Upregulation of Vasopressin V_2 and Aquaporin 2 in the Inner Medullary Collecting Duct of Cardiomyopathic Hamsters Is Attenuated by Enalapril Treatment

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Previous studies showed that aquaporin 2 (AQP2) is elevated in the kidney of the heart failure rat suggesting that an increased amount of AQP2 contributes to water retention in heart failure. We performed the present study to determine whether angiotensin II play a role in causing an increase in the expression of arginine vasopressin (AVP) V_2 and AQP2 mRNA in the kidney of the cardiomyopathic hamster. The expression of AVP V_2 and AQP2 mRNA in the inner medullary collecting duct (IMCD) was measured by competitive reverse transcriptase-polymerase chain reaction (RT-PCR) before and after treatment with an angiotensin-converting enzyme inhibitor, enalapril. Our results showed that the expression of AVP V_2 (0.53 ± 0.05 *v* 1.03 ± 0.15 amol/μg of total RNA, $P < .01$) and AQP2 mRNA (0.027 ± 0.002 *v* 0.036 ± 0.002 amol/μg of total RNA, $P < .05$) in the IMCD of the cardiomyopathic hamster is upregulated. Treating the cardiomyopathic hamster with enalapril for 7 days negated the changes. In situ hybridization experiments confirmed the intensity of the signals for both AVP V_2 and AQP2 mRNA was more intense in the IMCD of the cardiomyopathic hamster. Enalapril treatment reduced the signal intensity to a level comparable to the normal hamster. These results suggested that the increases in the expression of AVP V_2 and AQP2 mRNA are mediated by angiotensin II.

IMPAIRED WATER excretion and enhanced water reabsorption are associated with congestive heart failure.[1] The reason for enhanced water reabsorption in heart failure is partly related to an increase in arginine vasopressin (AVP) in the circulation[2-4] and partly to hypersensitivity of the renal tubules to AVP.[5] This notion is supported by studies that showed vasopressin antagonists reversed the defect in water excretion seen in heart failure rats[6] and cardiomyopathic hamsters.[4] Another possible cause for increased water reabsorption in heart failure may be accounted for by the upregulation of AVP V_2 receptors. The upregulation of AVP V_2 in the inner medullary collecting duct (IMCD) leads to an increase in the expression of AQP2 mRNA.[7] Nielsen et al[8] and Xu et al[9] have reported that in the heart failure animals the aquaporin–2 water channel (AQP2) mRNA and protein levels are upregulated. The immediate increase in AQP2 in the apical membrane of the collecting duct is mediated by the binding of AVP to the V_2 receptor to increase the formation of cyclic adenosine monophosphate (cAMP). In a longer term, vasopressin can also act as a transcription activator to increase in AQP2 mRNA and AQP2 water channel.[10]

Recent studies showed that the natriuretic and diuretic responses to atrial natriuretic factor (ANF) infusion were attenuated in cardiomyopathic hamsters.[11] When these hamsters were treated with enalapril for 7 days, the natriuetic and diuretic response to ANF infusion were restored to normal. These observations suggested that the expression of AVP V_2 and AQP2 mRNA in the IMCD of the kidney of cardiomyopathic hamsters might be affected by angiotensin-converting enzyme inhibition. The goal of the present study was to determine the role of angiotensin II in regulating the expression of AVP V_2 mRNA and AQP2 mRNA in the kidney of cardiomyopathic hamsters.

MATERIALS AND METHODS

Male cardiomyopathic hamsters and age-matched normal hamsters were purchased from Canadian Hybrid farms (Centerville, King's County, Nova Scotia). Hamsters used in these studies were 280 to 300 days old. Hamsters were divided into 4 groups. Groups 1 (n = 7) and 2 (n = 7) were normal and cardiomyopathic hamsters. Groups 3 (n = 7) and 4 (n = 7) were normal and cardiomyopathic hamsters that were treated with angiotensin-converting enzyme inhibitor, enalparil (2.5 mg/kg/d subcutaneously [SC]) for 1 week before studies were performed.

