Plasma adrenomedullin during acute changes in intravascular volume in hemodialysis patients

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Background. Adrenomedullin, is a potent vasorelaxant that is highly expressed in the adrenal medulla, kidney, heart and lung. Since there is indirect evidence that hypervolemia enhances the release of this peptide, we measured plasma adrenomedullin in 9 uremic patients on chronic dialysis treatment and in 10 healthy subjects matched for age and gender.

Methods. Measurements were performed in baseline conditions, after isotonic fluid subtraction (by isolated ultrafiltration) and during a 70° tilt. Tilt was performed in volume-depleted state, that is, after isolated ultrafiltration (UF). In the control experiment patients underwent sham UF (UF = 0) followed by a period of supine resting identical to the one they had spent in tilted position in the active experiment. Adrenomedullin was measured on pre-extracted plasma samples (Sep-Pak C-18 cartridges) by a specific RIA for human adrenomedullin 1-52.

Results. The average plasma adrenomedullin was 2.6 times higher ($P < 0.01$) in uremic patients (103 ± 8 pg/ml) than in healthy subjects (39 ± 7 pg/ml). After fluid subtraction (-2.6 ± 0.2 liter) adrenomedullin fell to $79. \pm 8$ pg/ml ($P = 0.02$) but remained well above the upper limit of the 95% CI in normal subjects (52 pg/ml). There was no relationship between adrenomedullin and ANF changes. In the control experiment sham UF did not modify plasma adrenomedullin. Tilt did not significantly change plasma adrenomedullin either in dialysis patients or healthy subjects.

Conclusions. Plasma adrenomedullin is markedly raised in uremic patients on dialysis, which confirms that the kidney has a major role in the clearance of this peptide. However, the fall in plasma adrenomedullin after isolated UF indicates that the plasma concentration of this peptide is influenced by the body fluid volume status. Whether or not adrenomedullin participates in the counter-regulatory response to fluid subtraction in uremic patients remains to be explored by specific antagonists of this substance.

Adrenomedullin is a 52 amino acid polypeptide isolated from human pheocromocytoma that is structurally homologous to calcitonin gene-related peptide (CGRP) [1]. Like CGRP, adrenomedullin is a potent vasorelaxant, and there is increasing evidence that this peptide may function as a paracrine and/or autocrine factor in the regulation of cardiovascular homeostasis [2–4]. Adrenomedullin is measurable in human plasma [5] and it has been found that the concentration of this substance is raised in clinical situations associated with altered extracellular volume control such as heart failure [6–14], chronic renal failure [14–18], hypertension [15, 18, 19] and primary hyperaldosteronism [20]. Circulating adrenomedullin is markedly raised in hemodialysis patients [16, 17], a phenomenon that has been attributed to reduced renal clearance of this peptide [17].

Extracellular volume undergoes cyclical changes in uremic patients on chronic hemodialysis, increasing gradually during the dialysis interval and decreasing rapidly during ultrafiltration-dialysis. Measuring the influence of volume stimuli on plasma adrenomedullin may provide useful information on the regulation of circulating levels of this peptide in dialysis patients. Therefore, in this study we sought to establish whether extracellular fluid volume removal by isolated ultrafiltration and tilt-induced central hypovolemia has a measurable influence on plasma adrenomedullin concentration in these patients.

METHODS

Patients

Nine uremic patients (6 males and 3 females; age 52 ± 14 years, mean $\pm$ SD) on regular dialysis treatment participated in the study. They had been on treatment for periods ranging from 1 to 16 years (5.5 ± 3.4, mean $\pm$ SD). All were being dialyzed by cuprophan filters by using a standard dialysate (Na 145 mmol/liter, K 1.5 mmol/liter, HCO_3 37 mmol/liter, Ca 1.50 to 1.75 mmol/liter, Mg 0.5 mmol/liter). Their Kt/Vs ranged from 1 to 1.6. Causes of renal failure were polycystic renal disease in three, rapidly progressing glomerulonephritis in one, chronic pyelonephritis in one, cortical necrosis in one, and undefined in three. Residual

renal function was negligible in all but one case (24 hr urine volume 500 ml). Four patients had mild to moderate left ventricular hypertrophy on echocardiography, but none had evidence of heart failure. All patients were judged to be at "dry weight" by standard clinical criteria [21]. Three patients were on antihypertensive treatment (Nifedipine slow release), and in these cases drug treatment was withdrawn at least one week before the study.

