samples were collected just after initiation of anesthesia (phase 0), just before commencing CPB (phase 1), 10 min before the end of CPB (phase 2), 60 min after CPB (phase 3), and 24 h after operation (phase 4). Urine volume (UV, ml/kg/h) and mean arterial pressure (MAP, mmHg) were expressed using the average data of each phase. Collected blood samples were centrifuged immediately and cells were separated from the plasma or serum, which were stored at -30 °C until assay. Urine samples were also stored at -30 °C until assay.

Plasma and urine osmolarity (Osm) were measured by the method of freezing point depression [10]. Plasma concentrations of VP, AM, ANP, BNP, and urinary concentrations of VP and aldosterone (ALD) were

Table 1 Clinical background of participating patients

No.	Age	Sex	Diagnosis	Qp/Qs	CTR (%)	Non-cardiac abnormalities
1	4y 1m	Female	ECD	4.2	59	-
2	1y	Female	DORV	1.1	55	-
3	8m	Female	MR	1.0	54	-
4	4y 4m	Female	ECD	2.1	55	-
5	6y 1m	Female	ASD	2.2	54	-
6	7m	Female	VSD	2.7	58	21 trisomy
7	1y 2m	Male	VSD, PS	1.7	56	-
8	11m	Male	VSD	3.2	60	-
9	1y 11m	Female	TOF	1.1	52	-
10	6y 4m	Female	AR, PS	1.0	55	-
11	9m	Female	VSD, PS, SAS	1.6	59	-
12	1y 3m	Male	TOF	1.2	60	21 trisomy
13	8m	Female	VSD	3.9	60	-

AR, aortic regurgitation; ASD, atrial septal defect; CTR, cardiothoracic ratio; DORV, double outlet right ventricle; ECD, endocardial cushion defect; MR, mitral regurgitation; PS, pulmonary stenosis; SAS, subaortic stenosis; TOF, tetralogy of Fallot; VSD, ventricular septal defect.

measured by radioimmunoassay using respective RIA kits. Serum concentrations of sodium (Na) and urinary concentrations of Na and creatinine (Cr) were measured by an autoanalyzer system. Urinary concentrations of Na, VP, and ALD were corrected for Cr to the excretion rate of Na, VP, and ALD, respectively.

All values are presented as mean \pm SEM. Differences between groups in each phase were analyzed for statistical significance by one-way analysis of variance (ANOVA) using StatView version 5 software (Abacus Concepts, Berkeley, CA, USA). Correlation between 2 variables was analyzed using total original data ($n = 65$) and the percent rise from the control (phase 0) to CPB (phase 2 and 3) period ($n = 26$). Stepwise multiple regression analysis was also performed, using plasma AM level as the dependent variable. The independent variables, which were considered as factors affecting plasma AM levels, included the following 11 factors: UV, MAP, plasma and urine Osm, serum and urinary Na, plasma and urinary VP, plasma ANP, plasma BNP, and urinary ALD. A P value less than 0.05 denoted the presence of a statistically significant difference.

Results

The anesthetic used in all patients was fentanyl, isoflurane, and nitrous oxide in oxygen. The average duration of operation and anesthesia was 235 ± 16 min and 333 ± 18 min, respectively. The average volume of blood loss during the total duration of surgery was 73 ± 25 ml; fluid replacement, predominantly consisting of lactated Ringer's solution, and saline and blood transfusion when appropriate, was used in all patients (average, 695 ± 73 ml; range, 250–1,135 ml) during the total duration of the anesthetic period. Average UV and urine excretion rates during the total duration of the anesthetic period were 563 ± 69 ml and 9.3 ± 0.9 ml/kg/h, respectively. Finally, average total in- and out-volume rates during anesthesia including CPB time were 13.7 ± 1.2 and 10.5 ± 0.9 ml/kg/h, respectively.

