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### Research Article

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## Upregulation of Aquaporin-2 Water Channel Expression in Chronic Heart Failure Rat

Ding-Li Xu,\* Pierre-Yves Martin,\* Mamiko Ohara,\* Judy St. John,\* Tim Pattison,\* Xianzhong Meng,† Ken Morris,\* Jin K. Kim,\* and Robert W. Schrier\*

\*Department of Medicine and the †Department of Surgery, University of Colorado Health Sciences Center, Denver, Colorado 80262

### Abstract

Aquaporin-2 (AQP2) mediates vasopressin-regulated collecting duct water permeability. Chronic heart failure (CHF) is characterized by abnormal renal water retention. We hypothesized that upregulation of aquaporin-2 water channel could account for the water retention in CHF. Male rats underwent either a left coronary artery ligation, a model of CHF, or were sham operated. 31–33 d after surgery, mean arterial pressure (MAP) and cardiac output were measured in conscious animals, and the animals were killed 24 h later. Cardiac output (CO) and plasma osmolality were significantly decreased and plasma vasopressin increased in the CHF as compared to the sham-operated rats. Both mRNA and protein AQP2 were significantly increased in the kidneys of the CHF rats. The effect of oral administration of a nonpeptide V2 vasopressin receptor antagonist, OPC 31260, was therefore investigated. OPC 31260 induced a significant increase in diuresis, decrease in urinary osmolality, and rise in plasma osmolality in the OPC 31260-treated CHF rats as compared to untreated CHF rats. The mRNA and protein AQP2 were significantly diminished in both cortex and inner medulla of the treated CHF rats. In conclusion, an early upregulation of AQP2 is present in CHF rats and this upregulation is inhibited by the administration of a V2 receptor antagonist. The results indicate a major role for vasopressin in the upregulation of AQP2 water channels and water retention in experimental CHF in the rat. (J. Clin. Invest. 1997; 99:1500–1505.) Key words: vasopressin • vasopressin-receptor antagonists • hyponatremia • water excretion • edema

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Address correspondence to Robert W. Schrier, M.D., Chairman, Department of Medicine, University of Colorado Health Sciences Center, 4200 East 9th Avenue, Denver, CO 80262. Phone: 303-315-7765; FAX: 303-315-7702; E-mail: pat.knox@uchsc.edu

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### Introduction

Water retention is characteristic of advanced congestive heart failure (CHF)<sup>1</sup> (1). Moreover, hyponatremia is a well-defined predictor of mortality of heart failure (2, 3). Plasma arginine vasopressin (AVP) concentrations have been found to be elevated in hyponatremic patients with CHF (4) and are not suppressed during an acute water loading (5). The hypothalamic messenger RNA for vasopressin has also been shown to be increased in experimental CHF (6). Recently, the aquaporin-2 (AQP2) water channel has been cloned and located in the principal cells of the collecting duct (7). Regulation of water transport across the renal principal cells depends on two mechanisms. (a) The rapid action of AVP to increase the water permeability of the apical membrane of principal cells by translocating the AQP2 water channels from cytosolic vesicles to the plasma membranes (8). This short-term effect is mediated by the V2 receptor-dependent increase of adenosine 3'5'-cAMP and may involve cAMP-dependent phosphorylation of AQP2 (9). (b) Long-term regulation of collecting duct water permeability is characterized by an increase in AQP2 mRNA and protein content during fluid restriction and AVP infusion into diabetes insipidus (Brattleboro) rats (10–12). Therefore, in a pathophysiological situation such as CHF, a chronic increase in plasma AVP concentration could upregulate the expression of AQP2.

This study was therefore undertaken to determine the AQP2 expression in a rat model of CHF induced by the ligation of the left coronary artery. For this purpose, AQP2 mRNA and protein were compared between sham-operated and decompensated CHF rats. Next the effect of a nonpeptide V2 vasopressin receptor antagonist (OPC 31260) was examined in sham-operated and CHF rats on AQP2 mRNA and protein, water excretion, and plasma osmolality.

### Methods

Male Sprague-Dawley rats (200–250 g; Sasco, Omaha, NE) were used for all experiments. The rats were housed in a controlled environment and kept in filter-top microisolators. Animals were allowed free access to tap water and food (ProLab 3000; Agway Inc., Syracuse, NY) that contained 0.44% sodium and 22.5% protein. The protocol was approved by the University of Colorado Health Sciences Center Animal Care and Use Committee.