The control group was formed by 10 healthy, normotensive subjects (7 males and 3 females, mean age 47 ± 10 years, ± SD, range 23 to 55 years) recruited from the medical and nursing staff.

Study protocol

The protocol of the study was in conformity with the ethical guidelines of our institution, and informed consent was obtained from each participant. Both the active and control experiments (see below) were performed midweek, after a short dialysis interval.

Dialysis patients

Active experiment. On the day of study, patients had their blood pressures and heart rates monitored at three minute intervals for 30 minutes while resting in supine position on a dialysis bed equipped with a scale. Baseline blood sampling was performed at the end of this period. Then isolated ultrafiltration [22] was started at a rate of about 20 ml/min. During this procedure no dialysate circulated through the filter and the ultrafiltrate was collected in a graduated cylinder. Ultrafiltration was interrupted when all the body wt excess accumulated during the preceding dialysis interval was removed. Blood sampling was performed again at the end of ultrafiltration. Arterial pressure and heart rate were measured three times with a three minute interval before sampling. Isolated ultrafiltration was followed by 180 minutes of isovolumic dialysis (with a standard dialysate, see above). Immediately after dialysis the response to tilting was tested. Tilting was carried out with patient lying on a motor-driven tilt table angled at 70°. Arterial pressures and heart rates were measured every two minutes while the final blood sample was taken either after 14 minutes or, if signs of intolerance to the maneuver had supervened, immediately before returning the patients to the supine position.

Control experiment. In the control experiment the inlet and the outlet of the dialysate compartment were accurately sealed to avoid ultrafiltration while the blood circulated through the filter (sham ultrafiltration). After sham ultrafiltration (which, case by case, lasted exactly as long as the isolated ultrafiltration in the active experiment), dialysis was carried out for 180 minutes. At the end of the dialysis session, patients remained in bed in a supine position for the same time they had been in a tilting position in the active experiment. Blood sampling was performed at the same time points as the active experiment.

Healthy subjects

Healthy subjects had their blood pressures and heart rates monitored at three minute intervals for 30 minutes while resting in a supine position with blood sampling at the end of this period. Tilting was then performed. Arterial pressure measurements and blood sampling were repeated after 1, 10 and 30 minutes of tilting.

Methods

Plasma cathecolamines and plasma renin activity (PRA) were measured by commercially available RIA methods (Amicyl-test™; Immunological Laboratories, Hamburg, Germany; and Renctk™, Sorin, Vercelli, Italy). Atrial natriuretic factor (ANF) and endothelin I were measured on pre-extracted plasma samples according to methods established in our laboratory [23, 24]. Plasma adrenomedullin was measured on pre-extracted (C-18 Sep-pak) plasma samples by a sensitive RIA employing an antibody against human adrenomedullin (Peninsula Laboratories, Merseyside, UK). This antibody does not cross react with human CGRP, endothelin I, ANP, BNP and CNP. All adrenomedullin plasma samples were processed in a single assay and the intra-assay variation of this RIA in our laboratory was 7%. Reverse-phase HPLC studies showed that plasma immunoreactive adrenomedullin emerged as a single peak at a position identical to that of authentic human adrenomedullin (1-52) in uremic subjects [16]. Furthermore, studies in our laboratory had also shown that the loss of peptide hormones with molecular weight exceeding 1000 Daltons (adrenomedullin molecular wt = 6,028) was negligible during isolated ultrafiltration performed by cuprophan filters [25].

Arterial pressure and heart rate measurements were performed by an automatic sphygmomanometer connected to an automatic recorder (Dinamap, model 1540; Critikon, Tampa, FL, USA).

Data analysis

Baseline arterial pressure and heart rate represent the average value of the last three measurements recorded during the supine rest. In dialysis patients, the average value of the three blood pressure and heart rate recordings (before blood sampling) at the end of isolated ultrafiltration and the measurement at the end of tilting (again, immediately before blood sampling) were considered for statistical analysis. In healthy subjects during the tilting procedure the hemodynamic measurements preceding each blood sampling were taken.