Several parameters were examined serially in this study (Figs. 1 and 2). UV markedly increased in phases 2 and 3, and recovered to the basal level (phase 0 and 1) in phase 4, whereas MAP diminished significantly only in phase 2. Plasma Osm increased in phase 2 and remained

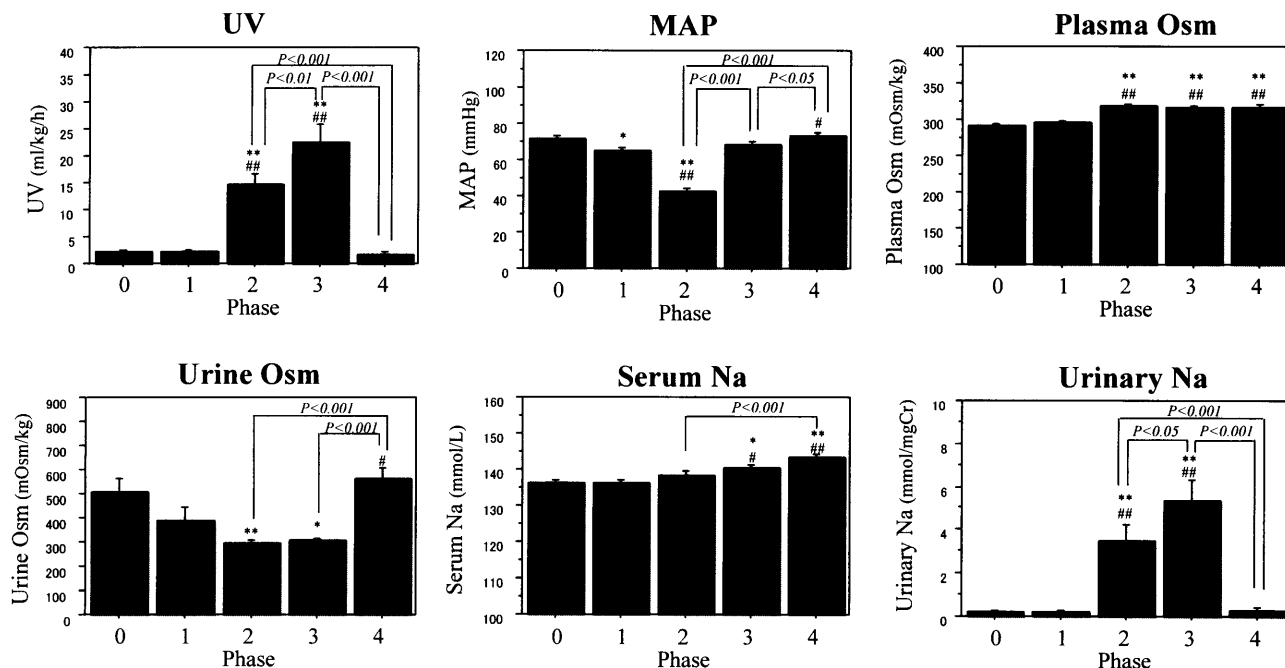


Fig. 1 Serial changes in urine volume (UV), mean arterial pressure (MAP), plasma osmolality (Osm), urine Osm, serum, and urinary concentrations of sodium (Na), before, during, and after cardiopulmonary bypass. Each phase is described in "Materials and Methods". * $P < 0.05$, ** $P < 0.001$ vs. phase 0; # $P < 0.05$, ## $P < 0.001$ vs. phase 1.

