Cardiac aquaporin expression in humans, rats, and mice

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Butler, Tanya L., Carol G. Au, Baoxue Yang, Jonathan R. Egan, Yee Mun Tan, Edna C. Hardeman, Kathryn N. North, A. S. Verkman, and David S. Winlaw. Cardiac aquaporin expression in humans, rats, and mice. Am J Physiol Heart Circ Physiol 291: H705-H713, 2006. First published March 31, 2006; doi:10.1152/ajpheart.00090.2006.—Water accumulation in the heart is important in ischemia-reperfusion injury and operations performed by using cardiopulmonary bypass, with cardiac dysfunction associated with myocardial edema being the principal determinant of clinical outcome. As an initial step in determining the role of aquaporin (AQP) water channels in myocardial edema, we have assessed the myocardial expression of AQPs in humans, rats, and mice. RT-PCR revealed expression of AQP-1, -4, -6, -7, -8, and -11 transcripts in the mouse heart. AQP-1, -6, -7, and -11 mRNAs were found in the rat heart as well as low levels of AQP-4 and -9. Human hearts contained AQP-1, -3, -4, -5, -7, -9, -10, and -11 mRNAs. AQP-1 protein expression was confirmed by Western blot analysis in all three species. AQP-4 protein was detected in the mouse heart but not in the rat or human heart. To determine the potential functional consequences of myocardial AQP expression, water permeability was measured in plasma membrane vesicles from myocardial cells of wild-type versus various AQP knockout mice. Water permeability was reduced by AQP-1 knockout but not by AQP-4 or AQP-8 knockout. With the use of a model of isolated rat heart perfusion, it was found that osmotic and ischemic stresses are not associated with the development of myocardial edema, with expan-

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multiple AQPs at transcript level but corresponding protein expression of AQP-1 and AQP-4 only. In addition, we note species differences in expression of AQP-4 protein, which is present at high levels only in the mouse heart. Functional studies demonstrate that AQP-1 facilitates water transport in vitro but that osmotic stress does not involve the regulation of cardiac AQP-1 transcript or protein.

MATERIALS AND METHODS

Human heart and skeletal muscle specimens. The Human Ethics Committee at Children’s Hospital at Westmead approved the use of tissue for this study. Human ventricular myocardium was obtained fresh from the operating theater. Tissue normally discarded as part of corrective surgery for children with structural heart disease was used. Ventricular myocardium was most commonly obtained from the right ventricular outflow tract in children undergoing repair for tetralogy of Fallot. Samples from left ventricular (LV) outflow tract and interventricular septum were also available. The tissue was obtained while the patient was on cardiopulmonary bypass after cardiopлегic arrest of the heart. Specimens were immediately frozen in liquid nitrogen after resection, with a warm ischemia time of ~30 s. Additional cardiac tissue was obtained from donor hearts that were not used for transplantation. Human skeletal muscle was obtained from a bank of tissue that included muscle with normal histology derived from children undergoing amputation for osteosarcoma.

Rat and mouse tissues. The Animal Ethics Committee at Children’s Hospital at Westmead approved the use of tissue for this study. Heart and skeletal muscle was harvested from adult Wistar rats and CD1 wild-type, AQP-1 knockout (−/−) (24), AQP-4−/− (23), and AQP-8−/− (53) mice under deep anesthesia. Organs were removed and immediately frozen in liquid nitrogen.

Cardiac myocyte preparations. Primary cultures of cardiac myocytes were prepared from the hearts of neonatal Wistar rats by using the Worthington Cardiomyocyte Isolation System (Worthington Biochemical). Cells were prepared from 2-day-old rats following the manufacturer’s instructions. The cells were plated onto plastic culture dishes coated with Type IV Collagen (Sigma) at 7.5 μg/ml and harvested at various times between 0 and 4 days of culture. Spontaneous beating of the cells was observed from day 1 of the culture. Isolation and culture of cardiac myocytes using this method produces cultures that are >90% pure, with the major cellular contaminant being fibroblasts (49).

Subcellular fractionation. Mitochondrial and sarcolemmal membrane fractions were prepared from wild-type mouse skeletal muscle and heart by using OptiPrep gradients (Axis-Shield, Oslo, Norway) following the manufacturer’s methods. Fresh tissue was finely minced and then dispersed in ice-cold buffer [250 mM sucrose, 10 mM HEPES (pH 7.4), 1 mM EDTA, and protease inhibitor cocktail] with a dounce homogenizer. Nuclei and whole cells were removed by centrifugation at 700 g for 10 min (×2). The supernatant was then centrifuged for 10 min at 16,000 g to produce a mitochondrial pellet. The supernatant of this spin was retained as the cytoplasmic/membrane fraction. The mitochondrial pellet was washed twice with homogenization buffer. Each fraction was separately added to 20% iodoxanol in homogenizing buffer and centrifuged at 300,000 g for 2 h at 15°C to produce a continuous gradient. Fractions were retained, and the membranes were concentrated with further centrifugation at 100,000 g for 30 min. Proteins were solubilized in an equal concentration of 5× SDS sample buffer [240 mM Tris·HCl (pH 6.8), 6 mM EDTA, 6% SDS, 0.01% bromophenol blue, and 3% mercaptoethanol] before Western analysis was performed.