Biochemical measurements were corrected for hemoconcentration by multiplying each measurement by the Prb/Prx ratio, where Prb represents the serum proteins concentration of the baseline sample and Prx the concentration of the sample.
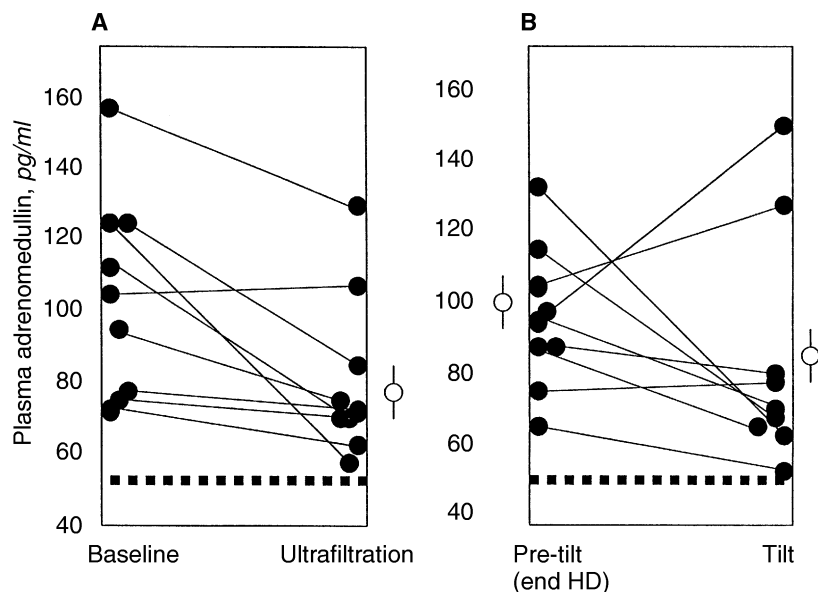
Fig. 1. **In the active experiment, the changes in plasma adrenomedullin concentrations induced by isolated ultrafiltration (*A*) and by tilt (*B*).** The dotted line indicates the upper limit of the 95% CI in healthy subjects (52 pg/ml).

Data are presented as mean ± SEM. The response to tilt in healthy subjects was analyzed by one way ANOVA followed by the Newman Keuls test for multiple comparisons. Because in dialysis patients we planned two sets of paired comparisons (paired *t*-tests for baseline vs. post-ultrafiltration and pre-tilt vs. post-tilt), we considered only differences with $P < 0.025$ (Bonferroni inequality) to be statistically significant. The relationship between paired variables was tested by the least squares method.

RESULTS

Baseline plasma adrenomedullin was 2.6 times higher ($P < 0.01$) in uremic patients (103 ± 8 pg/ml) than in healthy subjects (39 ± 7 pg/ml). In both groups adrenomedullin was independent of age, arterial pressure and heart rate.

Adrenomedullin in dialysis patients

Active experiment: Isolated ultrafiltration (Table 1 and Fig. 1). No patient had syncopal episodes or vomited during the procedure. The volume of ultrafiltrate removed ranged from 1.8 to 3.5 liters (average 2.6 ± 0.2 liter), which produced a 34% increase in serum proteins concentration. Mean arterial pressure fell significantly ($P < 0.01$) during UF while the heart rate showed a small rise (NS). Plasma norepinephrine, PRA and plasma antidiuretic hormone (ADH) rose significantly ($P < 0.01$) while ANF changed in the opposite direction (NS). Plasma endothelin was little affected by UF. As shown in Figure 1A, plasma adrenomedullin concentration decreased significantly after UF (from 103 ± 9 pg/ml to 79 ± 8 pg/ml, $P < 0.02$), the average decrease being $20\% \pm 7\%$ (range 0.7% to -35%), but on average it remained at levels 1.5 higher than the upper limit of the 95% CI in healthy subjects (52 pg/ml).

Changes in plasma adrenomedullin and ANF were much more pronounced after correction of the data for hemoconcentration (Table 1). The fall in plasma adrenomedullin was unrelated to changes in mean arterial pressure, plasma norepinephrine ($r = -0.32$), PRA ($r = -0.06$), ANF ($r = 0.0$), endothelin ($r = 0.0$) and ADH ($r = 0.67$, $P = 0.068$).

Active experiment: Tilt. Only five patients were able to maintain the tilt position longer than 10 minutes. The average tilt tolerance was 7.8 ± 1.8 minutes (range 1 to 14 min). Tilt caused an 8 mm Hg fall in MAP associated with a significant rise in heart rate and in plasma catecholamines (Table 1). PRA, plasma Endothelin and plasma ANF were unaffected by tilt. Plasma adrenomedullin after tilt fell in all but three patients (pre-tilt 98 ± 7, tilt 84 ± 11), but on average these changes failed to attain the threshold of statistical significance (Fig. 1B).

