Congestive heart failure in rats is associated with increased collecting duct vasopressin sensitivity and vasopressin type 2 receptor reexternalization

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Brød L, Müllertz KM, Torp M, Nielsen J, Græbe M, Hadrup N, Nielsen S, Christensen S, Jonassen TE. Congestive heart failure in rats is associated with increased collecting duct vasopressin sensitivity and vasopressin type 2 receptor reexternalization. *Am J Physiol Renal Physiol* 305: F1547–F1554, 2013. First published October 2, 2013; doi:10.1152/ajprenal.00461.2012.—A number of studies have shown that rats with congestive heart failure (CHF) have increased protein levels of the vasopressin (AVP)-regulated water channel aquaporin-2 (AQP2) even during conditions with unchanged circulating levels of AVP, suggesting an increase in the sensitivity of the AVP type 2 (V2) receptor in experimental CHF. The present study was aimed at investigating AVP signaling in rats with moderate CHF (left ventricular end diastolic pressure >10 mmHg; normal plasma AVP levels) induced by ligation of the left anterior descending coronary artery. Sham-operated rats were used as controls. Western blotting analyses revealed an increased abundance of AQP2 in renal cortex (+33 ± 9% of sham; P < 0.05) and in inner medulla (IM) (+54 ± 15% of sham; P < 0.05) in CHF rats compared with sham-operated controls. Dose-response studies on isolated collecting ducts (CDs) showed an increased accumulation of cAMP in response to AVP in CHF rats compared with controls. V2 receptor surface-binding studies in isolated IMCDs showed a marked and comparable AVP-induced V2 receptor internalization in response to AVP in both CHF and control rats. As expected V2 receptor surface binding remained low after AVP challenge in control rats. In contrast to this, V2 receptor surface binding returned to pre-AVP levels within 30 min in the CHF rats, indicating an obtained recycling ability of the V2 receptor in CHF. Together the results indicate the presence of an increased AVP sensitivity in the CDs from CHF rats, associated with an acquired recycling ability of the V2 receptor.

AQP2

**CONGESTIVE HEART FAILURE (CHF)** is associated with sodium and water retention and in severe cases associated with excessive water retention because of increased levels of circulating vasopressin (AVP), resulting in dilutional hyponatremia. Severe CHF with dilutional hyponatremia has a poor prognosis (3). The dilutional hyponatremia is most likely the result of increased circulating levels of AVP, and it has been shown that treatment with an AVP type 2 receptor (V2 receptor) antagonist increases plasma sodium concentration in CHF patients with dilutional hyponatremia (28).

It is well described that AVP regulates renal collecting duct (CD) water permeability through stimulation of aquaporin-2 (AQP2) (21, 22). Experimental studies in CHF rats have shown an association between increased plasma AVP levels, hyponatremia and an increased protein expression, and a membrane targeting of AQP2 (23, 38), thereby confirming a central role for AQP2 in dysregulation of CD water reabsorption and development of hyponatremia. However, we have in previous studies been able to show increased protein levels of AQP2 in CHF in rats with unchanged plasma sodium concentrations and where circulating levels of AVP were within the normal physiological range and unchanged compared with control animals (10). This finding suggests that CHF may have an exaggerated CD water reabsorption, even in the presence of normal circulating levels of AVP.

In contrast to CHF, other animal models with impaired water handling such as liver cirrhosis (4, 8, 9, 13, 15), nephrotic syndrome (1), and rats subjected to combined water loading and V2 receptor agonist treatment (6, 7) show an “escape” from the effect of AVP on the CDs. This escape phenomenon is characterized by a marked downregulation of AQP2 despite sustained elevated plasma levels of AVP. At the cellular level the principal cells of the CDs, in both cirrhosis and the combination of water loading and V2 receptor agonist treatment, are associated with a decreased cAMP production in response to AVP stimulation (4, 6). Thus, conditions associated with an AVP escape are characterized by an ability to prevent water retention and hyponatremia in the presence of increased plasma AVP concentrations by mechanisms that include an uncoupling of V2 receptor signaling in the CDs. Therefore, it is of particular interest to study the mechanisms responsible for increased AQP2 protein levels in CHF to expand the knowledge of excessive water retention and the fatal consequences associated with this in CHF.