1. Abbreviations used in this paper: AQP2, aquaporin-2; AVP, arginine vasopressin; CO, cardiac output; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; LVMI, left ventricular myocardium infarct; MAP, mean arterial pressure.

### Experimental heart failure

The coronary-ligated rat model of CHF was prepared for the study using standard methods (13, 14). Briefly, rats underwent left coronary artery ligation or sham operation. Each rat was anesthetized with a mixture of ketamine hydrochloride (100 mg/kg) and xylazine (10 mg/kg) intramuscularly. After anesthesia, animals were placed in the supine position, intubated, and ventilated by a positive pressure ventilator for rodents (RSP 1002; Kent Scientific Corporation, Litchfield, CT). A left thoracotomy was performed in the fourth intercostal space to expose the heart and the pericardium was gently opened. The left coronary artery was ligated between the left atrial appendage and the right ventricular outflow tract with a 6-0 silk suture. The chest was then closed in layers and the air was evacuated by slight lateral pressure of the thorax. Using this method there is a 30–40% mortality within the first 24 h after the operation due to acute heart failure. Sham operations were performed in which the pericardium was opened, but no suture was inserted around the coronary artery.

### Experimental protocol

31 or 33 d after surgery, the rats were studied according to the following protocols.

**Protocol I: AQP2 gene expression in experimental heart failure rats model.** This protocol included a total of 13 rats (6 sham-operated and 7 CHF rats). On the day 33 after the surgery, rats were anesthetized as described above, and the right carotid artery and jugular veins were cannulated with polyethylene catheters (PE 50-Intramedic; Clay Adams, Becton Dickinson and Co., Parsippany, NJ). The catheters were tunneled subcutaneously through the back of the neck and exteriorized. After 1 d of recovery from anesthesia, the conscious rats were placed in a small, well ventilated Plexiglas chamber and fluxed with room air. Cardiac output (CO) was then measured using the standard indocyanine green dye technique as previously described (15, 16). In brief, 5 µg of green dye were injected into a jugular catheter while blood was simultaneously pumped through the shunt from carotid artery to jugular vein at 3 ml/min through a densitometer cuvette. This signal was imputed to a computer, physiograph, and oscilloscope to generate a dye curve. The arterial pressure signal was imputed to a physiograph and microcomputer. After the hemodynamic evaluation, rats were allowed to recover for 24 h and then decapitated. Trunk blood was collected and kidneys were harvested. The CHF rats included in the study were those with a left ventricle myocardium infarct (LVMI) size  $\geq 20\%$ . These animals had compensated CHF. In preliminary studies it was found that animals with LVMI size  $< 20\%$  did not exhibit CHF. For the determination of the infarct size, rat heart was harvested and rinsed in cold phosphate-buffered saline. Then, the right and left ventricles were weighed. The heart was fixed in 3% formaldehyde and subsequently the left ventricles were cut from apex to base into five transverse slices. The fraction of the left ventricle that was infarcted was measured and expressed as a percentage of the entire ventricular area thus providing an approximate estimate of the extent of left ventricular infarction (13, 17). All infarctions were transmural and involved only the free wall of the left ventricle.

**Protocol II: Effects of V2 vasopressin receptor antagonist, OPC 31260, on AQP2 gene expression in experimental CHF rat model.** This protocol included 10 CHF rats (LVMI  $\geq 20\%$ ) and 6 sham-operated rats. On the day 31 after surgery, rats were housed individually in metabolic cage. Measurements of water and food intake, body weight, and urine volume were taken on day 34 after surgery. Placebo or OPC 31260 (generously provided by Otsuka America Pharmacy, Rockville, MD) was administered orally (30 mg/kg/24 hours) and a 24-h balance study was then conducted. In the balance experiments animals were housed individually in metabolic cages (Nalgene metabolic cages; Nalge Company, Rochester, NY). These cages provide a good separation of the urine and the feces with the combination of a collecting funnel and a separating cone in the lower chamber. After the collection, the rats received the same dose of OPC 31260 and

were decapitated 3 h later. Trunk blood was collected and the kidneys were harvested.

Plasma sodium levels were determined using a Beckman CX3 (Beckman Instruments, Fullerton, CA). Plasma osmolalities were determined by freezing point depression (Advanced Instruments Inc., Needham Heights, MA). Plasma arginine vasopressin (AVP) concentrations were measured by radioimmunoassay as previously described (18). Rat AVP antibody (No. 2849) for AVP radioimmunoassay was generously provided by Dr. Jacques Durr (Bay Pines, FL).