The hamsters were anesthetized with phenobarbital (50 mg/kg intraperitoneal [IP]), and kidneys were removed and cut into half in ice-cold phosphate-buffered saline (PBS). The IMCD was isolated by a method described previously.[12] The renal papillary tissues were minced and digested in RPMI-1640 medium (Sigma-Aldrich Canada, Oakville, Ontario, Canada) containing collagenase (1.5 mg/mL) (United States Biochemical, Cleveland, OH) for 30 minutes at 37°C. An equal volume RPMI-1640 medium containing 10% fetal calf serum (FCS) was added to stop digestion. The mixture was then centrifuged and the resulting pellet was resuspended in 10 mL of RPMI-1640 containing 10% FCS and fractionated in percoll (specific gravity, 1.07) for 20 minutes at 2,000 rpm. The papillary collecting duct cells were found at the top of the percoll layer. The IMCD cells obtained were confirmed by histologic and biochemical methods.

Competitive reverse transcriptase-polymerase chain reaction (RT-PCR) method was used to measure the expression of AVP V_2 and AQP2 mRNA in the IMCD of the kidney. For RNA quantification by competitive RT-PCR, RT reactions were performed with a constant amount of target RNA with the corresponding RNA competitor. A single set of primers was used to amplify both target and added competitor of known concentration. Competitors for AVP V_2 mRNA were synthesized with the sense primer 5′-AGC AAC AGC AGC CAG GAG GAA C-3′ and the antisense primer 5′-GGC CCA GCA ATC AAA CAC CCG CCA GGA TCA TGT AGG AGG AGG-3′ resulting in a amplification product of 355 bp length. The competitive PCR for

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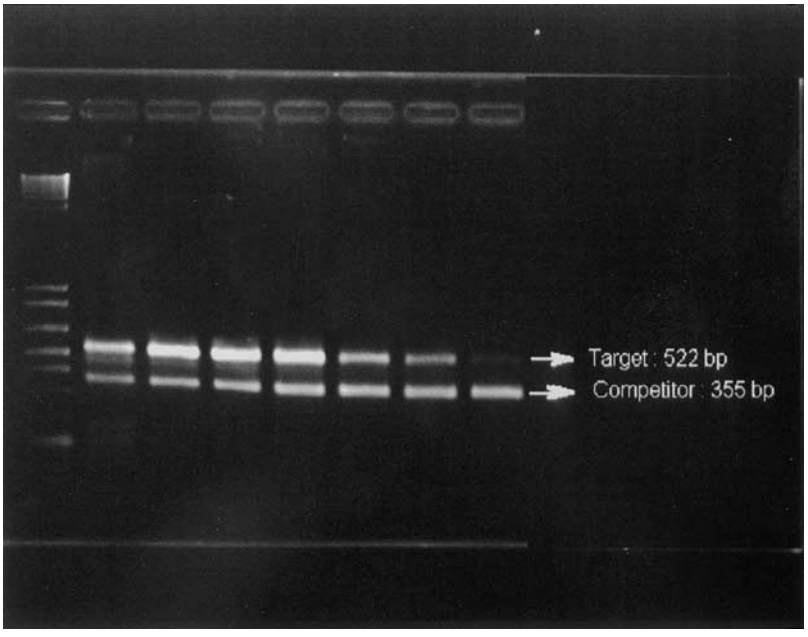

Fig 1. Competitive RT-PCR analysis of vasopressin V_2 with different amounts of competitor. Agarose gel stained with ethidium bromide is shown.

AVP V_2 mRNA was performed with the sense primer 5′-AGC AAC AGC CAG GAG GAA-3′ and the antisense primer 5′-GGC CCA GCA ATC AAA CAC CC-3′ resulting in a PCR fragment of 522 bp. Competitors for AQP2 were synthesized with the sense primer 5′-TCC TTC CTT CGA GCT GCC TT-3′ and the antisense primer 5′-ACG TTC CTC CCA GTC GGT GTC AGG GGT CCG ATC CAG AAG A-3′ producing a 404-bp fragment. The competitive PCR for AQP2 was performed with the sense primer 5′-TCC TTC CTT CGA GCT GCC TT-3′ and the antisense primer 5′-ACG TTC CTC CCA GTC GGT GT-3′ resulting in a PCR product of 504 bp length. PCR was performed in a Perkin Elmer GeneAMP PCR system (Norwalk, CT). The PCR products were analyzed in 1.5% agarose gel followed by staining with ethidium bromide. A representative gel is shown in Fig 1. Appropriate bands were scanned and quantitated by computer densitometry (AlphaImager; Alpha Innotech, San Leandra, CA).

