Urinary excretion of aquaporin-2 water channel exaggerated dependent upon vasopressin in congestive heart failure

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Background. Impaired water excretion occurs in patients with congestive heart failure. The present study was undertaken to determine whether urinary excretion of aquaporin-2 (AQP-2) water channel is exaggerated in patients with congestive heart failure dependent upon arginine vasopressin (AVP).

Methods. Sixty-five patients with congestive heart failure and eight age- and gender-matched control subjects were examined. The patients were divided into four groups according to the criteria of New York Heart Association (NYHA). Plasma AVP levels, urinary excretion of AQP-2, and cardiac index were determined.

Results. Plasma AVP levels were progressively increased following the severity of NYHA class in the patients with congestive heart failure. Cardiac index was inversely decreased, and there was a negative correlation between plasma AVP levels and cardiac index ($r = -0.430, P < 0.02$). Urinary excretion of AQP-2 was $187.3 \pm 50.2$ fmol/mg creatinine in the control subjects. It was markedly increased in the patients. Urinary excretion of AQP-2 was $1144.4 \pm 257.5$ and $990.5 \pm 176.0$ fmol/mg creatinine in the patients with NYHA class III and class IV, respectively, values significantly greater than the control subjects ($P < 0.05$). Urinary excretion of AQP-2 had a positive correlation with plasma AVP levels ($r = 0.280, P < 0.02$).

Conclusion. The present study indicates that exaggerated urinary excretion of AQP-2 is dependent on baroreceptor-mediated release of AVP in patients with congestive heart failure.

Impaired water excretion occurs in patients with congestive heart failure [1, 2]. In these clinical settings there is hypervolemic hyponatremia to various extents. Baroreceptor-mediated, nonsuppressible release of arginine vasopressin (AVP) is found despite hypo-osmolality, which should reduce AVP release to undetectable levels [3–7]. The administration of AVP V2 receptor antagonists determined the involvement of AVP in the impairment in water excretion in rats with heart failure [8, 9].

Aquaporin-2 (AQP-2) is an AVP-regulated water channel of collecting duct cells [10, 11]. In response to AVP, AQP-2 is translocated from cytoplasmic vesicles to apical plasma membranes, and increases water permeability [12–14]. Exogenous and endogenous AVP increases the expression of AQP-2 mRNA in the kidney [11, 12, 15, 16]. AQP-2 is in part excreted into the urine [17], which is measurable by radioimmunoassay (RIA), Western blot, and enzyme-linked immunosorbent assay (ELISA) using a specific antibody against AQP-2 [17–19]. There is a positive correlation between plasma AVP levels and urinary excretion of AQP-2 in normal subjects [20]. Augmentation in urinary excretion of AQP-2 is found in various disorders of impaired water excretion, including syndrome of inappropriate secretion of antidiuretic hormone (SIADH), hypopituitarism, mineralocorticoid-responsive hyponatremia of the elderly (MRHE), liver cirrhosis, and heart failure [21–25].

The present study was undertaken to determine whether urinary excretion of AQP-2 is increased in patients with congestive heart failure. Furthermore, the relationship between plasma AVP levels and urinary excretion of AQP-2 was analyzed.

METHODS

Subjects

Sixty-five patients with congestive heart failure were examined from January 2002 to December 2003. They were 51 males and 14 females, with the ages ranging from 30 to 83 years ($62.0 \pm 12.0$ years, mean $\pm$ SD). Because of varying symptoms closely linked to congestive heart failure the patients were admitted to the Cardiac Care Unit and the Cardiovascular Ward of Jichi Medical School Omiya Medical Center. The patients were divided into four subgroups according to the criteria of New York Heart Association (NYHA): NYHA class I,
15 patients; NYHA class II, 17 patients; NYHA class III, 15 patients; and NYHA class IV, 18 patients. The patients included acute and chronic heart failure and acute exacerbation of chronic heart failure; that is, five patients with hypertensive heart failure, 20 patients with ischemic heart disease, 15 patients with dilated cardiomyopathy, six patients with valvular disease, and 13 patients with miscellaneous causes. Sixteen patients had diabetes mellitus. However, patients with congestive heart failure were excluded if they had complicated with other edematous diseases or advanced renal diseases with serum creatinine levels more than 176.8 μmol/L. After the hospitalization, ultrasonic cardiogram was examined in all the patients, and Swan-Ganz catheterization was carried out in 30 of 65 patients to determine the cardiac hemodynamics. Eight age- and gender-matched control subjects (39 to 75 years) served as a control. On the admission, blood collections were made to determine serum sodium, plasma osmolality, and plasma AVP concentration while the subjects rested recumbent on the bed. Two-hour urine collections were made through bladder catheter to measure urinary osmolality, urine creatinine, and urinary excretion of AQP-2. The present study was approved by the Ethical Committee of Jichi Medical School for Human Study. We obtained informed consent from the subjects who joined into the present protocol.