### Northern blot analysis

Total RNA from the whole kidney or from the cortex and inner medulla that were dissected from each kidney was isolated by a simplified guanidinium thiocyanate protocol (RNAzol-B; Teltest, Inc., Friendswood, TX). The total RNA (5–20 µg depending of the sample used) was then size fractionated on a 1.0% agarose-6% formaldehyde gel and subsequently transferred to a nylon membrane and ultraviolet crosslinked. The blots were prehybridized in Rapid-Hyb buffer (Amersham, Arlington Heights, IL) for 20 min at 65°C before the addition of the labeled probe. The probe used for AQP2 consisted of a 850-bp segment from the rat gene. Briefly, a 850-bp PCR product (kindly provided by I. Teitelbaum, Denver, CO) was subcloned into the PCR II vector (Invitrogen, San Diego, CA). The subsequent product was amplified and sequenced for verification. This probe was labeled using [<sup>32</sup>P]dCTP and the Red-prime random primer labeling kit (Amersham) denatured by boiling for 5 min and added to the prehybridization solution. The membranes were hybridized at 65°C for 2 h and then washed twice with 2× SSC and 0.1% SDS for 20 min at room temperature and twice at 65°C for 30 min with 1× SSC and 0.1% SDS. The membranes were exposed to Kodak-X-OMAT film (Eastman Kodak Inc., Rochester, NY) overnight at –70°C with an intensifying screen. Membranes were then stripped and hybridized to a 1.2-kb cDNA fragment of human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene (American Type Culture Collection, Rockville, MD), washed, and analyzed as for AQP2. Figures are representative of three separate blots with different animals.

### Western blotting analysis

The rabbit polyclonal antibody against AQP2 was prepared by Genemed Biotechnologies Inc., (South San Francisco, CA) using a synthetic peptide (CELHSPQSLPRGSKA) from the COOH terminus of AQP2 (19). The peptide was conjugated to keyhole limpet hemocyanin (KHL) by a cysteine sulhydryl linkage. Test bleedings were screened by ELISA. Final titers were reported to be  $> 100,000$ . Western blots were performed as previously described (20). Briefly, the renal inner medulla was glass homogenized in a lysis buffer (50 mM B-glycerophosphate, 100 mM Na<sub>3</sub>VO<sub>4</sub>, 2 mM MgCl<sub>2</sub>, 1 mM EGTA, and 0.5% Triton-X100, 1 mM DTT) containing protease inhibitors (20 mM pepstatin, 20 mM leupeptin, 1,000 U/ml aprotinin, and 1 mM PMSF). Particulate (pellet) and cytosolic (supernatant) fraction from the cortex were separated after homogenization in a sucrose buffer (250 mM sucrose, 50 mM Hepes, 1 mM EDTA, and 1 mM EGTA) containing the same protease inhibitors and centrifuged at 100,000 g for 60 min. Protein concentration was determined

**Table I. CO, MAP, and Plasma AVP Concentration in CHF and Control Rats (Mean  $\pm$  SEM)**

Group	n	AVP	CO	
			pg/ml	liter/min
Sham operated	6	3.22 $\pm$ 1.29	0.161 $\pm$ 0.025	118 $\pm$ 4
Control				
CHF	7	11.11 $\pm$ 2.71	0.089 $\pm$ 0.012	105 $\pm$ 5
P value		< 0.05	< 0.05	NS

NS, nonsignificant.

for each sample using the Bradford method (Bio-Rad Laboratories, Richmond, CA). The proteins were separated on denaturing SDS/12.5% polyacrylamide gels by electrophoresis. Proteins were then transferred to a polyvinylidene difluoride (PVDF) membrane (Millipore Corp., Bedford, MA) by wet electroblotting for 90 min. Blots were blocked overnight at 4°C with 5% nonfat dry milk in TBS-T, pH 7.5 (20 mM Tris base, 137 mM NaCl, 0.1% Tween 20). Blots were incubated with AQP2 antibody (1:500 dilution) for 90 min at room temperature and then washed and incubated with the second antibody (donkey anti-rabbit immunoglobulin G conjugated with horseradish peroxidase, diluted 1:1,500; Amersham) for 1 h. Control immunoelectrophoresis with preimmune sera gave no labeling. Subsequent detection of the specific proteins was carried out by enhanced chemiluminescence (ECL) (Amersham), according to the manufacturer's instructions. Prestained protein markers (Sigma Chemical Co., St. Louis, MO) were used for molecular weight determinations. Densitometry results are reported as integrated values (area X density of the band) and expressed in percentage as compared to controls (100%). Equal protein loading was checked by Coomassie blue staining of the membranes. Figures are representative of three separated blots with different animals.

