Effects of terlipressin on the aquaretic system: evidence of antidiuretic effects

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Krag A, Bendtsen F, Pedersen EB, Holstein-Rathlou N-H, Møller S. Effects of terlipressin on the aquaretic system: evidence of antidiuretic effects. Am J Physiol Renal Physiol 295: F1295–F1300, 2008. First published August 27, 2008; doi:10.1152/ajprenal.90407.2008.—The vasopressin analog terlipressin is believed to cause vasoconstriction selectively by V1 receptor stimulation. However, a possible antidiuretic effect by V2 receptor stimulation has never been ruled out. Twenty-two patients with ascites, including seven with refractory ascites, were included. The subjects were studied during a 400 ml/h oral water load before and after infusion of 2 mg of terlipressin (18 patients) or placebo infusion (4 patients). Effects on the V2 receptors were assessed by evaluating aquaporin (AQP)2 excretion, free water clearance (CH2O), urine osmolality (Uosm), and fractional distal water excretion (ΔFeH2O). After terlipressin the excretion of AQP2 increased by 89% [144 ng/mmol creatinine, 95% confidence interval (CI) 73–214 ng/mmol creatinine, P = 0.001], CH2O decreased 1.05 ml/min (from 0.17 to −0.89 ml/min, P = 0.001), and ΔFeH2O decreased 37% (19 vs. 12; 95% CI 2–11, P = 0.01). Uosm increased by 27% (93 mosmol/kgH2O, 95% CI 23–164 mosmol/kgH2O, P = 0.02). Plasma sodium decreased 1.1 mmol/l (P < 0.01). An increase in AQP2 excretion and a decrease in CH2O and distal water excretion after terlipressin despite water loading is a clear indication of activation of the antidiuretic system (V2 receptor effect).

THE VASOPRESSIN ANALOG TERLIPRESSIN is widely used in the treatment of type 1 hepatorenal syndrome (HRS) (15, 22). Patients with HRS are characterized by avid sodium and water retention. Terlipressin is believed to selectively cause vasoconstriction by stimulation of V1 receptors (9). The V1 receptors are predominantly located in the smooth muscles of the arterial vasculature in the splanchnic region (6). Terlipressin (triglycyllysine vasopressin) is an analog of the natural arginine vasopressin (AVP), which has affinity for both V1 and V2 receptors (24). AVP-induced V2 receptor stimulation mediates water transport in the renal collecting ducts by increasing the number of aquaporin-2 water channels (AQP2) in the apical plasma membrane (2). V2 receptors are located on the principal cells in collecting ducts in the kidney (24). V2 receptor stimulation activates adenyl cyclase, which increases intracellular cAMP concentration (2). Increased intracellular cAMP stimulates phosphorylation of the AQP2 protein, which is then translocated from intracellular vesicles to the apical membrane (2, 16). AVP regulates AQP2 in both an acute and a chronic manner. Short-term regulation by AVP involves trafficking of vesicles with AQP2 molecules to the apical membrane. cAMP-stimulated transcription of the AQP2 gene is the long-term regulation of AQP2 (2, 16). Release of AVP from posterior pituitary gland is controlled by osmoreceptors in the anterior hypothalamus and arterial baroreceptors. AQP2 is recycled and endocytosed when cAMP levels decrease; however, a proportion is excreted in the urine (2, 8, 21). It has been shown that urine AQP2 (U-AQP2) reflects the action of AVP on the collecting ducts (20). It is a very dynamic system, and changes in AQP2 appear within minutes after relevant stimuli (20).

In a previous study on renal effects of terlipressin (9), we observed a decrease in free water clearance (CH2O) in the terlipressin group, despite oral water loading and increased glomerular filtration rate (GFR). An antidiuretic effect of terlipressin, which is a potential clinically relevant side effect in relation to treatment of HRS, has never been investigated.