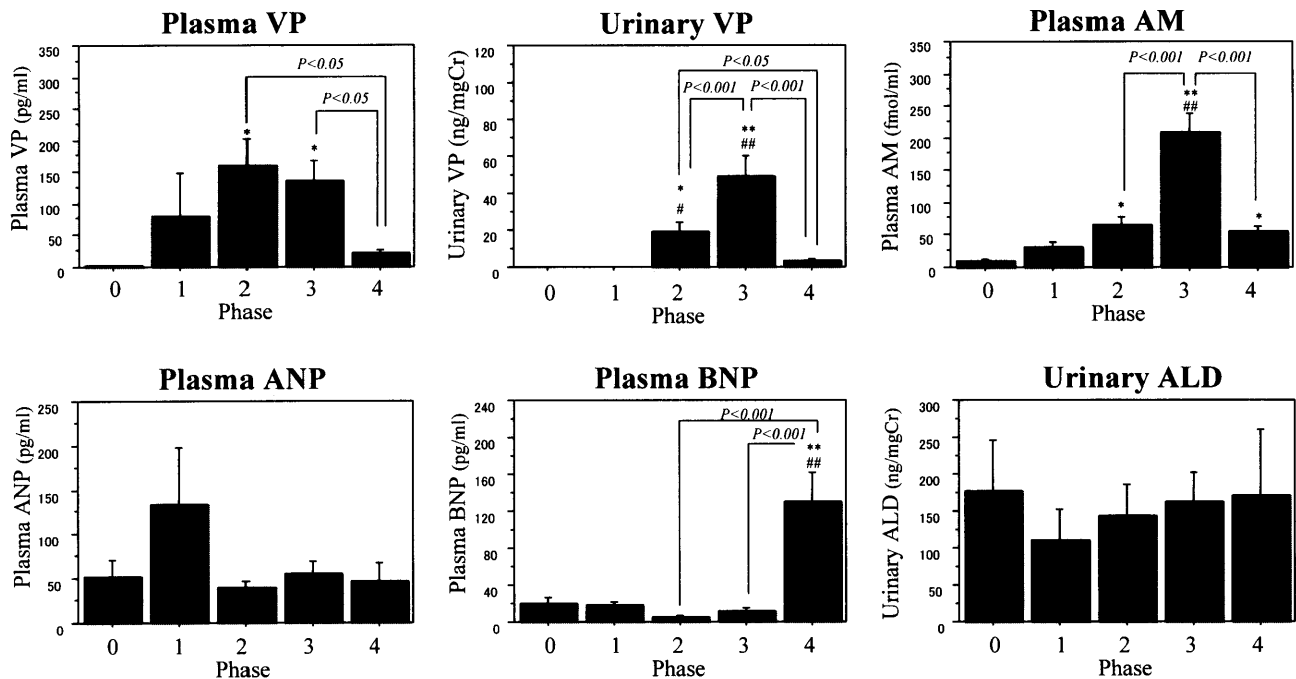


Fig. 2 Serial changes in plasma and urinary concentrations of vasopressin (VP), plasma concentrations of adrenomedullin (AM), atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), and urinary concentration of aldosterone (ALD) before, during, and after cardiopulmonary bypass. Each phase is described in "Materials and Methods". * $P < 0.05$, ** $P < 0.001$ vs. phase 0; # $P < 0.05$, ## $P < 0.001$ vs. phase 1.

persistently high in phase 4, although urine Osm significantly decreased in both phases 2 and 3. Serum concentration of Na increased significantly from phase 3, but urinary Na excretion showed a transient increase, reaching a peak level in phase 3 during the observation period (Fig. 1). Plasma VP significantly increased in phases 2 and 3 and returned to the basal level in phase 4, whereas urinary concentration of VP increased only in phase 3 (Fig. 2). Plasma AM showed a transient increase, reaching a peak level in phase 3; the pattern paralleled the changes in urinary Na and VP. Although plasma ANP (Fig. 2) and urinary ALD concentrations did not change significantly during the study, those of BNP markedly increased in phase 4 (Fig. 2).

The simple correlation coefficients describing the relationship between various parameters that were examined in the present study using the entire sampled data ($n = 65$) are shown in Table 2. Plasma AM levels correlated significantly with urinary concentration of VP, Na, UV, and plasma Osm (Table 2); weakly though significantly with serum Na and plasma VP; and

significantly and negatively with urine Osm. Plasma VP levels correlated significantly with plasma Osm, urinary VP, UV, and urinary Na excretion, but negatively with MAP and urine Osm. In contrast, there were only few variables that showed high correlation with plasma ANP, BNP, and urinary ALD during the entire period of this study. The results of linear regression analyses between plasma AM and urinary VP, urinary Na excretion, UV, and plasma Osm are shown in Fig. 3. Stepwise regression analysis indicated that urine volume was the most important determinant of plasma AM (Table 3). Finally, to determine the dynamic change in each of these parameters during CPB, we examined the correlation between each 2 variables using the percent rise from control to CPB period. Plasma AM showed a high correlation with plasma BNP, whereas plasma ANP correlated with urine volume (Fig. 4).