Paraffin sections were prepared by fixing the kidney overnight in 4% formaldehyde-PBS, dehydrated through successive baths of ethanol (75%, 95% and 100% at 10 minutes each) and toluene (2X 30 minutes each), and embedded in 3 successive baths of Paraplast (Oxford Labcare, Division of Sherwood Medical, St Louis, MO) at 58°C. Paraffin sections were cut (8 μm), mounted on poly-L-lysinated slides, dried overnight, and stored at 4°C until used for in situ hybridization (ISH) experiments. ISH was performed with digoxigenin (DIG) 3′-end labeled cDNA probe (Boehringer, Mannheim, Mannheim, Germany). The cDNA was prepared by PCR amplifications of total RNA obtained from IMCD using primers listed above. ISH was performed according to methods described by Baumgart et al.[13] Visualization of the ISH signal was performed using a Zeiss Axioskop 2 light microscope. Digital images were captured using a monochrome CCD camera (Pentamax; Princeton Instruments, Trenton NJ).

Plasma AVP and angiotensin were determined by radioimmunoassay after extraction with C_{18} Sep-Pak (Water Associates, Milford, MA) using commercially prepared kits purchased from Peninsula Laboratory (Belmont, CA). Plasma sodium concentrations were measured with IL 943 flame photometer (Instrumentation Laboratory, Lexington, MA).

Data are expressed as means ± SEM. Unpaired Student's *t* tests were performed to determine differences between groups. A *P* value less than .05 was considered statistically significant.

RESULTS

Table 1 summarizes the hematocrit, body weight, heart weight and plasma AVP, and angiotensin levels in all groups. Hematocrit was higher in the normal hamster than those seen in the cardiomyoapthic hamster. Cardiomyopathic hamsters had a lower body weight compared with normal hamsters. The differences in weight between cardiomyopathic and normal hamsters remained the same after 7 days of enalpril treatment. The

Table 1. Hematocrit, Body Weight, Heart Weight, and Hormonal Activity for all Groups

	Normal Untreated	Cardiomyopathic Untreated	Normal Enalapril-Treated	Cardiomyopathic Enalapril-Treated
Hematocrit (%)	56 ± 1.5	50 ± 0.5*	55 ± 1.9	52 ± 016†
Body weight (g)	156 ± 2.2	134 ± 4.9*	151 ± 1.5	124 ± 2.8*
Heart weight (g)	0.67 ± 0.02	0.87 ± 0.04*	0.58 ± 0.02	0.74 ± 0.04*
Heart weight body weight ratio	0.00423 ± 0.0001	0.0065 ± 0.0003*	0.0038 ± 0.0001	0.0060 ± 0.0003*
Plasma sodium (mmol/L)	144 ± 1.3	145 ± 2.0	140 ± 1	140 ± 1
Plasma vasopressin (pg/mL)	3.1 ± 0.7	7.1 ± 1.7†	2.6 ± 0.4	3.8 ± 0.5‡
Plasma angiotensin II (pg/mL)	76 ± 12	153 ± 23*	39 ± 7†	77 ± 7*§

*$P < .01$, †$P < .05$ compared with corresponding normal hamsters.

‡$P < .05$, §$P < .01$ compared with corresponding cardiomyopathic hamsters.

Table 2. Effect of Enalapril Treatment on the Expression of Vasopressin V_2 and AQP2 mRNA

	Normal Untreated	Cardiomyopathic Untreated	Normal Enalapril-Treated	Cardiomyopathic Enalapril-Treated
Vasopressin V_2mRNA amol/μg of total RNA	0.53 ± 0.05	1.03 ± 0.15*	0.57 ± 0.15	0.58 ± 012
AQP2 mRNA amol/μg of total RNA	0.027 ± 0.002	0.036 ± 0.002†	0.025 ± 0.001	0.028 ± 0.001

*$P < .01$, †$P < .05$ compared with corresponding normal hamsters.