Because CHF apparently is associated with an increased CD sensitivity to AVP resulting in increased AQP2 levels despite normal circulating levels of AVP, it seems logical to examine whether cAMP production in response to AVP, in isolated CD from rats with experimentally induced CHF, is increased compared with control animals.

Stimulation of the V2 receptor by AVP also triggers signals for receptor internalization (24, 27, 31), a process including G protein-coupled receptor kinases, receptor phosphorylation, and the endocytotic machinery of β-arrestins and clathrins (27). Upon ligand-induced receptor internalization, G-coupled receptors are most commonly recycled to the plasma membrane. However, previous studies have shown that this may not be the case for the V2 receptor (2, 5, 11, 12). Consequently, it could be of interest to examine whether experimentally induced CHF is associated with changes in receptor internalization and recycling abilities that could contribute to the apparent increased AVP sensitivity in CDs.
The present study was therefore aimed at elucidating AVP signaling and AQP2 regulation in CHF rats, with special emphasis on possible changes in signal transduction after AVP stimulation as well as on a possible role of altered V2 receptor internalization/recycling properties in isolated CDs from CHF rats.

METHODS

Animals and Physical Environment

Female Wistar rats (250–300 g body wt; Charles River, Hannover, Germany) were housed in a temperature (22–24°C)- and moisture (40–70%)-controlled room with a 12:12-h light-dark cycle (lights on from 6:00 A.M. to 6:00 P.M.) and with free access to tap water and standard rat chow. Animals were treated with buprenorphine (0.1 mg/kg sc) three times a day for two consecutive days after all surgical procedures. The animal studies were conducted following the guidelines for the care and handling of laboratory animals established by the Danish government. Specifically, the study was approved by the Danish Animal Experiments Inspectorate under the Ministry of Food, Agriculture, and Fisheries as well as approved locally by the Department of Experimental Animals at the University of Copenhagen, Denmark.

Model of CHF

CHF was induced by ligation of the left anterior descending coronary artery (LAD) under isoflurane anesthesia (10, 23, 29). Sham-operated rats were used as controls. Three weeks after surgery, CHF was evaluated by measurement of left ventricular end diastolic pressure (LVEDP) using a Millar pressure catheter SPR-407 (10). LAD-ligated rats with LVEDP >10 mmHg were included in the study as CHF rats.

Plasma AVP Concentrations

A separate series of CHF rats was permanently instrumented (isoflurane anesthesia) with medical-grade Tygon catheters in the femoral artery 2 wk after the initial CHF or sham operation (14, 26). Blood samples were collected under unstressed conditions 4 wk after the initial operation, and plasma AVP was measured by radioimmunoassay as previously described (4, 17).

CD Preparations

Cortical CDs (CCD) and CDs from the inner stripe outer medullary (ISOMCD) were isolated by microdissection, whereas inner medullary CDs (IMCD) were obtained by a combined digestion/centrifugation method originally described by Stokes and coworkers (31).

Microdissection. During isoflurane anesthesia, the left kidney was perfused in vivo with collagenase solution, and CCDs and ISOMCDs were microdissected and used for the measurement of AVP-induced cAMP accumulations as described previously (4).