Discussion

Changes in the endocrine system under CPB have

Table 2 Simple correlation coefficients between various parameters

	P-AM (fmol/ml)	P-VP (pg/ml)	P-ANP (pg/ml)	P-BNP (pg/ml)	P-Osm (mOsm/kg)	S-Na (mmol/L)	UV (ml/kg/h)	U-VP (ng/mgCr)	U-ALD (ng/mgCr)	U-Osm (mOsm/kg)	U-Na (mmol/mgCr)	MAP (mmHg)
P-AM (fmol/ml)	1.00	0.26*	-0.01	-0.02	0.40*	0.32*	0.44†	0.53†	-0.05	-0.25*	0.44†	-0.06
P-VP (pg/ml)	-	1.00	-0.10	-0.15	0.41†	0.09	0.34*	0.40†	-0.03	-0.24*	0.29*	-0.25*
P-ANP (pg/ml)	-	-	1.00	0.04	-0.26*	-0.16	-0.10	-0.06	-0.11	0.03	-0.09	-0.08
P-BNP (pg/ml)	-	-	-	1.00	0.09	0.31*	-0.25*	-0.15	0.35*	0.18	-0.24	0.28*
P-Osm (mOsm/kg)	-	-	-	-	1.00	0.60†	0.44†	0.40*	-0.10	-0.04	0.42†	-0.21
S-Na (mmol/L)	-	-	-	-	-	1.00	0.11	0.15	-0.12	0.31*	0.19	0.19
UV (ml/kg/h)	-	-	-	-	-	-	1.00	0.66†	0.06	-0.43†	0.82†	-0.24
U-VP (ng/mgCr)	-	-	-	-	-	-	-	1.00	0.00	-0.28*	0.67	0.04
U-ALD (ng/mgCr)	-	-	-	-	-	-	-	-	1.00	-0.31*	-0.00	-0.06
U-Osm (mOsm/kg)	-	-	-	-	-	-	-	-	-	1.00	-0.39*	0.44†
U-Na (mmol/mgCr)	-	-	-	-	-	-	-	-	-	-	1.00	-0.20
MAP (mmHg)	-	-	-	-	-	-	-	-	-	-	-	1.00

Simple correlation between 2 variables was analyzed using the entire sampled data ($n = 65$) before, during, and after cardiopulmonary bypass. * $P < 0.05$, † $P < 0.001$. Abbreviations, see text. P-, plasma; S-, serum; U-, urine.

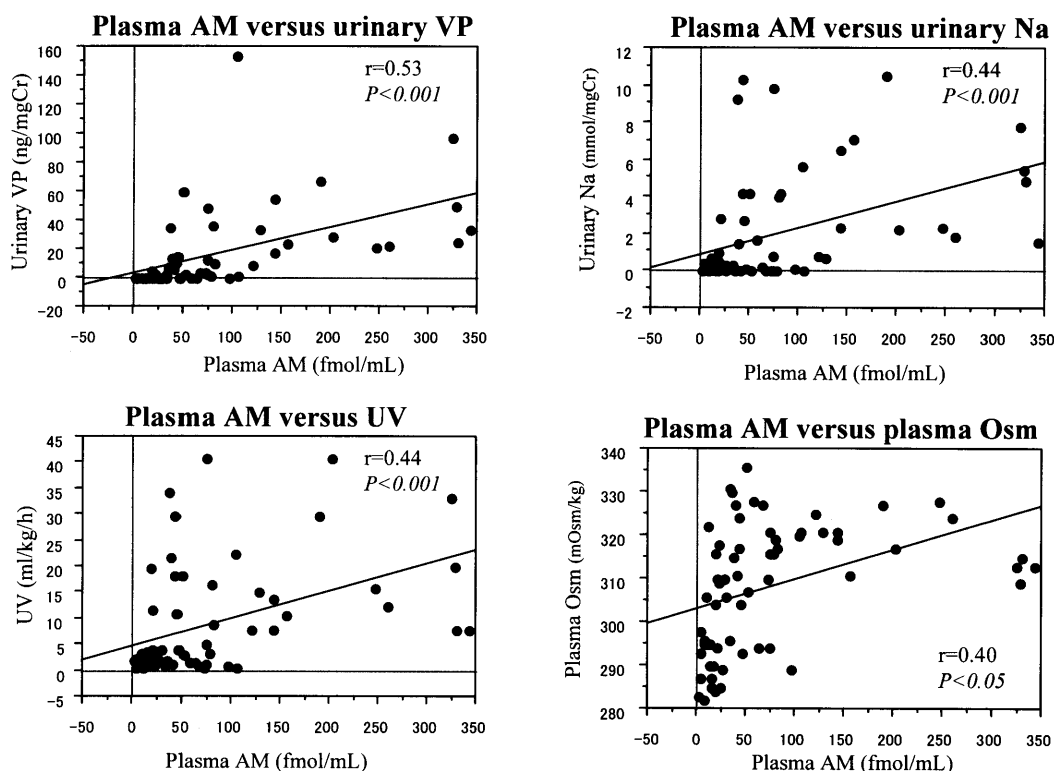


Fig. 3 Correlation between plasma adrenomedullin (AM) concentration and urinary vasopressin (VP), urinary sodium (Na), urine volume (UV), and plasma osmolality (Osm) using the entire number of sampled data ($n = 65$).