We hypothesized that terlipressin has affinity to V2 receptors and interferes with the aquaretic system. Therefore, we aimed at investigating the effects of terlipressin on AQP2 excretion and renal water handling in patients with cirrhosis and ascites during oral water loading.

MATERIALS AND METHODS

Patients. Twenty-two patients between 18 and 75 yr of age with alcoholic cirrhosis and ascites, including seven with refractory ascites, four of which had type 2 HRS according to current criteria (22), were included. The subjects were studied during a 400 ml/h oral water load before and after infusion of 2 mg of terlipressin (18 patients) or placebo infusion (4 patients).

None of the patients had experienced gastrointestinal bleeding within the last week before the study or had signs of insulin-dependent diabetes, acute or chronic intrinsic renal or cardiovascular disease, arterial hypertension, abnormal electrocardiogram (ECG) (apart from QT prolongation), or any acute medical conditions such as infections or acute heart or lung disease. Furthermore, alcohol abstinence for 6 wk was required. A negative history of arterial hypertension and cardiac or pulmonary disease, a normal clinical examination apart from signs of portal hypertension and cirrhosis, and a normal ECG excluded preexisting cardiac diseases. Diuretics and beta-blockers were discontinued 3 days before the investigations. None of the patients was receiving any other drugs that could interfere with

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A 5 ml/h constant infusion of 51Cr-EDTA was given as a rapid intravenous bolus injection with 6.5 MBq of 51Cr-EDTA, followed by a constant infusion at 8 ml/h (Kivex P 300 pump, Hoersholm, Denmark) prepared in 60 ml of isotonic saline containing 16 MBq of 51Cr-EDTA and urine osmolality (Uosm). V1 receptor effects were evaluated by mmol/day sodium diet.

The study was approved by the Danish Medicines Agency (EudraCT no. 2004.000568-29), and the Regional Ethics Committee also approved the study (KF 02-059/04); all subjects gave informed consent.

Methods. Effects on the V2 receptors were assessed by evaluating AQP2 excretion, \( C_{\text{AQP2}} \), urine volume (\( V_u \)), osmolar clearance (\( C_{\text{osmol}} \)), and urine osmolality (\( U_{\text{osmol}} \)). V1 receptor effects were evaluated by changes in mean arterial pressure (MAP).

The patients were studied at 9:00 AM after a 9-h fast. At 10:00 PM the previous day, an oral dose of 300 mg of lithium carbonate was administered. An oral water load of 200 ml of tap water was given every half-hour from 9:00 AM to the end of the clearance periods. The patients were in the supine position throughout the investigation. All patients had a bladder catheter placed before the clearance periods to ensure correct urine sampling.

51Cr-EDTA was used to determine GFR. Infusion of tracers was prepared in 60 ml of isotonic saline containing 16 MBq of 51Cr-EDTA (GE-Healthcare, Hilleroed, Denmark). At 9:00 AM, a priming dose of 8 ml of the 51Cr-EDTA solution was given as a rapid intravenous bolus injection together with 6.5 MBq of 51Cr-EDTA, followed by a constant infusion at 8 ml/h (Kivex P 300 pump, Hoersholm, Denmark) for 3.5 h, in total 15 MBq.

\( V_u \) was recorded, and samples were assayed for lithium, sodium, osmolality, and 51Cr-EDTA. A gamma counter (Wallac 1480 WIZARD 3, Turku, Finland) assessed the radioactivity of 51Cr-EDTA in the samples. The samplings were repeated at 30-min intervals, resulting in three clearance periods after equilibration and before intervention. Blood and urine were collected at the end of each clearance period. Thereafter, patients with nonrefractory ascites were randomized to bolus infusion of 2 mg of terlipressin (Ferring Pharmaceuticals, Copenhagen, Denmark) or 10 ml of isotonic saline, whereas all patients with refractory ascites received 2 mg of terlipressin. Urine and blood samplings for renal function tests were then repeated for three more clearance periods of 30 min.