IMCD cell suspension. The peritoneum was bathed for 1 min in ice-cold Hanks’ solution (isoflurane anesthesia), the kidneys were rapidly removed, and the inner medulla (IM) was dissected and digested in Hanks’ solution containing 0.12 U/ml collagenase A (37°C, 95% O2-5% CO2 aeration). IMCDs were obtained by repeated centrifugation (2 min, 2,500 g) of the collected supernatants, and protein concentration of the IMCD cell suspension was measured (Pierce BCA protein kit). During the setup of the method, it was tested whether the repeated centrifugations were able to separate the IMCDs from the other inner medullary structures (i.e., the thin descending and ascending limb of the loop of Henle) and blood vessels (vasa recta). The IMCD enrichment method applied was to conduct Western blot analysis to identify the presence of AQP1 (specific for thin limb of Henle and vasa recta showing) on samples generated from pellets and supernatant. These analyses identified a well-defined signal of AQP1 in the samples generated from the pellets and a very weak signal in samples made of the supernatants, confirming that method generated a suspension rich in IMCD with only minor amounts of thin limbs and vasa recta (data not shown). For details regarding the Western Blot analyses, see below. The IMCD cell suspension was used both for V2 receptor binding/recycling studies and for studies of AVP-induced cAMP accumulations.

AVP V2 Receptor Binding/Recycling

The IMCD cell suspension was loaded (45–50 μg protein) on MultiScreen-FC plates and incubated (20 min, 37°C and 5% CO2) with or without the presence of 10−7 M AVP (in DMEM/F-12), allowing V2 receptor sequestration. Stimulation was terminated by washing (D-PBS with 5 mM acetic acid); fresh DMEM/F-12 was added, and V2 receptor recycling was allowed (37°C and 5% CO2, 0, 15, 30, and 120 min). V2 receptors on the cell surface were detected after labeling with 10 nM [3H]AVP (35–85 Ci/mmol, NET-800; PerkinElmer). Radioactivity was measured using MicroScint 20 (Packard) and the Topcounter-NXT (Packard) (33).

Nonspecific binding was minimized by precoating the Multi-Screen-FC plates in 0.3% polyethylenimine (PEI) (in D-PBS, 2 h, room temperature). Saturation studies were obtained by incubating IMCDs in the presence of [3H]AVP concentrations ranging from 10 to 50 nM.

AVP Stimulated cAMP Accumulation in CDs

CDs were incubated and analyzed using the commercial cAMP Enzyme Immunoassay kit (Cayman Chemicals) as previously described (4). cAMP levels were expressed relative to length of the isolated CDs (CCD and ISOMCD) or by protein amount in the given CD sample (IMCD).

Western Blotting

The right kidney was removed, and the cortex, outer medulla (OM), and inner medulla were isolated and immediately frozen in liquid nitrogen and stored at −80°C until processing for membrane fractionation. The samples were homogenized using a tissue homogenizer (Ultra-Turrax T8; Ika, Staufen, Germany) in 9 ml ice-cold homogenization buffer containing 300 mM sucrose, 25 mM imidazol, 1 mM EDTA-disodium salt, and the following protease inhibitors: 0.1 mg/ml Pefabloc buffer, 4 μg/ml leupeptin buffer, and phosphatase inhibitors (184 μg/ml sodium orthovanadate buffer, 1.05 mg/ml sodium fluoride buffer, and 82 ng/ml okadecic acid buffer; pH was adjusted to 7.2 with 0.1 M HCl).

After centrifugation (4,000 g, 4°C, 15 min) the supernatant was isolated, and the protein concentration was measured by use of a commercial kit (Pierce BCA Protein Assay Reagent Kit catalog no. 23226; Pierce, Rockford, IL). All samples were diluted to a final protein concentration of 1 μg/μl adding sample buffer (in the final solution: 486 mM Tris·HCl pH 6.8, 8.7% glycerol, 104 mM SDS, and 0.0875 mM bromphenol blue), dithiothreitol (25 mM in the final solution), and homogenizing buffer. Finally, the samples were solubilized at 90°C for 10 min. The samples were run on 12% polyacrylamide gels, and the proteins were electrotransferred to polyvinylidene difluoride membranes (Millipore, Bedford, MA). After unspecific binding sites were blocked (60 min in PBS-T buffer containing 5% milk), the membranes were probed overnight at 4°C with the appropriate primary antibody. The labeling was visualized with horseradish peroxidase-conjugated secondary antibody (P448; diluted 1:3,000; Dako, Glostrup, Denmark) using an enhanced chemiluminescence system (ECL+; Amersham). For AQP1 (data not shown) and AQP2 the 29- and 35- to 50-kDa bands, corresponding to nonglycosylated and glycosylated protein, respectively, and for β-arrestin2 a 48-kDa band were scanned by a FluorX multimager (Bio-Rad Laboratories). Densitometry of individual bands was quantitated using the software AJP-Renal Physiol • doi:10.1152/ajprenal.00461.2012 • www.ajprenal.org
Table 1. LVEDP and pAVP concentrations (in a subset of animals chronically instrumented with femoral arterial catheters for collection of blood samples in the conscious state during unstressed conditions) in CHF rats and sham-operated control rats.