heart weight was significantly higher in the cardiomyopathic hamster, and this was not affected by enalapril treatment. Plasma AVP and angiotensin II were increased in the untreated cardiomyopathic hamsters compared with its age-matched control. Following enalapril treatment, there was a significant decrease in plasma angiotensin II levels in both cardiomyopathic (153 ± 23 *v* 77 ± 7 pg/mL, $P < .01$) and control hamsters (76 ± 12 *v* 39 ± 7 pg/mL, $P < .05$). Plasma AVP levels were lowered in normal hamsters (3.1 ± 0.7 *v* 2.6 ± 0.4 pg/mL, not significant [NS]) with enalparil treatments, but decreased significantly in cardiomyopathic hamsters (7.1 ± 1.7 *v* 3.8 ± 0.5 pg/mL, $P < .05$).

Table 2 summarizes the effect of enalapril on the expression of AVP V_2 and AQP2 mRNA in the IMCD of the normal and cardiomyopathic hamsters. In the untreated hamsters, the expression of AVP V_2 mRNA was significantly higher in cardiomyopathic hamsters compared with normal hamsters (0.53 ± 0.05 amol/μg of total RNA *v* 1.03 ± 0.15 amol/μg of total RNA, $P < .01$). This increase in the expression of AVP V_2 mRNA correlated with the heart weight to body weight ratio ($y = 193.2X - 0.265$, $r = .71$, $P < .01$). This relationship is illustrated in Fig 2. These data suggested that an increase in AVP V_2 mRNA correlated with the severity of heart failure. Enalapril treatment prevented the increase in the expression of AVP V_2 mRNA found in the cardiomyopathic hamsters (1.03 ± 0.15 *v* 0.58 ± amol/μg of total RNA, $P < .01$). An increase in the expression of AQP2 mRNA was also seen in untreated cardiomyopathic hamsters when compared with untreated normal hamsters (0.027 ± 0.002 *v* 0.036 ± 0.002 amol/μg of total RNA, $P < .05$). Figure 3 shows the relationship between the expression of AQP2 mRNA and the heart weight to body weight ratio. A significant correlation was found between these 2 parameters ($y = 5.589X + 0.0048$, $r = .69$, $P < .01$). Enalapril treatment abolished the increase in the expression of AQP2 mRNA (0.025 ± 0.001 *v* 0.028 ± 0.001 amol/μg of total RNA, NS).

The RT-PCR data were confirmed by ISH studies. Figure 4 shows the intensity of the AVP V_2 mRNA signal detected in the IMCD of normal and cardiomyopathic hamsters. Figure 4A shows the signals found in the normal hamster, which were less intense than those seen in the cardiomyopathic hamster (Fig 4B). Enalapril treatment normalized the increased signal in the cardiomyopathic hamsters (Fig 4C). The specificity of the probe was confirmed by competitive hybridization, omission of the probe in the hybridization mixture, and pretreatment of the tissue sections with Ribonuclease A (0.1 mg/mL) (Sigma Chemicals). Signal was not detected with Ribonuclease A treatment (Fig 4D).

Figure 5 shows the AQP2 mRNA signal detected in the IMCD of the normal and cardiomyopathic hamsters. The signals for AQP2 mRNA were higher in cardiomyopathic hamster (Fig 5B) than those seen in the normal hamster (Fig 5A). The intense signals seen in the cardiomyopathic hamster were abolished by enalapril (Fig 5C).

DISCUSSION

Plasma AVP levels are elevated in patients with congestive heart failure,[2] as well as in animal models with experimental heart failure.[3,4,14] Circulating AVP levels in cardiomyopathic hamters are elevated to twice that of age-matched controls similar to those reported previously.[4] The increase in AVP release are caused by nonosmotic stimulation, increased synthesis, or decreased metabolism and degradation. The increase in circulating AVP can play a significant role in water retention in congestive heart failure. The importance of AVP in promot-

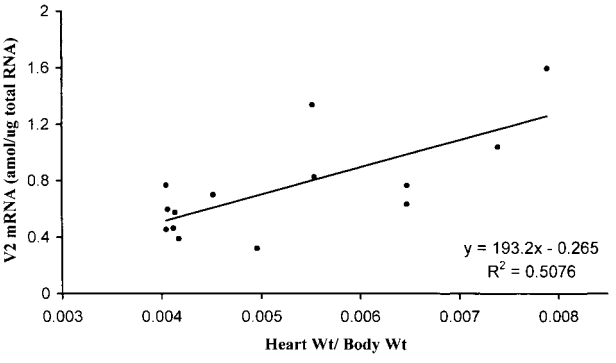

Fig 2. The relationship between the expression of AVP V_2 mRNA in the IMCD and the heart weight to body weight ratio is shown. These data suggested that an increase in AVP V_2 mRNA correlated with the severity of heart failure.