Measurements
Blood was collected in chilled tubes containing ethylenediaminetetraacetic acid (EDTA)-Na₂ (1 mg per mL blood) and centrifuged at 3000 rpm at 4°C for 15 minutes. The supernatants were decanted and frozen at −20°C until the time of assay for plasma AVP. The collected urine samples were also frozen at −20°C until the time of assay for AQP-2. Plasma AVP was measured by RIA using AVP RIA kits (Mitsubishi Chemistry, Tokyo, Japan) [8]. AQP-2 was measured by ELISA [19, 21]. Plasma osmolality and urine osmolality were measured by freezing-point depression (Model 3W2) (Advanced Instruments, Needham Height, MA, USA). The normal value of plasma AVP is 0.2 to 2.2 pmol/L. That of urinary excretion of AQP-2 is 153.3 ± 28.1 fmol/mg creatinine.

Statistical analysis
All values were expressed as the means ± SEM. They were analyzed by Fisher’s t test to compare the difference. Simple linear regression analysis was performed to calculate correlations. The statistical package of StatView was employed for the present analysis. A P value of less than 0.05 was considered significant.

RESULTS
Table 1 shows serum sodium, plasma osmolality, urine osmolality and others in the patients with congestive
heart failure and the control subjects. Both serum sodium and plasma osmolality seemed likely to decrease following the upper classes of NYHA, but they were not statistically different from those in the control subjects. Also, there was no difference in urine osmolality among all the groups. The ratio of urine osmolality/plasma osmolality was not significantly different among any subgroup (data not shown). In most of the patients with NYHA class III and class IV medication with several drugs, including diuretics, had been performed persistently, and thus this may affect the present results at the hospitalization.

Plasma AVP levels and cardiac index in the patients with congestive heart failure and the control subjects are depicted in Figure 1. Plasma AVP levels were increased gradually in association with the upper classes of NYHA in the patients. The levels were elevated to 17.2 ± 3.5 and 29.4 ± 5.8 pmol/L in the subgroups of NYHA class III and class IV, respectively, values significantly higher than that in the control subjects (P < 0.05). Cardiac index was gradually decreased according to the severity of NYHA class (Fig. 1). Plasma AVP levels had a negative correlation with the cardiac index (r = −0.430, P < 0.02). We also determined pulmonary capillary wedge pressure (PCWP) and central venous pressure (CVP) in the patients. Both PCWP and CVP became greater according to the upper classes of NYHA (data not shown). However, there was no significant correlation between plasma AVP levels and PCWP (r = 0.260, P = 0.126), and between plasma AVP levels and CVP (r = 0.190, P = 0.345).

Figure 2 shows urinary excretion of AQP-2 in the patients with congestive heart failure and the control subjects. In the control subjects urinary excretion of AQP-2 was 187.3 ± 50.2 fmol/mg creatinine. It was remarkably increased in the patients with congestive heart failure. Urinary excretion of AQP-2 was 1144.4 ± 257.5 and 990.5 ± 176.0 fmol/mg creatinine in the subgroups of NYHA class III and class IV, respectively, which were significantly greater than that in the control subjects (P < 0.02).

Figure 3 depicts the relationship between plasma AVP levels and urinary excretion of AQP-2 in the patients with congestive heart failure. Urinary excretion of AQP-2 had a positive correlation with plasma AVP levels (r = 0.280, P < 0.02). The analysis was also carried out excluding the patients with NYHA class IV, because varying pathologic and/or medicating factors might influence the condition in those with NYHA class IV. Again, the positive correlation between them was evident and its significance became greater (r = 0.328, P < 0.02). However, urinary
excretion of AQP-2 did not correlate with urinary sodium excretion and urine osmolality (data not shown).