been estimated in several previous studies [11]. CPB, as well as anesthesia and surgery, induces a peculiar endocrinological state caused by a variety of factors such as anxiety, pain, hypoxia, acidosis, change in systemic arterial blood pressure, sodium loading, and administration of diuretics and other drugs [11]. In the present study, we investigated the changes in various hormonal factors and metabolites under CPB and stable anesthesia in pediatric cardiac surgery. Given that the ratio of priming volume of CPB to estimated circulating volume in children is far higher than that in adults, it is suggested that the CPB procedure induces comparatively marked hemodynamic changes in children. Our data demonstrated that plasma AM concentration correlated significantly with UV. Taking into consideration the effects of small doses of dopamine, furosemide, and mannitol administered during anesthesia on the diuretic state, our results suggest that AM plays an important role in fluid homeostasis

in the presence of marked changes in fluid balance induced by CPB in pediatric cardiac surgery.

Previous studies have shown that patients with heart failure often have high plasma AM levels, which tend to increase with worsening of heart failure [12-14]. Such increase in plasma AM is thought to be the result of enhanced production of AM, based on the fact that increased extracellular volume causes extension of cardiac myocytes or shear stress of cardiac muscle cells [15]. In our study, we did not examine for differences in the basal level of AM based on the severity of heart failure, because patients with severe heart failure and cyanotic conditions were excluded from the study. In contrast with the above mentioned previous reports, other previous studies have reported the relationship between AM and diuresis or other hormone substances. Because the prognosis of patients with heart disease partly depends on fluid control during cardiac surgery, it is important to understand the relationship among various hormones and their contribution to fluid homeostasis under CPB.

Several studies have examined the effect of cardiac surgery or CPB on plasma AM [16-18]. The results of these studies, together with our findings, indicate that plasma AM levels increase during CPB. However, the interpretation of this increase is controversial. Nagata and coworkers [16] reported that the highest increase in plasma AM occurred at weaning from bypass in 10 adult patients with cardiac disease, and that such rise correlated significantly with aortic cross-clamp time. They suggested that the potent vasodilatory effect of AM might play a role in cardiovascular regulation during and after surgery with CPB. Inoue and colleagues [17] measured plasma AM

Table 3 Multivariate stepwise regression analysis using plasma AM concentration as the dependent variable

Factor selected	Standardized regression coefficient	Partial correlation coefficient	Multiple correlation coefficient
UV (ml/kg/h)	0.602	0.580**	
Plasma BNP (pg/ml)	0.248	0.266*	
Urinary ALD (ng/mgCr)	-0.180	-0.202	0.586**

Stepwise multiple regression analysis was performed with plasma AM concentration as the dependent variable, using the entire sampled data. Independent variables included the eleven parameters examined in this study. * $P < 0.05$ and ** $P < 0.001$.

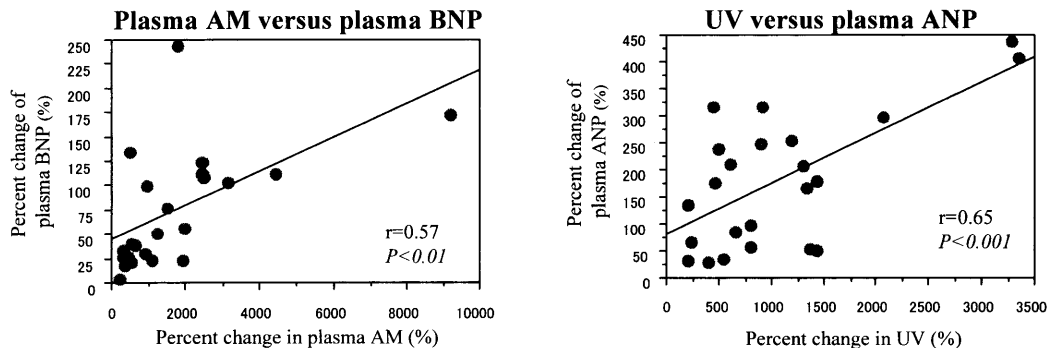


Fig. 4 Correlation between plasma adrenomedullin (AM) and brain natriuretic peptide (BNP), urine volume (UV), and plasma atrial natriuretic peptide (ANP). Plots were expressed as percentage of values under CPB (phase 2 and 3) relative to control period (phase 0).