Plasma and urinary lithium concentrations were measured by atomic absorption spectrophotometry (Perkin Elmer 2380). Sodium in plasma and urine was measured by flame emission spectrophotometry (Perkin Elmer 2380). Plasma and urinary osmolarities were measured by the method of freezing point depression (Osmomat 030-D, Gonotec, Berlin, Germany).

U-AQP2 was measured by radioimmunoassay as previously described (20). Urine samples were centrifuged for 5 min at 3,000 rpm. Supernatant (125–3,000 μl) was freeze dried and kept frozen at −20°C until being assayed. Rabbit anti-AQP2 antibody for radioimmunoassay was obtained from Sören Nielsen (Water and Salt Research Centre, Institute of Anatomy, Aarhus University, Aarhus, Denmark). The minimal detection level was 32 pg/tube. The coefficients of variation were 11.7% (interassay) and 5.9% (intra-assay).

Urine CAMP (U-cAMP) was measured with a kit obtained from R & D Systems (Minneapolis, MN). The minimal detection level was 12.5 pmol/tube. The coefficients of variation were 6.9% (interassay) and 5.3% (intra-assay).

AVP was extracted from plasma with C18 Sep-Pak (Water Associates, Milford, MA) and subsequently determined by radioimmunoassay (19). The antibody against AVP was a gift from Prof. Jacques Dür (Bay Pines VA Health Care System; Bay Pines, FL, and the University of South Florida, College of Medicine, Tampa, FL). The minimal detection level was 0.5 pmol/l. The coefficients of variation were 13% (interassay) and 9% (intra-assay).

Plasma concentrations of norepinephrine were determined by high-performance liquid chromatography, as described elsewhere (13). The intra-assay and interassay coefficients of variation were 8% and 9%, respectively. The plasma renin concentration was determined by commercially available two-site immunoradiometric assay (DGR International, Hamburg, Germany). The mean plasma concentration of renin in 536 healthy subjects was 26 pg/ml (range 5.2–33.4 pg/ml) (14). Aldosterone was measured with a commercial radioimmunoassay kit (DSL-8600, Diagnostic Systems Laboratories, Webster, TX). The mean morning plasma concentration in 73 healthy adults in the supine position was 192 pmol/l (range 80–450 pmol/l) (14).

Table 1. Demographic, clinical, and biochemical characteristics of 22 patients with cirrhosis and ascites

<table>
<thead>
<tr>
<th></th>
<th>Terlipressin Group (n = 18)</th>
<th>Placebo Group (n = 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>56.4±8.1</td>
<td>57.5±9.5</td>
</tr>
<tr>
<td>Sex, M/F</td>
<td>11/7</td>
<td>1/3</td>
</tr>
<tr>
<td>Child-Pugh score</td>
<td>9.6±2.0</td>
<td>7.8±1.5</td>
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<tr>
<td>MELD score</td>
<td>11.2±6.0</td>
<td>5.5±2.4</td>
</tr>
<tr>
<td>Ascites grade 1/2/3</td>
<td>5/6/7</td>
<td>2/1/0</td>
</tr>
<tr>
<td>Spironolactone, mg/day</td>
<td>200 (0–400)</td>
<td>100 (50–200)</td>
</tr>
<tr>
<td>Furosemide, mg/day</td>
<td>80 (0–320)</td>
<td>80 (0–160)</td>
</tr>
</tbody>
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Values are means ± SD or medians (range) for n subjects. Reference intervals are in parentheses. M, male; F, female; MELD, Model for End-Stage Liver Disease; HR, heart rate; MAP, mean arterial pressure. Comparison between terlipressin group and placebo group: *P < 0.05.