<table>
<thead>
<tr>
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<th>LVEDP, mmHg</th>
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<th>pAVP, pg/ml</th>
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<tr>
<td>Sham</td>
<td>4.8 ± 0.3</td>
<td>31</td>
<td>3.5 ± 1.3</td>
<td>6</td>
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<tr>
<td>CHF</td>
<td>15.0 ± 1.1*</td>
<td>32</td>
<td>1.9 ± 0.8</td>
<td>10</td>
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Values are means ± SE; n, no. of rats. LVEDP, left ventricular end diastolic pressure; pAVP, plasma vasopressin; CHF, congestive heart failure. *P < 0.05 vs. sham.

In the present study, Western blot analyses of AQP2 protein levels in the cortex, OM, and inner medulla showed that both the cortical and inner medullary (IM) levels of AQP2 protein were increased in CHF rats compared with sham-operated controls [cortical: CHF 1.33 ± 0.09 (n = 6) vs. sham 1.00 ± 0.02 (n = 7), P < 0.05 and IM: CHF 1.54 ± 0.15 (n = 8) vs. sham 1.00 ± 0.07 (n = 6), P < 0.05; see Fig. 1, A and C]. No difference was found analyzing AQP2 protein expression in OM (Fig. 1B). The finding of increased AQP2 protein levels in kidneys from CHF is in line with previous reports (10, 23, 38), and, like in the study by Lütken et al. (20), the upregulation seemed segmental, i.e., upregulation in the inner medulla and cortex but not in the OM.

**AVP Induced cAMP Accumulation**

It is well described that AVP regulates the AQP2 water channel through V₂ receptor activation and cAMP accumulation. Isolated CDs were therefore used for AVP/cAMP dose-response studies to test whether CHF is associated with altered AVP sensitivity. Measurement of cAMP accumulation in response to AVP stimulation (10⁻¹⁰, 10⁻⁹, 10⁻⁸, and 10⁻⁶ M, 30°C, 20 min) showed both increased cAMP levels during baseline conditions and increased maximal cAMP accumulation in response to AVP in CCDs (Fig. 2A). Similarly, the AVP-mediated cAMP accumulation was significantly increased in the IMCD (Fig. 2E), whereas there were no differences either in baseline levels or in AVP-stimulated cAMP in OMCD (Fig. 2C). The potency of AVP against the V₂ receptor evaluated through EC₅₀ was as follows: CCD: CHF 2.9 nM vs. sham 1.1 nM; OMCD: CHF 1.3 nM vs. sham 2.0 nM; IMCD: CHF 10.0 nM vs. sham: 45.3 nM. Consequently, CHF was not
associated with changes in the potency of the AVP against the V2 receptor. However, it should be noticed that the potency obtained in the IMCD suspension from both CHF and sham was lower than what was found in the microdissected CCD and OMCDs. The cAMP accumulation in response to AVP in the presence of the unspecific phosphodiesterase inhibitor isobutyl methylxanthine (IBMX) was also increased in both the CCD (Fig. 2B) and IMCD (Fig. 2F). This suggests that the increased cAMP accumulation found in the CCDs and IMCDs is because of increased cAMP production and not an alteration in cAMP turnover. Together these data suggest that CHF rats have increased AVP sensitivity in CCD and IMCD, but not in OMCD, which seems to match the findings of increased AQP2 levels in CCDs and IMCDs but not in OMCDs.