Control experiment. There were no significant changes in plasma adrenomedullin either after sham UF or bed resting (for a period identical to that spent in tilt position in the active experiment; Table 2). Adrenomedullin changes during sham UF were significantly less pronounced than during active UF ($P = 0.02$). During sham ultrafiltration there was a significant increase in plasma catecholamines ($P < 0.01$), an expected effect of extracorporeal blood cooling [26, 27].

Healthy subjects

Response to tilt. Mean arterial pressure was well maintained during early tilt and showed a 8 mm Hg decrease at the end of the test while the mean heart rate showed an immediate and steady increase ($P < 0.01$; Table 3 and Fig. 2). Plasma norepinephrine ($P < 0.01$) and PRA ($P = 0.048$, NS) displayed the expected rise. Plasma adrenomedullin was affected very little by tilt (one way ANOVA, $P = 0.56$).

Table 1. Hemodynamic and metabolic measurements in the active experiment in hemodialysis patients

	Baseline	End-UF	Pre-tilt (end HD)	Tilt
MAP *mm Hg*	95 ± 7	75 ± 7[a]	82 ± 7	68 ± 7[b]
Heart rate *beats/min*	79 ± 2	81 ± 3	89 ± 3	105 ± 4[a]
Total proteins *g/dl*	6.6 ± 0.2	9.0 ± 0.3[a]	8.4 ± 0.3	8.6 ± 0.3
BUN *mg/dl*	136 ± 3	117 ± 14	64 ± 4	64 ± 4
Adrenomedullin *pg/ml* (corrected for hemoconcentration)	103 ± 9	79 ± 8[b] (58 ± 7)[a]	98 ± 7 (77 ± 5)	84 ± 11 (67 ± 10)
ANF *pg/ml* (corrected for hemoconcentration)	95 ± 15	90 ± 13 (65 ± 10[a])	82 ± 9 (76 ± 8)	82 ± 7 (81 ± 6)
PRA *ng/ml/h*	8.1 ± 2.0	25.3 ± 9.8[a]	28.9 ± 12.2	30.1 ± 11.7
Norepinephrine *pg/ml*	324 ± 37	825 ± 82[a]	529 ± 68	903 ± 115[a]
Epinephrine *pg/ml*	26 ± 4	74 ± 16[b]	41 ± 11	97 ± 20[a]
ADH *pg/ml*	4.1 ± 0.3	17.8 ± 6.1[a]	5.3 ± 0.8	18.6 ± 5.7[a]
Endothelin *pg/ml*	15.1 ± 1.5	15.6 ± 1.8	16.8 ± 1.3	15.8 ± 1.3

Data are mean ± SEM. Abbreviations are: UF, ultrafiltration; HD, hemodialysis; MAP, mean arterial pressure; BUN, blood urea nitrogen; ANF, atrial natriuretic peptide; PRA, plasma renin activity; ADH, antidiuretic hormone.
[a] $P < 0.01$ and [b] $P < 0.025$ (Ultrafiltration vs. Baseline and Tilt vs. pre-Tilt)

Table 2. Control study of hemodialysis patients

	Baseline	Sham-UF	(Pre-recumbency) end HD	Recumbency
MAP *mm Hg*	97 ± 5	90 ± 6	91 ± 5	93 ± 5
Heart rate *beats min*	75 ± 4	73 ± 4	85 ± 4	85 ± 3
BUN *mg/dl*	144 ± 16	145 ± 15	66 ± 8	67 ± 8
Total proteins *g/dl*	7.2 ± 0.2	6.9 ± 0.2	6.8 ± 0.2	7.0 ± 0.2
Adrenomedullin	103 ± 18	101 ± 18	91 ± 19	94 ± 39
PRA *ng/ml/h*	3.1 ± 0.9	4.2 ± 1.1	4.1 ± 1.3	3.9 ± 1.1
Norepinephrine *pg/ml*	501 ± 0.91	645 ± 88[a]	529 ± 87	539 ± 65
Epinephrine *pg/ml*	19 ± 4	34 ± 6[a]	28 ± 5	43 ± 10
Endothelin *pg/ml*	11.1 ± 1.2	10.6 ± 1.2	10.2 ± 1.0	9.9 ± 0.9
ADH *pg/ml*	2.3 ± 0.4	2.0 ± 0.2	2.0 ± 0.4	1.7 ± 0.4

In this study patients underwent sham ultrafiltration followed by isovolumic hemodialysis, and then remained supine in the dialysis bed for a period identical to that of the tilt study (**methods** section). Data are mean ± SEM.
[a] $P < 0.01$ vs. baseline
Abbreviations are in Table 1.