![Fig. 1. Urine aquaporin (AQP2) excretion in milligrams per millimole of creatinine at baseline and in three 30-min clearance periods after 2-mg terlipressin infusion. *P < 0.05, **P < 0.01 compared with baseline.](http://ajprenal.org/2008/11/F1296/10.1152/ajprenal.00568-2008)
Calculations. Clearance during steady state was calculated by the standard formula \( C_x = U_x \times P_x / V_x \), where \( C_x \) is renal clearance of substance \( x \), \( U_x \) is concentration in urine of substance \( x \), \( V \) is urine flow rate, and \( P_x \) is mean plasma concentration of substance \( x \) in the clearance period. Lithium clearance (\( C_{Li} \)) is used as a marker of proximal delivery of fluid to the distal nephron segments based on the assumption that lithium is filtered freely across the glomerulus and reabsorbed in proportion to sodium and water in the proximal tubules and that no reabsorption or secretion takes place in the distal nephron segments. The excretion fraction (\( FE \)) of substance is calculated as \( FE = V_x / C_x \). 

Statistics. Results are expressed as means ± SD or medians (range). Paired differences are expressed as mean changes and 95% confidence interval for the difference (95% CI). Concentrations of vasoactive substances are additionally shown as medians and total range due to nonnormal distribution. Statistical analyses were performed by unpaired Student’s \( t \)-test or the Mann-Whitney test and paired Student’s \( t \)-test or the Wilcoxon test, as appropriate. All reported \( P \) values are two tailed, with values <0.05 considered statistically significant. The SPSS 10.1 statistical package (SPSS, Chicago, IL) was applied throughout.

RESULTS

Demographic, clinical, and biochemical variables are shown in Table 1. Terlipressin induced an increase in MAP of 18 ± 12 mmHg (\( P < 0.001 \)) vs. –0.5 ± 10.8 mmHg in the placebo group (\( P = 0.03 \)), and GFR increased by 17 ± 23 ml/min (\( P < 0.01 \)) vs. –3 ± 4 ml/min in the placebo group (\( P < 0.01 \)).

Figure 1 shows that U-AQP2 given as nanograms per millimole of creatinine gradually increased after terlipressin infusion but not after placebo infusion. U-AQP2 increased significantly already in the first clearance period after terlipressin, from 160 ± 82 to 217 ± 91 ng/mmol creatinine (\( P = 0.02 \)) and continued to increase throughout the second (\( P = 0.005 \)) and third (\( P = 0.001 \)) periods. The same pattern was seen when U-AQP2 excretion was given as a rate in nanograms per minute (Fig. 2); however, the difference only reached significance in the third clearance period. The overall change, from baseline to the third period, in U-AQP2 excretion in nanograms per millimole of creatinine was an increase of 89% (144 ng/mmol creatinine, 95% CI 73–214 ng/mmol creatinine; \( P = 0.001 \)). (Table 2, Fig. 1). We observed no change in cAMP excretion (Table 2).

Figure 3 shows the change in \( C_{H_2O} \) in response to terlipressin. In concordance with the change in AQP2 excretion, \( C_{H_2O} \) decreased below zero throughout the clearance periods after terlipressin.

From baseline to the third period, \( C_{H_2O} \) decreased 62% (1.05 ml/min, 95% CI 0.48–1.62 ml/min; \( P = 0.001 \)) (Table 2) (Fig. 3). \( C_{Li} \), which is a measure of delivery of fluid to the distal nephron segments, increased significantly after terlipressin (Table 2). In the terlipressin group \( DFeH_2O \) decreased 37% (19% vs. 12%; 95% CI 2–11%; \( P = 0.01 \)) (Fig. 4); similarly,
absolute distal water reabsorption increased ($P = 0.02$) (Table 2). Plasma sodium decreased a mean of 1.1 mmol/l (95% CI 0.4–1.8 mmol/l; $P < 0.01$) (Table 2); the individual values are shown in Fig. 5. Urinary volume and fractional water excretion were unchanged (Table 2). $C_{\text{OSM}}$ increased by 48% (0.84 ml/min, 95% CI 0.17–1.52 ml/min; $P = 0.02$) and $U_{\text{osm}}$ increased by 27% (93 mosmol/kgH$_2$O, 95% CI 23–164 mosmol/kgH$_2$O, $P = 0.02$) after terlipressin (Table 2). Plasma osmolality remained constant at 271 mosmol/kgH$_2$O in both groups (Table 2). Because of cross-reaction between AVP and terlipressin in the assay used, the values after terlipressin were above the upper detection limit for this assay in all patients who received terlipressin (Table 2). Therefore, the results only reflect that all patients received terlipressin in this group.