**Binding Characteristics of [3H]AVP to V2 Receptor in IMCDs**

V2 receptor binding experiments were conducted for optimization of [3H]AVP ligand binding to rat kidney IMCD V2 receptors. Nonspecific binding was minimized by precoating the MultiScreen-FC plates in 0.3% PEI. At a [3H]AVP ligand concentration of 10 nM, ligand depletion could be neglected (<10% of total AVP is bound) (Fig. 3A), and maximal binding of radioligand at incubation at 4°C (to secure lack of receptor...
binding was shown to decrease with prolonged binding time. In addition, nonspecific binding was determined by incubating IMCDs with increasing concentrations of [3H]AVP (10–7 M, 20 min, 37°C) at 4°C for varying time periods (0, 1, 2, and 17 h). These results confirmed significant ligand-induced V2 receptor internalization in both sham and CHF rats.

By removing the agonist from the system, it was possible to examine potential receptor recycling. Decreased [3H]AVP surface binding (55 ± 8% of baseline level, P < 0.05) was found in the sham group up to 2 h after ligand stimulation (Fig. 4B), indicating that in sham rats the V2 receptor was not recycled to internalization during incubation with the [3H]-labeled ligand) was reached after 17 h (Fig. 3B). In addition, nonspecific binding was shown to decrease with prolonged binding time (data not shown). Finally, a linear correlation was demonstrated between tissue protein (IMCD) and [3H]AVP binding for protein concentrations up to 75 µg (r² = 0.9951; Fig. 3C).

Consequently, we decided that it was possible to use incubation with 10 nM [3H]AVP for 17 h at 4°C (with an IMCD protein concentration between 30 and 50 µg/sample) to measure the amount of surface-bound V2 receptors in a given sample after a given time of AVP or vehicle stimulation, and also after a given time period after the ligand has been removed (by washing) from the system. This will make it possible to quantify the degree of both receptor internalization and recycling after ligand stimulation.

**AVP Induced Internalization of V2 Receptor in Isolated IMCDs**

V2 receptor-binding studies conducted on freshly isolated IMCDs showed similar total surface binding of [3H]AVP in CHF and sham rats (CHF 3.9 ± 0.5 cpm/µg protein vs. sham 3.5 ± 0.4 cpm/µg protein, P = 0.58; Fig. 4A). Incubation of IMCDs with AVP (10–7 M, 20 min, 37°C) induced receptor internalization in both groups as shown by a marked reduction in [3H]AVP surface binding 15 min after termination of AVP stimulation (%binding/vehicle binding: CHF 54 ± 9%, sham 67 ± 8%, P < 0.05 vs. baseline for both CHF and sham; Fig. 4B). These results confirmed significant ligand-induced V2 receptor internalization in both sham and CHF rats.

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the surface. This finding is in agreement with previous results obtained from V_2 receptor-transfected HEK cells (12) and in LLC-PK1a cells transfected with a V_2 receptor-green fluorescent protein complex (2, 5).

In contrast and to our great surprise, we found that [^{3}H]AVP surface binding in IMCDs from CHF rats was returned to baseline levels 30 min after termination of ligand stimulation, indicating recycling of V_2 receptor in CHF rats. Consequently, IMCD from CHF rats with normal circulating levels of AVP seems not only to have increased sensitivity to AVP and increased AQP2 protein levels but seems also to have profound changes in the recycling ability of the V_2 receptor.

Western Blotting of β-Arrestin2

As mentioned, β-arrestins are known to play a central role in the internalization of G protein-coupled receptors by facilitating the recruitment of receptors to clathrine-mediated endocytosis (27). Western blotting of inner medullary β-arrestin2 protein expression was conducted and in agreement with the unchanged V_2 receptor internalization comparing CHF and sham rats, protein expression of β-arrestin2 was also unchanged (Fig. 5).

