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. ± 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 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 ± 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 ± 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, ± so, 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 (Amicyle-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 hemocencentration 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.
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% ± 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, $P = 0.56$).
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, 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 intravenously 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 lipopolysaccharide, 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 cuprophane 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

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

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

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. 

* P < 0.01 and b P < 0.025 (Ultrafiltration vs. Baseline and Tilt vs. pre-Tilt)

### Table 2. Control study of hemodialysis patients

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Sham-UF</th>
<th>(Pre-recumbency)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(end HD)</td>
<td>Recumbency</td>
<td></td>
</tr>
<tr>
<td>MAP mm Hg</td>
<td>95 ± 5</td>
<td>90 ± 6</td>
<td>91 ± 5</td>
</tr>
<tr>
<td>Heart rate bpm/min</td>
<td>75 ± 4</td>
<td>73 ± 4</td>
<td>85 ± 4</td>
</tr>
<tr>
<td>BUN mg/dl</td>
<td>144 ± 16</td>
<td>145 ± 15</td>
<td>66 ± 8</td>
</tr>
<tr>
<td>Total proteins g/dl</td>
<td>7.2 ± 0.2</td>
<td>6.9 ± 0.2</td>
<td>6.8 ± 0.2</td>
</tr>
<tr>
<td>Adrenomedullin</td>
<td>103 ± 18</td>
<td>101 ± 18</td>
<td>91 ± 19</td>
</tr>
<tr>
<td>PRA ng/ml/h</td>
<td>3.1 ± 0.9</td>
<td>4.2 ± 1.1</td>
<td>4.1 ± 1.3</td>
</tr>
<tr>
<td>Norepinephrine pg/ml</td>
<td>501 ± 0.9</td>
<td>645 ± 88*</td>
<td>529 ± 87</td>
</tr>
<tr>
<td>Epinephrine pg/ml</td>
<td>19 ± 4</td>
<td>34 ± 6*</td>
<td>28 ± 5</td>
</tr>
<tr>
<td>ADH pg/ml</td>
<td>11.1 ± 1.2</td>
<td>10.6 ± 1.2</td>
<td>10.2 ± 1.0</td>
</tr>
<tr>
<td>Endothelin pg/ml</td>
<td>2.3 ± 0.4</td>
<td>2.0 ± 0.2</td>
<td>2.0 ± 0.4</td>
</tr>
</tbody>
</table>

In this study patients underwent sham ultrafiltration followed by isoosmotic 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.

* P < 0.01 vs. baseline

Abbreviations are in Table 1.

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

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

Abbreviations are in Table 1.

* P < 0.01 vs. baseline
that changes in plasma concentration are likely to under-
estimate 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 pa-
tients.

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 accumu-
lates 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 hy-
povolemia is an inadequate stimulus to switch off the
release of adrenomedullin. However, it should be recog-
nized that in dialysis patients the short duration of tilt
(average 8 min) in comparison with the half-life of ad-
renomedullin (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 ad-
renomedullin 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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