In the placebo group no statistically significant changes were observed. However, there was a clear tendency toward an increase in CH$_2$O and a decrease in Uosm in response to oral water alone (Table 2).

**DISCUSSION**

The main findings of this study were that terlipressin 1) induces AQP2-mediated antidiuresis and 2) induces a decrease in plasma sodium.

![Free water clearance after terlipressin](image1)

**Fig. 3.** Free water clearance at baseline and in three 30-min clearance periods after 2-mg terlipressin infusion. **$P < 0.01$** compared with baseline.

![Distal fractional water excretion](image2)

**Fig. 4.** Distal fractional water excretion at baseline and in the clearance period from 60 to 90 min after 2-mg terlipressin infusion. **$P < 0.01$** compared with baseline.

![Plasma sodium](image3)

**Fig. 5.** Plasma sodium in millimoles per liter at baseline and at 90 min after 2-mg terlipressin infusion. **$P < 0.01$** compared with baseline.

To our knowledge this is the first study to investigate the effect of terlipressin on AQP2-mediated water retention. In the first human study with terlipressin in five healthy subjects and in two early rat studies a decrease in diuresis was observed after terlipressin; however, these findings were never explored further (3, 10, 17). This lack of interest in antidiuresis was probably because people focused on the effects on bleeding. However, today terlipressin has become a standard treatment in HRS in several countries, and an antidiuretic effect in these patients might be an important side effect.

AVP and terlipressin are very similar in structure. AVP is a peptide that consists of nine amino acids (Cys-Tyr-Phe-Gln-Asn-Cys-Pro-Arg-Gly), with the cysteine residues forming a sulfur bridge. Terlipressin is a synthetic 12-amino acid peptide (1-triglycyl-8-lysine-vasopressin) derived from the natural hormone lysine-vasopressin. The amino acid sequence of terlipressin acetate is Gly-Gly-Gly-c[Cys-Tyr-Phe-Gln-Asn-Cys]-Pro-Lys-Gly-NH$_2$. With respect to the molecular structure it is not surprising that terlipressin also has affinity to V$_2$ receptors and can induce antidiuresis.

We observed an increase in $U_{\text{osm}}$ and a decrease in CH$_2$O in the terlipressin group. Theoretically, this could be the result of V$_2$ receptor-mediated water reabsorption in the collecting ducts or could be due to decreased delivery of fluid to the distal part of the nephron. However, since there was a significant increase in GFR, and most importantly in C$_{\text{cl}}$, which reflects increased delivery of fluid to the distal nephron segments, we conclude that the increase in $U_{\text{osm}}$ and the decrease in CH$_2$O must be due to an increased water reabsorption in the distal nephron segments, including the collecting ducts. This suggests that the V$_2$ receptor stimulation was intense, which corresponds with the observed increase in AQP2 excretion and in absolute distal water reabsorption. In contrast, terlipressin increases sodium excretion without affecting the distal sodium reabsorption (9).

We observed an absolute decrease in mean plasma sodium of 1.1 mmol/l 90 min after terlipressin. This is not only statistically significant but may well be clinically significant if the effect is sustained. However, it should be noted that the changes in plasma sodium were not based on a tonicity balance calculation, which may provide a better description...
of the dynamics in the system (11). A major drawback in these patients would be the immense sodium and water retention with tense ascites in which estimations of total body water would be inaccurate and hamper such calculations.