#### Preparation of tissue for immunocytochemistry

Tissue blocks prepared from the kidney, inner medulla, and cortex were postfixed with 4% paraformaldehyde for 2 h and then infiltrated with Acrylamide and embedded in OCT and frozen in liquid nitrogen and stored at -80°C until further procedures were performed. Cryosections (6  $\mu$ m) cut on Tissue TEK cryostat were collected on charged slides. After washing in PBS, the cryosections were first incubated with 0.1% Triton X for solubilizing the membranes, 0.5% BSA for blocking nonspecific binding, and then with the antibody against AQP2 (1:500) for 1 h at 37°C. The labeling was visualized using fluorescein conjugated goat anti-rabbit, diluted 1:100 (Jackson Immuno Research Laboratories, Inc., West Grove, PA) and incubated for 1 h at 37°C. Between each of these procedures, samples were washed in PBS, 3  $\times$  5 min at room temperature. Micrographs were taken on Zeiss Axiophot IF. Microscopes at an original magnification of 400, using Kodak Ektachrome 400 slide film (Eastman Kodak Inc.).

#### Statistical analysis

Statistical analysis of CO, MAP, plasma sodium, osmolality, AVP concentration, AQP2/ GAPDH density ratio, and density of immunoblots results were performed using unpaired and paired *t* tests or ANOVA tests followed by Student-Newman-Keuls multiple comparisons test. Results are expressed as mean  $\pm$  SEM.  $P < 0.05$  was considered significant.

## Results

*MAP, CO, and plasma AVP concentration in rats included in the protocol 1.* MAP and CO were lower in the CHF (LVMI  $\geq$  20%) as compared to controls, but the fall in MAP did not reach statistical significance (Table I). Plasma AVP concentrations were significantly increased in the CHF rats as compared to the control animals.

*AQP2 expression in sham-operated versus CHF rats.* Northern blot analysis from whole kidney total RNA demonstrated a transcript at 1.4 kb, which expresses AQP2 mRNA. AQP2 mRNA was significantly increased in CHF rats: AQP2/GAPDH density ratio  $1.779 \pm 0.072$  ( $n = 5$ ), vs. sham-operated rats ( $n = 5$ )  $1.385 \pm 0.23$ ,  $P < 0.05$  (Fig. 1).

Protein extracts from the inner medulla were immunoblotted. Immunoblots showed a band at 29 kD, indicating AQP2, as well as a high molecular weight broad band between 36–45 kD, which represents the glycosylated protein form of AQP2. Immunoreactivity of the AQP2 protein (29 kD) was increased significantly in the CHF versus control rats:  $202.2 \pm 24.9\%$  ( $n = 5$ ) vs.  $100.0 \pm 9.9\%$  ( $n = 5$ ),  $P < 0.05$  (Fig. 2). The glycosylated fraction increased in parallel with the 29-kD band.

*Effects of OPC 31260 on plasma sodium, osmolality, urine volume, and AVP concentration in experimental heart failure rats.* Oral administration of OPC 31260 significantly increased plasma osmolality and urinary volume in CHF rats as compared to CHF rats treated with vehicle or sham-operated rats. Plasma AVP concentrations in the CHF rats treated with OPC 31260 showed no further increase when compared to CHF rats treated with vehicle, both maintaining higher plasma AVP

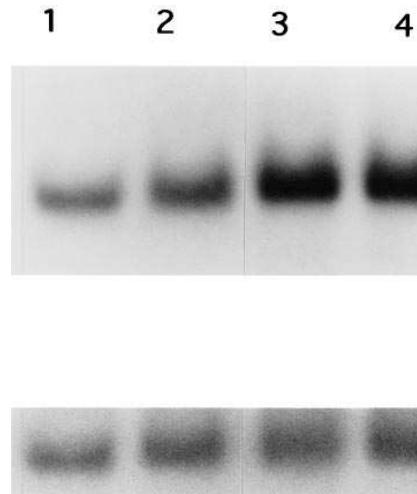


Figure 1. AQP2 mRNA expression in CHF rats. Northern blot analysis of 20  $\mu$ g of total RNA from whole kidney. (Lanes 1 and 2) Sham-operated rats. (Lanes 3 and 4) CHF rats. Northern blot hybridization shows that AQP2 mRNA are detected at 1.4 kb. Hybridization to GAPDH cDNA is also shown. AQP2 expression is increased in CHF rats. All the Northern blots are representative of three separate experiments.