Table 3. Hemodynamic and neurohumoral response to tilt in healthy subjects

	Baseline	Tilt 1 min	Tilt 10 min	Tilt 30 min
MAP *mm Hg*	89 ± 3	94 ± 3	92 ± 3	81 ± 6
Heart rate *beats/min*	63 ± 3	75 ± 4	78 ± 4	77 ± 3[a]
Adrenomedullin *pg/ml*	39 ± 7	48 ± 4	49 ± 6	46 ± 6
PRA *ng/ml/h*	0.9 ± 0.2	0.9 ± 0.2	1.7 ± 0.7	3.1 ± 1.9
Norepinephrine *pg/ml*	175 ± 21	218 ± 28	232 ± 29	291 ± 24[a]
Epinephrine *pg/ml*	23 ± 7	31 ± 7	34 ± 7	45 ± 5[a]
ANF *pg/ml*	13 ± 1	12 ± 1	10 ± 2	10 ± 1
ADH *pg/ml*	7 ± 2	3 ± 0.3	6 ± 1	20 ± 11[a]
Endothelin *pg/ml*	0.7 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	0.8 ± 0.1

Abbreviations are in Table 1.
[a] $P < 0.01$ vs. baseline

endothelial and vascular smooth cells), the adrenal medulla, lung and kidney. There are several lines of evidence suggesting that this vasodilator is involved in cardiovascular and extracellular volume control. Injected intrarenally in the dog it has a clear-cut natriuretic effect [28], during high salt diets it is more intensely expressed in the ventricle of salt sensitive than in Dahl resistant rats [29], and it is actively secreted by the human heart [9]. The regulation of adrenomedullin production is complex because it is influenced by circulating hormones and growth factors [30, 31] as well as by cytokines, IL-1α, TNF and lipopolysaccaride, which additively stimulate its synthesis [32]. The plasma half-life of adrenomedullin is about 20 minutes [33] and most likely the kidney as well as the lungs are important sites of adrenomedullin clearance. Because the plasma concentration of this substance and of the related compound, proadrenomedullin N-terminal 20 peptide (PAMP) [34], is strictly related to creatinine clearance, reduced renal extraction is considered to be the main determinant of the high plasma concentration in patients with chronic renal failure [14–18], particularly in hemodialysis patients [16, 17] where adrenomedullin is poorly removed by dialysis treatment [17]. The influence of isotonic fluid removal (by isolated ultrafiltration) on plasma adrenomedullin in dialysis patients has not been investigated.

Extracellular volume subtraction by isolated ultrafiltration has been often applied to study the influence of body fluid volume status on cardiovascular hormonal factors in dialysis patients [23, 25, 35, 36]. We felt that this procedure required an appropriate control study because changes in plasma concentration during ultrafiltration may be influenced by adsorption phenomena, by the generation of cytokines promoted by the contact of the blood with the cuprophan membrane (see above) [32], and by extracorporeal blood cooling [26, 27]. To circumvent this problem we performed a control study including sham ultrafiltration. The fact that adrenomedullin showed a clear-cut reduction after isolated ultrafiltration while it remained unchanged after sham ultrafiltration clearly indicates that the body fluid volume status *per se* has an independent effect on the plasma concentration of this peptide. It should be noted
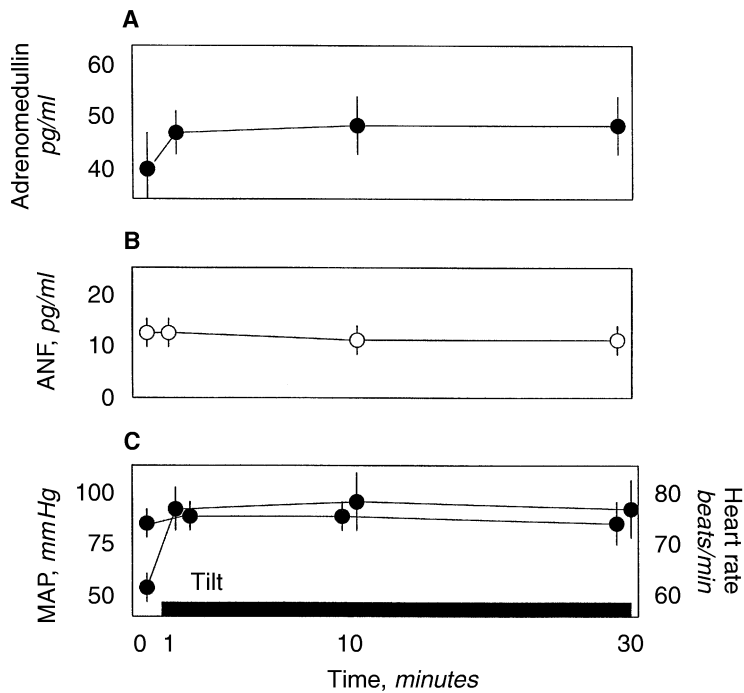
By the same token, plasma ANF remained by-and-large unchanged.