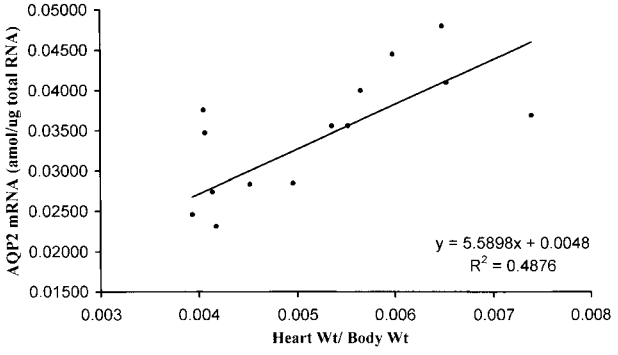

Fig 3. The relationship between body weight to heart weight ratio and the expression of AQP2 mRNA in the IMCD of the cardiomyopathic hamster is shown.

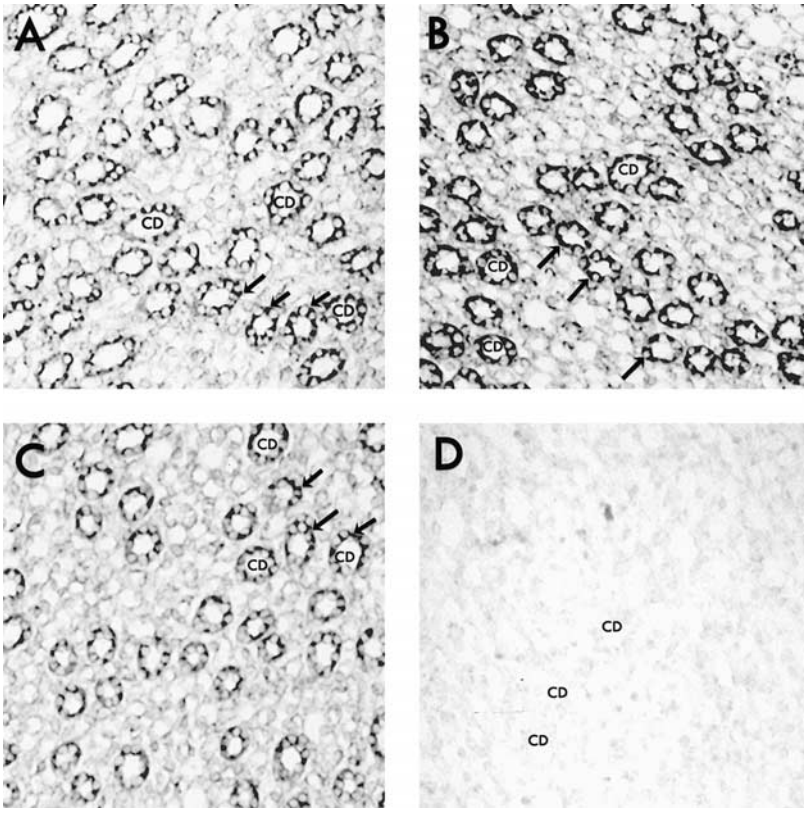

Fig 4. ISH studies for the detection of AVP V_2 mRNA signals in the IMCD of (A) normal, (B) cardiomyopathic hamsters, and (C) cardiomyopathic hamsters treated with enalapril are shown. (D) Shows the effect of RNAase treatment on detecting V_2 mRNA signal.