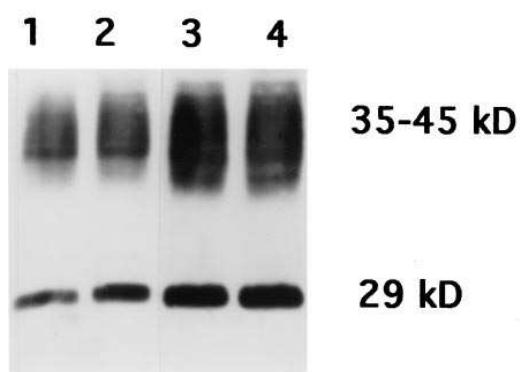


Figure 2. AQP2 protein expression in CHF rats. Western blot analysis for AQP2 protein expressed in rat kidney medulla using a polyclonal antibody against AQP2. 10  $\mu$ g of protein extract was loaded in each lane. (Lanes 1 and 2) Sham-operated rats. (Lanes 3 and 4) CHF rats. Two bands are detectable: a band of 29 kD and a broader band of 35–45 kD corresponding to the predicted molecular mass of AQP2 and its glycosylated form. Immunoreactivity for AQP2 is increased in CHF rats in both bands as compared to sham-operated rats. All the immunoblots are representative of three separate experiments.

Table II. Analysis of Plasma and Urine Parameters from Sham-operated and CHF Rats Treated with V2 Receptor Antagonist OPC-31260 or Vehicle (Mean  $\pm$  SEM)

	Sham operated	CHF + vehicle	CHF + OPC31260
<i>n</i>	6	5	5
PAVP (pg/ml)	3.33 $\pm$ 0.85	11.97 $\pm$ 3.49*	14.47 $\pm$ 3.73*
PNa (mmol/liter)	137 $\pm$ 0.4	134 $\pm$ 2.2	142 $\pm$ 2.9
POsm (mOsm/kg H <sub>2</sub> O)	295 $\pm$ 1.0	289 $\pm$ 0.7 <sup>†</sup>	297 $\pm$ 3.1**
UOsm (mOsm/kg H <sub>2</sub> O)	1453 $\pm$ 150	2316 $\pm$ 347 <sup>‡</sup>	346 $\pm$ 79 <sup>  </sup>
Food intake (g)	14.45 $\pm$ 3.92	15.98 $\pm$ 2.45	17.9 $\pm$ 2.14
Urine volume (ml/24 h)	18.0 $\pm$ 3.9	18.0 $\pm$ 3.4	34.0 $\pm$ 5.9 <sup>§</sup>
Body weight (g)	403 $\pm$ 20	375 $\pm$ 15	384 $\pm$ 21

\**P* < 0.05, <sup>†</sup>*P* < 0.01 CHF vs. sham operated; <sup>‡</sup>*P* < 0.05 CHF + OPC 31260 vs. CHF + vehicle and sham operated; <sup>||</sup>*P* < 0.001 CHF + OPC 31260 vs. CHF + vehicle and sham operated; \*\**P* < 0.01 CHF + OPC 31260 vs. CHF + vehicle. PAVP, plasma vasopressin; POsm, plasma osmolality; UOsm, urinary osmolality.

concentrations than the sham-operated rats. Urine osmolality was significantly increased in CHF rats as compared to sham-operated rats and OPC 31260 significantly decreased urinary osmolality in the CHF rats. Food intake and body weights were similar in the three groups (Table II).

Northern blot analysis showed an increase of AQP2 mRNA expression in kidney inner medulla of CHF rats as compared to sham-operated rats (AQP2/GAPDH density ratio 3.164  $\pm$  0.146 vs. 1.673  $\pm$  0.093, *n* = 3, in each group; *P* < 0.05), and this increase was abolished by the administration of OPC 31260 (2.341  $\pm$  0.225 vs. 3.164  $\pm$  0.146 for treated vs. untreated CHF rats, *n* = 3 in each group; *P* < 0.05). The increase was also present in cortex of CHF rats as compared to sham-operated rats (0.570  $\pm$  0.045 vs. 0.326  $\pm$  0.115, *n* = 3 in each group; *P* < 0.05) and was also suppressed by OPC 31260 administration to CHF rats (0.221  $\pm$  0.056 vs. 0.570  $\pm$  0.045 for treated vs. untreated CHF rats, *n* = 3 in each group; *P* < 0.05;