DISCUSSION

The present study confirms previous studies (10, 23, 38) showing that CHF in rats induced by left anterior coronary ligation is associated with increased protein levels of the AVP-regulated water channel AQP2. The upregulation was found in both the cortex and the inner medulla, but not in the OM. In addition, cAMP accumulation in response to AVP was significantly increased in isolated cortical and inner medullary, but not outer medullary, CDs from CHF rats compared with the response in isolated CDs from sham-operated controls. CHF in the rat model was not associated with increased circulating levels of AVP. Consequently, the results strongly suggest increased AVP sensitivity in the cortical and IMCD in rats with CHF. The increased cAMP accumulation in response to AVP can either be explained by an increased production or a decreased turnover. The studies in the isolated CDs did not indicate decreased turnover, since the increased cAMP accumulation was present both in the presence and absence of phosphodiesterase inhibition with IBMX. Consequently, CHF is associated with an increased cAMP production in the CDs in response to V_2 receptor stimulation. By conducting binding studies and experiments specifically aimed at evaluating potential recycling abilities of the V_2 receptor, we found that the V_2 receptor in isolated IMCD from the sham-operated controls did not recycle to the receptor surface after AVP stimulation, as previously reported in transfected cell lines (2, 5, 12). In contrast to this, CHF was associated with fast recycling of the V_2 receptors. The mechanisms responsible for this finding are unknown but point together with the increased AVP sensitivity within the cortical and IMCD to inappropriate increased activity in the AVP signaling pathway in the CDs. This induces increased AQP2 protein levels, which, as previously shown (29), are associated with an increased aquaretic response to selective V_2 receptor blockade in vivo, indicating that CHF in rats is associated with increased AVP-stimulated renal water reabsorption. Consequently, the present study confirms previous findings (10, 29) indicating that CHF, even in the absence of increased circulating levels of AVP, have increased AVP-mediated renal water reabsorption that eventually will result in whole body volume expansion and development of hyponatremia.

This increased AVP sensitivity in CHF is in contrast to other edema-forming conditions such as liver cirrhosis and nephrotic syndrome. Experimental models of liver cirrhosis (8, 9, 13, 15) as well as nephrotic syndrome (1) have shown decreased expression of AQP2, even in stages of the disease where plasma AVP levels were increased, indicating a decreased AVP sensitivity (AVP escape) in these models. We have previously shown that the decreased AQP2 protein levels in cirrhotic rats are associated with significantly attenuated AVP-induced cAMP accumulations in CDs (4), suggesting that cirrhosis is associated with an uncoupling of the V_2 receptor signaling cascade, which potentially could be conceived as a protective mechanism to avoid excessive water retention. The present study clearly shows that this is not the case in CHF. This is interesting since AQP2 downregulation as seen in cirrhosis would be an appropriate physiological escape mechanism for avoiding excessive water retention. Why such a mechanism is absent in CHF is unsolved, but, with the prospective studies of CHF showing the development of hyponatremia as an independent predictor of mortality (3), it is clear that an understanding of the mechanisms responsible for the increased AVP sensitivity in CHF would be of significant clinical importance.