ing water retention in congestive heart failure was highlighted by experiments with vasopressin V_2 antagonist. In these studies, V_2 receptor antagonists reversed the impairment in water excretion found in dogs[3] and rats[6] with induced cardiac failure. Studies in cardiomyopathic hamsters also showed that V_2 receptor antagonists restored the kidney to respond appropriately to ANP-induced natriuresis and diuresis. Furthermore, AVP-induced cAMP accumulation in the IMCD of cardiomyopathic hamsters was exaggerated when compared with age-matched controls.[12] This can be due to hypersensitivity of the IMCD to AVP, or alternatively, to the upregulation of AVP V_2 receptors in congestive heart failure. It is not clear from these previous studies whether vasopressin V_2 receptors are upregulated in the collecting duct of the kidney. In the present study, we quantified the AVP V_2 receptors mRNA in the IMCD by a RT-PCR method and found that the message for this receptor was significantly elevated compared with its age-matched controls. In addition, this increase in the expression of AVP V_2 mRNA correlated with the heart weight to body weight ratio, suggesting that the increase in the expression of AVP V_2 mRNA is related to the severity of heart failure. Increasing the number of vasopressin V_2 in the collecting duct of the kidney during congestive heart failure can contribute to excessive water reabsorption by the renal tubules.

One reason for the increase in AVP V_2 mRNA in the IMCD of the cardiomyopathic hamster can be due to hormonal stimulation. It is known that several hormonal systems are activated in congestive heart failure to compensate for the reduction in cardiac output. Systems that are thought to play an important role in this condition include the renin angiotensin system, the sympathetic nervous system, and AVP. In the present report, we examined the effect of the renin angiotensin system in promoting an increase in the expression of AVP V_2 mRNA in the IMCD of cardiomyopathic hamsters. We blocked the effect of angiotensin by treating cardiomyopathic hamsters with an angiotensin-converting enzyme inhibitor, enalapril. The increase in AVP V_2 mRNA found in cardiomyopathic hamsters was abolished after receiving enalapril treatment for 7 days. The RT-PCR results were affirmed by the ISH. These observations suggested that the increase in the expression of AVP V_2 mRNA seen in cardiomyopathic hamsters could be due to enhancing angiotensin activity. This conclusion is supported by our measurements of plasma angiotensin II levels. Plasma angiotensin II levels were significantly higher in cardiomyopathic hamsters, but were reduced after enalapril treatment. The reduction in angiotensin II levels correlates with the decrease in AVP V_2 mRNA expression in the IMCD. Further evidence to support the notion that angiotensin II regulates the expression of AVP V_2 mRNA was demonstrated by in vitro studies (unpublished observation). In these studies, angiotenisn II induced an increase in the expression of AVP V_2 mRNA in the IMCD of a rat. Thus, these data are consistent with the suggestion that an increase in angiotensin activity leads to upregulation in the expression of AVP V_2 mRNA in the IMCD of the cardiomyopahic hamster. Plasma AVP levels were also reduced after enalapril treatment, suggesting that AVP may also play a role in modulating the expression of AVP V_2 mRNA in the IMCD, and this remains to be determined.