Western blot analysis. For Western analysis of whole cell lysates, frozen tissue was homogenized in ice-cold lysis buffer [50 mM Tris (pH 8.0), 150 mM NaCl, 0.1% SDS, 1% NP-40, and 1 mM PMSF] containing protease inhibitor cocktail (Sigma-Aldrich) using a dounce homogenizer on ice. The homogenate was briefly spun at 1,000 g to pellet cell debris. Protein concentrations were determined with Bio-Rad protein assay dye reagent concentrate (Hercules). The homogenates were mixed with an equal volume of 5× SDS sample buffer before being loaded onto 12% SDS-PAGE gels.

Electrophoresis was followed by transfer to polyvinylidene fluoride Immobilon-P membranes (Millipore Australia, Sydney, NSW, Australia). Membranes were blocked and then incubated with AQP-1 (AQP11-A, 1:1,000 dilution, Alpha Diagnostic, San Antonio, TX), AQP-3 (sc-9885, 1:500 dilution, Santa Cruz Biotechnology, Santa Cruz, CA), AQP-4 (sc-9888, 1:1,000 dilution, Santa Cruz Biotechnology), AQP-5 (1:500 dilution, Merck), AQP-7 (sc-28625, 1:500 dilution, Santa Cruz Biotechnology), mitochondrial porin (1:3,000 dilution, Molecular Probes, Invitrogen), β-dystroglycan (1:250 dilution, Novocastra, Newcastle Upon Tyne, UK), cardiac/skeletal actin (5C5, 1:3,000 dilution, Sigma-Aldrich), or vascular endothelial (VE)-cadherin (sc-6458, 1:1,000 dilution, Santa Cruz Biotechnology) antibodies. Secondary antibodies were horseradish peroxidase-conjugated donkey anti-goat IgG (Santa Cruz Biotechnology), donkey anti-rabbit IgG (Amersham Biosciences), or sheep anti-mouse IgG (Amersham Biosciences), and the signal was visualized using enhanced chemiluminescence (Amersham Biosciences).

Immunofluorescence. Cryosections 8-μm thick were fixed with 3% formaldehyde in PBS, washed, and incubated in blocking buffer (PBS containing 2% serum). AQP-4 (sc-20812; 1:50 dilution, Santa Cruz Biotechnology), AQP-1 (AQP11-A, 1:1,000 dilution, Alpha Diagnostic), or α-sarcomeric actinin (EA-53, 1:400 dilution, Sigma) antibodies were applied for 1 h. Samples were washed and then incubated with Cy3-conjugated donkey anti-goat or donkey anti-rabbit IgG (1:250 dilution, Jackson ImmunoResearch, West Grove, PA). Samples were washed and mounted by using Shandon Immu-mount.

Tissues used for semithin sections and dual labeling were processed according to Griffiths et al. (8), with modifications. Tissues were fixed in 4% formaldehyde solution for 30 min before being transferred to 20% sucrose-polyvinylpyrrolidone solution for overnight infiltration. Samples were subsequently frozen in liquid nitrogen. Semithin sections of 0.5–1 μm were cut by using a Leica Ultracut UCT ultratome equipped with a Leica EM FC6 cryochamber at −60°C. Sections were labeled with AQP-1 and Alexa Fluor 488-conjugated donkey anti-rabbit IgG (Molecular Probes, Invitrogen). Colabeling was performed with fluorescin-conjugated isoelectin B4 (20 μg/ml, Vector, Burlingame, CA) to identify endothelial cells.

RT-PCR. Total RNA was extracted from tissues using Tri Reagent (Molecular Research Center, Cincinnati, OH), followed by cDNA synthesis using oligo dT15 and reverse transcriptase (Superscript III, Invitrogen). PCR amplifications of cDNA were performed using Taq DNA polymerase (Invitrogen). RNA from tissues known to express each AQP was included as positive controls. Negative controls included no-RT controls in which reverse transcriptase was omitted and there were no template controls in the PCR. All were appropriately negative. Primer sequences for rat AQP-1 to -9 and AQP-11 and human AQP-1 to -11 have been published previously (45). These and the mouse primers used to screen for AQP transcript are listed in Table 1.