DISCUSSION

The main finding in this study is that in dialysis patients, the raised plasma adrenomedullin concentration depends in part on the extracellular volume expansion generated during the dialysis interval.

Adrenomedullin is a potent vasodilator that is highly expressed in the cardiovascular system (cardiac myocytes,

Fig. 2. Adrenonemedullin and atrial natriuretic factor (ANF) response to tilt in healthy subjects. The mean arterial pressures (MAP) and heart rates are shown. Data represent mean ± SEM.

that changes in plasma concentration are likely to underestimate the reduction in adrenomedullin release elicited by acute volume subtraction. Indeed, ultrafiltration causes an important degree of hemoconcentration that tends to attenuate the fall in plasma adrenomedullin levels. The influence of fluid subtraction on plasma adrenomedullin was much more profound when the data were corrected for hemoconcentration (Table 2). Yet, plasma adrenomedullin after ultrafiltration remained at higher than normal levels, suggesting that reduced renal clearance has a major role in determining high plasma adrenomedullin in dialysis patients.

There is evidence in humans that adrenomedullin, like ANF, is directly secreted by the left ventricle and that its plasma concentration is related to left ventricular end diastolic pressure in patients with heart failure [9]. Thus, the heart may be an important source of adrenomedullin in dialysis patients. We have been careful in excluding from the study those patients with heart failure or with obvious fluid overload. The fluid excess in our patients was just the normal fluid excess that the typical dialysis patient accumulates between two midweek dialyses. If the heart is a source of adrenomedullin in dialysis patients, then the relationship between this substance and intracardiac pressures also must be evident in the normal-high range of intracardiac pressures, that is, in the range found in these patients before dialysis [37]. In the present study the effect of ultrafiltration on plasma adrenomedullin was higher than that on plasma ANF and there was no relationship between these two vasodilators. This phenomenon indicates that, like in a rat model of heart failure [12] where the lung may

be another site for increased adrenomedullin release, the control mechanism(s) of these two peptides are differently regulated in uremic humans.

While fluid removal produced a well defined reduction in plasma adrenomedullin, central hypovolemia by tilt had a much less strong (and statistically unsignificant) influence. To our knowledge this is the first study to test the effect of tilt on plasma adrenomedullin. The fact that this maneuver did not produce changes in the circulating levels of this peptide in normal subjects nor potentiated the effect of ultrafiltration in dialysis patients suggests that central hypovolemia is an inadequate stimulus to switch off the release of adrenomedullin. However, it should be recognized that in dialysis patients the short duration of tilt (average 8 min) in comparison with the half-life of adrenomedullin (20 min) might have attenuated a tilt-induced decrease in the plasma concentration of this peptide. As in our study, tilt had no influence on plasma ANF in healthy individuals [38].

In theory a reduction in circulating adrenomedullin could participate in the cardiovascular response to fluid removal. In other words, the decrease in plasma adrenomedullin in response to ultrafiltration could contribute to the compensatory rise in vascular tone that occurs during this maneuver. The fact that adrenomedullin changes throughout ultrafiltration were unrelated to mean arterial pressure changes and to neurohumoral factors that have an established role in cardiovascular homeostasis would speak against such a possibility. Although our data are in line with recent infusion studies in humans showing that the plasma concentrations observed in pathophysiological conditions

do not influence arterial pressure [33], the possibility remains that plasma concentration does not reflect changes at the tissue level and that adrenomedullin influences vascular tone only by local mechanisms, that is, by acting in close proximity of the secretion site. This possibility should be explored in specifically designed intervention studies using adrenomedullin antagonists.

In conclusion, plasma adrenomedullin is markedly raised in uremic patients on hemodialysis, which indicates that the kidney has a major role in the clearance of this peptide. However, the fall in plasma adrenomedullin after isolated UF suggests that the plasma concentration of this peptide is dependent in part on the body fluid volume status. Whether adrenomedullin participates in the counter-regulatory response to fluid subtraction in uremic patients or not remains to be explored by specific antagonists of this substance.

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