Table 2 shows the changes in serum sodium, plasma osmolality, plasma AVP, urinary excretion of AQP-2, and cardiac index in the congestive heart failure patients with NYHA class III and class IV, in whom Swan-Ganz catheterization was carried out at the initial periods of hospitalization. Serum sodium and plasma osmolality did not alter statistically during the catheter insertion. Both plasma AVP levels and urinary excretion of AQP-2 were markedly reduced, and they were further decreased to 4.7±0.9 pmol/L and 215.0±54.5 fmol/mg creatinine at the discharge, respectively. Inversely, the cardiac index was significantly increased from 2.1±0.1 to 2.9±0.2 L/min/m² (P<0.001).

DISCUSSION

The present study clearly demonstrated that urinary excretion of AQP-2 was exaggerated in the patients with congestive heart failure compared with that in the control subjects. The more increase in urinary excretion of AQP-2 was found in the patients with more severe grade of NYHA class. The alteration was closely in concert with the elevation of plasma AVP levels. However, we could not show severe hyponatremia and hypoosmolality in the patients with congestive heart failure despite of nonsuppressible release of AVP.

Clinical and laboratory experiments have demonstrated that impaired ability to excrete a water load in patients with congestive heart failure [1, 2, 26]. Persistent elevation of plasma AVP levels has been shown despite hypo-osmolality, which should suppress the osmotic release of AVP [3–8, 27]. These pathologic states are linked to hyponatremia to a various extent. RIA technique enables us to reliably measure plasma AVP levels. In most clinical settings nonsuppressible levels of plasma AVP are estimated to be relatively high as compared to the reduced plasma osmolality [1–8]. In the present study the plasma AVP levels reached extraordinary high levels in the patients with congestive heart failure. The elevation of AVP release was closely associated with the afferent pathways of baroreceptors, which was stimulated by reduced effective circulatory blood volume [1–8]. Plasma AVP levels had a negative correlation with the cardiac index. As most of patients in NYHA class III and class IV took diuretics, it is possible that this diuretic therapy may increase the excretion of diluted urine and further stimulate AVP secretion. We wonder the elevation of plasma AVP could be involved in cardiac derangement through V1a receptors as well as V2 receptors in congestive heart failure. In fact, the recent study showed that the administration of nonpeptide V1a and V2 receptor antagonist preserves cardiac function in congestive heart failure [28].

The present study further demonstrated exaggerated urinary excretion of AQP-2 in the patients with congestive heart failure. AQP-2 is the AVP-regulated water channel of collecting duct cells [10, 11]. Short-term regulation by AVP means that AQP-2 has cellular trafficking from the cytoplasmic vesicles to the apical plasma membranes; and vice versa after withdrawal of AVP stimulation [12–14, 29]. Long-term regulation means that AVP also regulates transcription and protein synthesis of AQP-2 [11, 12, 15, 29]. Recently, we clarified that AQP-2 is partly excreted into the urine, which is approximately 3% of AQP-2 in the collecting duct cells [17, 30]. There is no difference in urinary excretion of AQP-2 among the varying ages, ranging 24 to 76 years [30]. There were positive correlations between plasma AVP levels and urinary excretion of AQP-2 in the patients with congestive heart failure as well as in normal subjects [20]. In the patients with congestive heart failure, urinary excretion of AQP-2 was not progressively increased in concert with the grade of NYHA. The levels were somewhat low in the class IV compared with the class III. We may consider that receptor occupancy of AVP affected the V2 action in the kidney. Also, renal damage caused by lowered organ blood supply, as well as a series of intensive therapy might affect urinary excretion of AQP-2 in severe patients with NYHA class IV. There were heterogenous patients with congestive heart failure, and they influenced no finding of hyponatremia resulted from altered impairment in water excretion. In fact, most of patients had already treated with several drugs, including loop diuretics, antihypertensive agents, and so on before the admission. The finding of the exaggerated urinary excretion of AQP-2 may be tightly linked with the up-regulation of AQP-2 mRNA expression in the kidneys in the experimental models of congestive heart failure [31, 32]. There are only two reports regarding urinary excretion of AQP-2 in congestive heart failure [22, 25]. Martin et al [22] reported that...
percent decrease in urinary excretion of AQP-2 was found in patients after administering the nonpeptide AVP receptor antagonist. Petersen et al [25] showed an increase in excretion of AQP-2 in urine in patients with congestive heart failure, which was suppressed by an acute water load. In contrast, urinary AQP-2 excretion is elevated independently of plasma AVP in patients with liver cirrhosis [33]. The circulatory distress of decreased effective circulatory blood volume is similarly found in liver cirrhosis as well as congestive heart failure, which activates afferent baroreceptor pathway. The possible mechanism to make this difference could not be elucidated and further study will be needed.