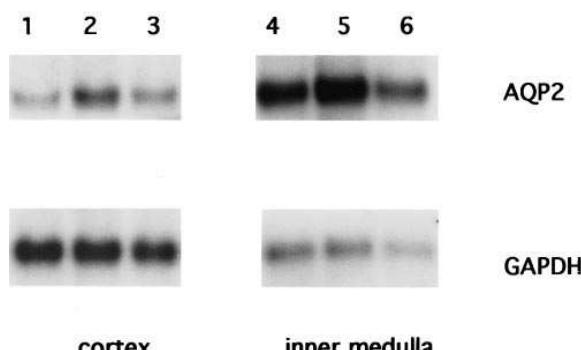


Figure 3. Effect of the V2 vasopressin-receptor antagonist, OPC 31260, on AQP2 mRNA expression in CHF rats. Northern blot analysis of total RNA extracted from cortex (10  $\mu$ g) and inner medulla (5  $\mu$ g) hybridized to AQP2 probe and GAPDH probe. (Lane 1) Cortex, sham-operated rat. (Lane 2) CHF rat + vehicle. (Lane 3) CHF rat + OPC 31260. (Lane 4) Inner medulla, sham-operated rat. (Lane 5) CHF rat + vehicle. (Lane 6) CHF rat + OPC 31260. In both cortex and inner medulla, AQP2 mRNA expression is increased in CHF rats, whereas CHF rats treated with OPC 31260 show a decrease in AQP2 as compared to CHF rats.

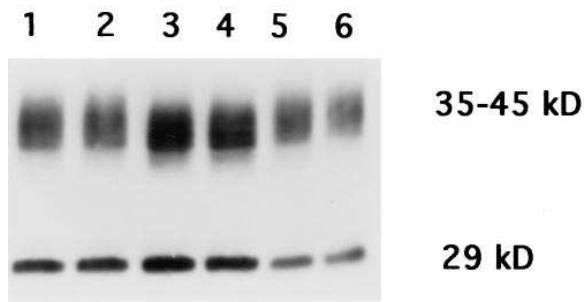


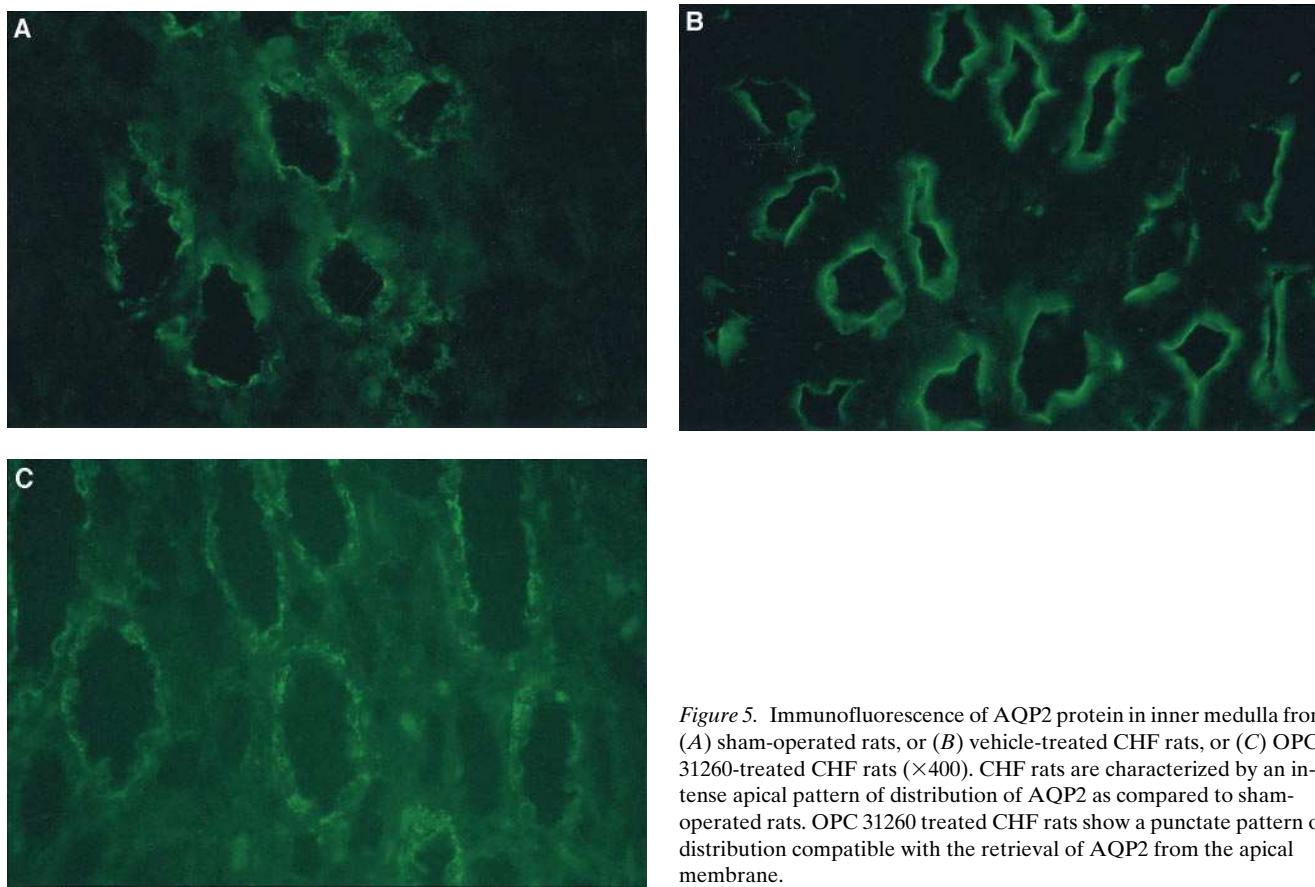
Figure 4. Effect of the V2 vasopressin-receptor antagonist, OPC 31260, on AQP2 protein expression in CHF rats. Western blot analysis for AQP2 protein expressed in rat kidney medulla using a polyclonal antibody against AQP2. 10  $\mu$ g of protein extract was loaded in each lane. (Lanes 1 and 2) Sham-operated rats. (Lanes 3 and 4) CHF rats + vehicle. (Lanes 5 and 6) CHF rats + OPC 31260. OPC 31260 decreases the increased immunoreactivity observed in CHF rats.