concentrations in blood samples taken from the radial artery and internal jugular bulb in 10 patients with coronary artery disease under mild hypothermic CPB. Their results suggested that overproduction of AM in the brain correlated with aortic cross-clamp time. In our study, although the highest increase in plasma AM was noted after CPB (phase 3), there was no significant correlation between plasma AM and aortic cross-clamp time (correlation between AM in phase 3 and cross-clamp time, $r = 0.22$; $P = 0.45$). Komai *et al.* [18] argued that pulmonary hypertension caused the blunted production of AM as a result of damaged pulmonary vasculature in children with cyanotic or high pulmonary blood flow associated with congenital heart disease. Because we selected subjects with mild cardiac disease without cyanosis (Q_p/Q_s , 2.1 ± 0.3), we did not compare pulmonary artery pressure with plasma AM levels. It should be noted that the above studies did not examine the relationship between AM and other hormones or metabolites, the levels of which may also be modified by CPB or cardiac surgery.

Anesthesia and surgical stress induce non-osmotic high VPemia. Although the blunted response of urine osmolarity to high VPemia is well documented, the precise underlying mechanism of such response has not yet been clarified. We recently demonstrated that plasma and urinary VP concentrations correlate with urinary level of aquaporin-2 (AQP2), a VP-regulated water channel protein [19], during surgery under general anesthesia, although a high VP-AQP2 system does not contribute to urine concentration [20, 21]. In the present study, we demonstrated that changes in plasma AM levels corresponded to fluid and electrolyte balance, especially urinary VP, Na, and UV under CPB. Although the diuretic and natriuretic effects of AM in the kidney are complimented by the central effects of AM, which inhibits water drinking, salt appetite, and VP [22], our results obtained under general anesthesia seem to favor a renal (peripheral) action of AM on diuresis. With respect to the relationship between AM and VP, Sato and colleagues [23] reported that AM is produced from renal tubular cell lines by V_2 receptor stimulation, but not by V_1 stimulation. Given that AM is localized in the cortical distal tubules and medullary collecting duct cells [2], we believe that VP-induced AM regulates fluid homeostasis under CPB, which produces extremely high VPemia.

Another interesting finding in our study is the relationship between plasma AM and BNP during CPB. Previous studies have shown high plasma ANP and BNP in

patients with heart failure, and found that plasma AM correlated with these natriuretic peptides [24]. Lainchbury *et al.* [25] examined the bioactivity and interaction between AM and BNP in patients with heart failure, and demonstrated that plasma AM levels closely correlated with plasma BNP concentrations. In the present study, although the actual survey values of plasma AM and BNP concentrations seemed unrelated, the percent rise of plasma AM during CPB showed a high correlation with that of plasma BNP, but not with that of ANP. Based on the results of previous studies and the present findings, it is feasible to suggest that AM induces potent diuresis in cooperation with BNP. In addition, our results showed that plasma BNP markedly increased 24 h after CPB, suggesting that BNP also contributes to diuresis in the late-phase after cardiac surgery. In contrast, plasma ANP increased transiently during CPB, and the percent rise of plasma ANP showed a high correlation with that of UV during CPB. Given that ANP is a significant diuretic substance and is clinically applied to induce diuresis, ANP may contribute to increased UV during CPB independent of AM. With regard to ALD, no clear changes in urinary ALD levels were observed in our study, although AM is thought to directly stimulate renin secretion via a cAMP-dependent mechanism in juxtaglomerular cells [26].

In conclusion, we demonstrated in the present study the serial changes in plasma AM and other parameters under CPB, a procedure associated with marked hemodynamic alterations. Our results suggest that AM plays a role in fluid homeostasis in cooperation with other hormones, especially VP and BNP. Although we cannot attribute all natriuresis to AM action, this peptide, a potent natriuretic substance, seems to be an important modulator of plasma volume and composition, and to contribute to natriuresis under CPB.

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