As noted earlier, urinary excretion of AQP-2 was closely in concert with plasma AVP levels in the patients with congestive heart failure. Similar results were obtained with disorders of impaired water excretion, such as SIADH, hypopituitarism, and MRHE [21, 24]. Enhancement in urinary excretion of AQP-2 is derived from the augmented antidiuretic action of AVP. However, maximal increase in plasma AVP levels in the patients with NYHA class IV was not associated with urinary excretion of AQP-2, because it was not the greatest in the NYHA class IV patients. This alteration could be interpreted by several possibilities. First, there might be renal escape from AVP-induced anti-diuresis [34, 35]. In the experimental SIADH rats the attenuated up-regulation of AQP-2 gene leads to the decrease in urinary concentrating ability [34, 35]. Second, renal tubular damage due to lowered organ blood supply might reduce the expressed AQP-2 in collecting duct cells in the NYHA class IV patients. Third, intensive therapies before and after the admission might alter either AVP release or renal response to AVP. In addition, we could not find a relationship between urine osmolality and urinary excretion of AQP-2.

There were some reasons. The 2-hour urine collection was made at hospitalization to measure urine osmolality and urinary excretion of AQP-2. The patients with NYHA class III and class IV had taken loop diuretics and other drugs before the emergency admission. Also, the intensive therapy for congestive heart failure was started immediately after the hospitalization in these patients. These treatments may affect the urinary parameters, and the relation of urinary AQP-2 excretion to urinary osmolality, though urinary AQP-2 excretion shows positive correlation with urinary osmolality in normal volunteers [30]. The alterations during therapeutic period were found in the patients with NYHA class III and class IV, in whom Swan-Ganz catheter was inserted. Urinary excretion of AQP-2 was markedly decreased in concert with the reduction in plasma AVP levels. It was further decreased to 213.0 fmol/mg creatinine (mean) at the discharge, that is, close to the normal range. This finding during the therapeutic period strengthened the profound relation between plasma AVP levels and urinary excretion of AQP-2 in congestive heart failure.

CONCLUSION

We demonstrated that exaggerated urinary excretion of AQP-2 was found in the patients with congestive heart failure. The extraordinary high levels of plasma AVP in the NYHA class IV patients could produce renal escape from AVP-induced anti-diuresis, and might be involved in cardiac derangement directly through V1a receptors. The present findings indicate that urinary excretion of AQP-2 is increased dependent upon baroreceptor-mediated AVP release in the patients with congestive heart failure.

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REFERENCES


Table 2. Changes in serum sodium, modified plasma osmolality, plasma arginine vasopressin (AVP), urinary excretion of aquaporin-2 (UAQ-P-2) and cardiac index in the New York Heart Association (NYHA) class III and class IV patients with congestive heart failure

<table>
<thead>
<tr>
<th></th>
<th>Serum sodium mmol/L</th>
<th>Modified plasma osmolality mmol/kg</th>
<th>Plasma AVP pmol/L</th>
<th>UAQ-P-2 fmol/mg creatinine</th>
<th>Cardiac index L/min/m²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Swan-Ganz catheter</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Insertion</td>
<td>141.0 ± 1.0 (21)</td>
<td>294.1 ± 1.1 (21)</td>
<td>25.3 ± 5.5 (21)</td>
<td>1008.3 ± 213.6 (17)</td>
<td>2.2 ± 0.1 (18)</td>
</tr>
<tr>
<td>Removal</td>
<td>141.9 ± 0.6 (21)</td>
<td>294.8 ± 1.2 (21)</td>
<td>7.2 ± 1.1 (21)</td>
<td>527.2 ± 155.2 (17)</td>
<td>2.9 ± 0.2 (18)</td>
</tr>
<tr>
<td>P value</td>
<td>NS</td>
<td>NS</td>
<td>&lt;0.01</td>
<td>&lt;0.05</td>
<td>&lt;0.001</td>
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</tbody>
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Modified plasma osmolality means the plasma osmolality, in which blood glucose level was calculated as 5.5 mmol/L in the diabetic patients. The number of patients was shown in parentheses. Values are means ± SEM.