The decrease in plasma sodium is the reflection of terlipressin’s ability to retain water as shown in this study and to induce natriuresis as previously shown (9). In studies in HRS terlipressin has been shown to improve plasma sodium, however, only in combination with albumin (18, 25). In the study by Ortega et al. (18) terlipressin alone did not improve serum sodium, in contrast to a significant increase in the patients who received terlipressin and albumin. Other studies support that terlipressin alone does not improve serum sodium (4, 5). This suggests that the improvements in hyponatremia observed in HRS studies are related to albumin alone and not to terlipressin. This is supported by a recent study in which severe hyponatremia was corrected by albumin infusion alone and associated with increased C\textsubscript{H\textsubscript{2}O} (7).

The present study investigated the acute effects of terlipressin, while treatment with terlipressin in HRS is usually for longer periods. It is well known that terlipressin in combination with albumin induces diuresis in HRS and is associated with an ~33% rate of complete response in terms of decreasing creatinine below 133 mmol/l (12, 23). Therefore, in responders the improved hemodynamics due to the effect of terlipressin on V\textsubscript{1} receptors and volume expansion with albumin may outweigh the antiuretic effects and increase diuresis during prolonged treatment. Furthermore, endogenous AVP levels, which were high at baseline, may decrease in long-term treatment. Terlipressin probably unloads the baroreceptor drive by increasing MAP and thereby decreases endogenous AVP release and reduces the overall antiuretic state.

Baseline U-AQP2 excretion was lower than observed in healthy subjects in a previous study (20), mean of 437 vs. 160 ng/mmol creatinine and mean of 3.9 vs. 1.0 ng/min, which is in accordance with recent published findings (1). AVP concentration at baseline in these patients was a mean of 2.4 pg/ml, which is elevated compared with a mean of 1.0 pg/ml in healthy subjects observed in a different study in which the same methods were used (20). P\textsubscript{osm} in the healthy control subjects was 284 mosmol/kgH\textsubscript{2}O compared with 272 mosmol/kgH\textsubscript{2}O in our patients. Under normal physiological circumstances a P\textsubscript{osm} of 272 mosmol/kgH\textsubscript{2}O would suppress AVP to an immeasurable level. The most likely explanation for AVP hypersecretion in cirrhosis is a nonosmotic baroreceptor-mediated release. This is probably an adaptive response to maintain MAP and arterial filling in order to ensure organ perfusion and oxygenation, which is more important than a stable P\textsubscript{osm}. In this situation the osmotic control of AVP release is overridden by the baroreceptor drive. Excretion of the second messenger (cAMP) in the AVP-AQP2 signaling pathway did not increase after terlipressin despite increased AQP2. cAMP is located intracellularly, and increased levels might not translate into increased urine levels with the observation period.

Implications. In patients with cirrhosis and ascites where aquaretics and natriuretics are desired, a combined V\textsubscript{1} and V\textsubscript{2} receptor effect is not optimal. Resolution of HRS demands improved GFR along with salt and water excretion. In responders in HRS treatment, it seems that terlipressin when combined with albumin acts in a balance between V\textsubscript{1} and V\textsubscript{2} receptor effects, where the beneficial effects of improved hemodynamics outweigh the V\textsubscript{2}-mediated antiuretic effects. This is probably not the case when terlipressin is used alone. In the 66% of patients who are partial responders or nonresponders to terlipressin and albumin (12, 23) V\textsubscript{2} effects could play a role in the lack of improvement in renal function. In patients with hyponatremia or oligouric HRS the antiuretic effects of terlipressin may be harmful and could be an explanation for the lack of response.

Conclusions. The increase in AQP2 excretion and the decrease in C\textsubscript{H\textsubscript{2}O} and distal water excretion after terlipressin despite water load are a clear indication of activation of the antidiuretic system. This response may influence the diuretic response and the ability to correct hyponatremia. The clinical significance in relation to treatment of HRS remains to be settled.

GRANTS

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