Fig. 3). Western blot analysis showed that the increase of AQP2 protein in CHF rats as compared to sham-operated rats (density 184.2  $\pm$  6.4% vs. 100  $\pm$  8.6%, *n* = 5 in each group; *P* < 0.05) was diminished in inner medulla by the administration of OPC 31260 (110.4  $\pm$  5.6% vs. 184.2  $\pm$  6.4% for untreated CHF rats, *n* = 5 in each group; Fig. 4). Similar results were noted in the particulate fraction of the cortex with OPC 31260 treatment (data not shown).

Immunocytochemistry confirms the effect of OPC 31260 in the CHF rats. Labeling of AQP2 by immunofluorescence was seen exclusively in the collecting duct tubules. Immunofluorescence was distributed in an apical pattern, as illustrated by an intense linear staining, in inner medulla of CHF rats (Fig. 5B) as compared to sham-operated rats (Fig. 5A). Administration of OPC 31260 to CHF rats resulted in a marked redistribution from the apical pattern to a punctate labeling consistent with retrieval of water channel from the plasma membrane (Fig. 5C).

## Discussion

Pretreatment hyponatremia is a common finding in advanced cardiac failure (2, 3). For many years the role of antidiuretic hormone in this hyponatremia associated with CHF was not known, mainly because the bioassay for AVP lacked the sensitivity to answer the question. Using a sensitive radioimmunoassay for AVP, however, heart failure patients with hypoosmolality of a degree sufficient to suppress AVP to undetectable levels in normal subjects were shown to have inappropriately high plasma concentrations of AVP (4). Subsequent studies have confirmed this nonosmotic stimulation of vasopressin in association with decompensated CHF, and have shown that the plasma vasopressin is not suppressible with an acute water load (5). An important role of AVP in the hyponatremia associated with CHF was further suggested by the demonstration of an increased expression of the mRNA for vasopressin in the hypothalamus of cardiac failure but not control animals (6). The pivotal importance of vasopressin-mediated water retention in experimental CHF has been documented by the use of antagonists to the V2 vasopressin receptor on the collecting duct. Initially, peptide V2 antagonists were shown to correct the impaired urinary dilution in response to an acute water load in a low cardiac-output model in



**Figure 5.** Immunofluorescence of AQP2 protein in inner medulla from (A) sham-operated rats, or (B) vehicle-treated CHF rats, or (C) OPC 31260-treated CHF rats ( $\times 400$ ). CHF rats are characterized by an intense apical pattern of distribution of AQP2 as compared to sham-operated rats. OPC 31260 treated CHF rats show a punctate pattern of distribution compatible with the retrieval of AQP2 from the apical membrane.

the rats (21). The nonpeptide V2 antagonist OPC 31260, the compound used in the present study, has been shown to normalize the response to an acute water load in conscious dogs with CHF secondary to rapid ventricular pacing (22) and rats with CHF-induced left coronary artery ligation (23).