CHF is associated with a neurohormonal activation, including increased sympathetic nerve activity and increased activity in the renin-angiotensin-aldosterone axis. Recent studies from our laboratory have shown that renal denervation (35) or vasopressin type 1 receptor (AT_1) receptor blockade with continuous losartan treatment (34) significantly reduces AVP-mediated cAMP production in isolated medullary thick ascending limbs of Henle from CHF rats. Likewise, we have previously shown that continuous losartan treatment normalizes...
AQP2 levels and significantly reduces the aquaretic response to selective V2 receptor blockade in CHF rats (29). In addition, a recent study by Lütken et al. (20) showed that candesartan treatment reduces the inner medullary AQP2 levels and decreases the apical targeting of APQ2 in CHF rats. Moreover, Wong and Tsui (36) demonstrated normalization of the increased inner medullary AQP2 expression in response to treatment with the angiotensin-converting enzyme (ACE) inhibitor enalapril in cardiomyopathic hamsters (36). In yet another study, Wong and Tsui (37) showed an increased inner medullary V2 receptor and AQP2 expression in response to ANG II stimulation in normal rats. Kwon and coworkers (18) showed that candesartan treatment in DDAVP-treated rats kept on a low-sodium diet was associated with a decreased urine concentration and decreased inner medullary AQP2 levels. This finding was recently confirmed in a study by Li and coworkers (19). Together these studies indicate a facilitating effect of ANG II on AVP-induced V2 receptor signaling. This hypothesis has recently been further supported by a study by Stegbauer and coworkers (30) showing decreased sensitivity to AVP in mice specifically lacking AT1 in CDs. Interestingly, Bauer and coworkers (30) showing decreased sensitivity to ANG II has recently been further supported by a study by Stegbauer and coworkers (30) showing decreased sensitivity to AVP in mice specifically lacking AT1 in CDs. Interestingly, the experiments revealed a recycling of surface V2 receptors for up to 2 h after ligand stimulation. In contrast, sham rats showed a sustained decrease in surface V2 receptors for up to 2 h after ligand stimulation. These findings confirm previous data indicating the absence of V2 receptor recycling after ligand-induced receptor internalization capability is unchanged in CHF. However, interestingly, the experiments revealed a recycling of surface-associated V2 receptor in isolated IMCDs from CHF rats 30 min after ligand (10^{-7} M AVP)-induced receptor internalization. In contrast, sham CD showed a sustained decrease in surface V2 receptors for up to 2 h after ligand stimulation. These findings confirm previous data indicating the absence of V2 receptor recycling after ligand-induced receptor internalization in transfected cell lines (2, 5, 11). Thus, interestingly, the current data do not give any information as to how CHF can induce recycling of the V2 receptor, except that it does not seem to involve changes in the protein levels of β-arrestin2. Further studies aimed at explaining the changed V2 receptor recycling properties in CHF are warranted.

In conclusion, the present study shows that increased AQP2 protein levels in CHF are associated with an increased AVP sensitivity, i.e., increased cAMP production in response to AVP stimulation in isolated cortical and IMCD and by an acquired V2 receptor recycling/externalization ability obtained in the IMCD.

**Perspectives**

Studies on the urinary excretion of the AQP2 water channel protein (25) have shown an increased excretion in CHF patients and a decreased excretion in cirrhotic patients compared with their respective controls. With the assumption that urinary AQP2 levels reflect the luminal targeting of the AQP2 water channel in vivo, these data strongly support our finding of an AVP escape in experimentally induced cirrhosis and on the contrary increased AVP sensitivity in experimentally induced CHF. The clinical relevance is further supported by the study by Xu and colleagues showing a positive correlation between renal AQP2 protein expression and urinary AQP2 protein excretion in rats with CHF (38, 39). Recent clinical trials have shown the potential of short-term treatment with tolvaptan to restore hyponatremia in severe CHF (28). However, one could argue that such increased AVP sensitivity in CHF patients is of less importance since the vast majority is treated with ACE inhibitors or AT1 receptor antagonists, which have been shown to restore AQP2 protein levels in experimental CHF. However, in many cases, the blockade of ANG II is most likely incomplete because of dose reduction applied to avoid hypotension and consequently it would make sense to investigate more into excess AVP-mediated water reabsorption in CHF patients even in those (the major part) where hyponatremia has not developed.

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the authors.

**AUTHOR CONTRIBUTIONS**

Author contributions: L.B., N.H., S.N., S.C., and T.E.J. conception and design of research; L.B., M.T., J.N., and M.G. performed experiments; L.B. analyzed data; L.B. and T.E.J. interpreted results of experiments; L.B. drafted manuscript; K.M.M., S.C., and T.E.J. edited and revised manuscript; T.E.J. prepared figures; T.E.J. approved final version of manuscript.

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