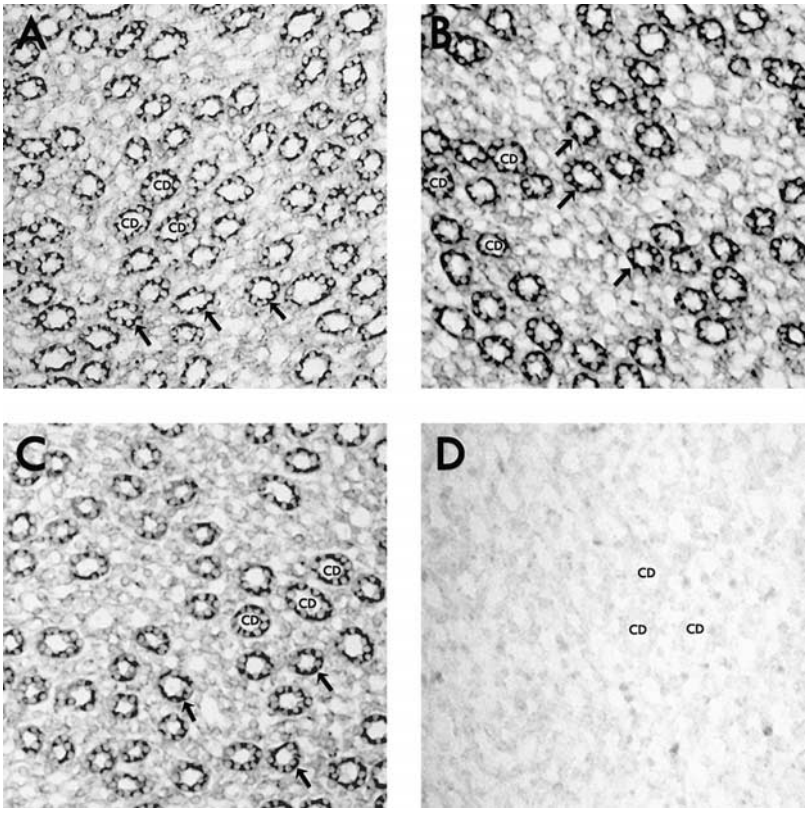

Fig 5. ISH studies for the detection of AQP2 mRNA signals in the IMCD of (A) normal, (B) cardiomyopathic hamsters, and (C) cardiomyopathic hamsters treated with enalapril are shown. (D) Shows that signals are not detected in slides treated with RNAase.

AQP2 is significantly elevated in experimental heart failure model.[8,9] The development of water retention in congestive heart failure rats was attributed to the increase in AQP2 expression in the kidney. In the present study, we also showed an increased in AQP2 mRNA in the kidney of the cardiomyopathic hamster, which is in agreement with previous reports. The sustained increase in AQP2 synthesis in congestive heart failure can be mediated by the AVP V_2 receptor.[9,15] Normally, activation of the AVP V_2 receptor by AVP will set off the production of cAMP that, in turn, triggers the insertion of the AQP2 into the apical membrane of the collecting duct. In congestive heart failure, both circulating AVP and AVP V_2 receptor are chronically elevated. This overactivation will increase local release of cAMP that can play an important role in the long-term regulation of AQP2. It has been shown that cAMP mediates V_2 agonists, and desamino[D-arginine] vasopressin (DDAVP) stimulated AQP2 mRNA accumulation in the kidney.[10] Thus, the increase in AQP2 mRNA expression seen in the IMCD of cardiomyopathic hamsters is probably due to cAMP accumulation secondary to elevation in AVP and AVP V_2 receptor.

Enalapril treatment attenuated the increase in AQP2 mRNA expression in the kidney. This suggests that angiotensin II has a direct effect in promoting an increase in the expression of AQP2 mRNA in the IMCD. A direct effect of angiotensin is unlikely because AVP V_2 receptor antagonist normalized the AQP2 expression in the kidney of heart failure rats.[9] Also, studies in Brattleboro rats showed that in the absence of antidiuretic hormone, the expression of AQP2 in the kidney is much lower than that seen in normal rats.[16,17] This suggests that AVP and AVP V_2 are important in regulating the expression of AQP2. An indirect effect of angiotensin II on the increase in the expression of AQP2 in the kidney is more plausible. In congestive heart failure, the following events probably occur to increase the expression of AQP2. Activation of the renin angiotensin system in heart failure stimulates an increase in both AVP and AVP V_2 mRNA expression that leads to a higher accumulation of cAMP in the kidney. This increase in cAMP accumulation by AVP can be linked to a significant increase in AQP2 mRNA expression, indicating that vasopressin receptor-signalling pathways regulate modulation of AQP2 mRNA levels.[17,18] Hence, after restoring the plasma AVP and AVP V_2 mRNA in the cardiomyopathic hamster to levels observed in the normal hamster with enalapril treatment, the increase in AQP2 mRNA in the cardiomyoapthic hamster was also normalized. These data further lend support to the notion that the vasopressin receptor-signalling pathway probably mediates the increase in the expression of AQP2 mRNA in cardiomyopathic hamsters.

In summary, the present study showed that AVP V_2 mRNA and AQP2 mRNA are significantly elevated in the IMCD of the cardiomyopathic hamster. The increase in the expression of AVP V_2 mRNA is mediated by angiotensin II, whereas, the increase in AQP2 mRNA is probably regulated through the vasopressin-receptor signaling pathways.

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