The most recent scientific advance in the area of renal water regulation is the cloning of the AQP2 water channel from the principal cells of the collecting duct (7). The present study examined whether the AQP2 water channels are upregulated in the experimental CHF in the rat. Upregulation, approximately twofold, was indeed demonstrated both at the level of mRNA and protein expression in this model of decompensated CHF. Aquaporin-2 gene 5'-flanking region has a cAMP response element (CRE) and it had been postulated that signals via V2 receptors may act on CRE and enhanced expression of AQP2 (24). In the present study, the increase in AQP2 mRNA suggests that the steady state tissue level of AQP2 mRNA has been altered either by an increase in transcription rate or by stabilization of the mRNA. In addition, the similar increase in inner medulla and cortex (where interstitial osmolality is isosmotic under all conditions) suggests that interstitial tonicity does not play a major role in this upregulation.

The cause of the upregulation of the AQP2 water channels observed in the CHF rats seemed most likely to be due to the increase in plasma AVP concentrations, which are significantly increased in CHF rats. In this regard, acute administration of AVP has been shown to increase the apical membrane localization of the AQP2 water channels (8, 25). Long-term administration of AVP or 24 h of water restriction has demonstrated

similar findings with an increase in the total amount of AQP2 water channels (10, 12, 19, 26). Vasopressin-independent factors could also regulate AQP2 expression as suggested by the downregulation of AQP2 protein in rats fed with a low protein diet (27) in rats on chronic lithium treatment (28) and in hypokalemic rats (29); however, upregulation of AQP2 independent of the vasopressin effect has not been yet demonstrated.

The definitive documentation of the role of AVP to mediate the upregulation of AQP2 water channels in experimental CHF necessitated the reversal of this effect by a V2 antagonist. The orally active, nonpeptide antagonist, OPC 31260, has 100 times the affinity for the V2 as compared to the V1 vasopressin receptor and also has no agonist effect in contrast to the earlier peptide V2 vasopressin antagonists (30). The antagonist was administered orally for 24 h to a group of CHF rats and compared to CHF rats receiving the vehicle. The V2 vasopressin antagonist downregulated the AQP2 water channels, redistributed the water channels from the apical membrane to the cytosol and increased urine flow in the CHF animals. This diuresis was associated with a normalization of plasma osmolality. This effect occurred in spite of increased plasma vasopressin concentrations as compared to the sham-operated rats.

The effect of orally active, nonpeptide V2 antagonist to downregulate water channels in CHF, as well as in other circumstances of arterial underfilling such as cirrhosis, and the syndrome of inappropriate secretion of antidiuretic hormone (SIADH) (31) provides the potential of aquauretic agents to treat chronic hyponatremia. Interestingly, 6 mo of treatment with OPC 31260 of the same CHF model used in the present

study was associated with improved survival as compared to untreated CHF animals (32). This finding is difficult to understand, but the combination of V1 and V2 vasopressin receptor antagonists in a single dose study has been shown to cause a more persistent increase in cardiac output and decrease in systemic vascular resistance in dogs with CHF as compared to treatment with the V1 antagonist alone (22). There are in vitro results in cultured vascular smooth muscle cells which demonstrate that a decrease in extracellular sodium, in a range that occurs in advanced CHF, is associated with increased cellular  $\text{Ca}^{2+}$  and enhanced shape change in response to vasoconstrictors (33). If this phenomenon occurs in vivo then V2 antagonists might not only treat hyponatremia in CHF but also enhanced cardiac performance and survival by reducing cardiac afterload in a manner similar to that observed with V1 antagonists (22).

In conclusion, the results of this study demonstrate an up-regulation of both the AQP2 mRNA and protein in experimental CHF. This effect on collecting duct water channels in CHF is associated with an increase in plasma vasopressin and can be reversed with an orally active, nonpeptide V2 vasopressin receptor antagonist. The therapeutic implications of these V2 antagonists, not only on hyponatremia, but on cardiac performance in patients with CHF are in need of study.

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