Quantitative RT-PCR was performed with Lux primers (Invitrogen) for rat AQP-1 (5′-CAACGTTGGTGAGCCATCGJOEJTG-3′; 5′-CCAGTGATTTGGGGAAG-3′), AQP-4 (5′-CATTAGGAGCGCGGTGAAGJEOJG-3′; 5′-TTGAGTTCCAGGCGCTTGTA-3′; 18S (5′-GACCTGGCAGATTGCAATACAGG-3′; 5′-GTAAGGTACGACGCTGTGAG-3′) the Platinum PCR SuperMix-UDG kit. All samples were run in duplicate. AQP levels were quantified during 45 cycles by using a Corbett ResearchRotor-Gene RG 3000A (Mortlake, Victoria, Australia), and analysis was performed by using Rotor-Gene Real Time Analysis Version 6.0 (Corbett Research). mRNA levels were quantified by using 18S rRNA to normalize the raw AQP-1 and AQP-4 signals.
**Table 1. Primers for AQP screen**

<table>
<thead>
<tr>
<th>AQP</th>
<th>Human</th>
<th>Rat</th>
<th>Mouse</th>
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<tbody>
<tr>
<td>AQP-1</td>
<td>F-5' GTTCAGGACACGCTGAAGGT 3'</td>
<td>F-5' CTTACCTGCAGACCTTTC 3'</td>
<td>F-5' TGGGTCTGCGGGCGACACTAG 3'</td>
</tr>
<tr>
<td>AQP-2</td>
<td>R-5' GAGGAGTCACTTGAACCTTTC 3'</td>
<td>R-5' TACGCTCACTCCACATTGCTTC 3'</td>
<td>R-5' GACCTGCTGAGCATTAGTAC 3'</td>
</tr>
<tr>
<td>AQP-3</td>
<td>F-5' GGGGACATTTGTTGAAATAGC 3'</td>
<td>F-5' ACCGATGCCTGAAATAGAAG 3'</td>
<td>R-5' ACCGATGCCTGAAATAGAAG 3'</td>
</tr>
<tr>
<td>AQP-4</td>
<td>R-5' AGCAGGGACATTGAAATAGC 3'</td>
<td>R-5' TCCGAGGGACATTGAAATAGC 3'</td>
<td>F-5' TGGGTCTGCGGGCGACACTAG 3'</td>
</tr>
<tr>
<td>AQP-5</td>
<td>F-5' GGATTTCTGCCTGACGCTTCT 3'</td>
<td>F-5' CTTGCGGAGGCGCTTCTCAGC 3'</td>
<td>R-5' ACCGATGCCTGAAATAGAAG 3'</td>
</tr>
<tr>
<td>AQP-6</td>
<td>R-5' CTCAAAAGGCGACCATGAT 3'</td>
<td>R-5' CTCAAAAGGCGACCATGAT 3'</td>
<td>F-5' TGGGTCTGCGGGCGACACTAG 3'</td>
</tr>
<tr>
<td>AQP-7</td>
<td>F-5' CGGCGGAGCAGTCTTCGAGG 3'</td>
<td>R-5' CGGCGGAGCAGTCTTCGAGG 3'</td>
<td>F-5' TGGGTCTGCGGGCGACACTAG 3'</td>
</tr>
<tr>
<td>AQP-8</td>
<td>R-5' ATGCGGACAGAAAGTCTGGT 3'</td>
<td>R-5' ATGCGGACAGAAAGTCTGGT 3'</td>
<td>R-5' ATGCGGACAGAAAGTCTGGT 3'</td>
</tr>
<tr>
<td>AQP-9</td>
<td>F-5' GGGGACATTGAAATAGC 3'</td>
<td>F-5' GGGGACATTGAAATAGC 3'</td>
<td>R-5' ATGCGGACAGAAAGTCTGGT 3'</td>
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<tr>
<td>AQP-10</td>
<td>R-5' CTTCCTCCACACCTGGTCAGT 3'</td>
<td>R-5' CTTCCTCCACACCTGGTCAGT 3'</td>
<td>R-5' ATGCGGACAGAAAGTCTGGT 3'</td>
</tr>
<tr>
<td>AQP-11</td>
<td>F-5' CAGCAGGACACGCTGAAGGT 3'</td>
<td>R-5' CAGCAGGACACGCTGAAGGT 3'</td>
<td>R-5' ATGCGGACAGAAAGTCTGGT 3'</td>
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AQP, aquaporin; F, forward; R, reverse.

**Water permeability measurements.** Hearts from three mice were poised and homogenized, and membrane fractions were collected by centrifugation through a discontinuous sucrose gradient as described previously (50). Membrane fractions were pelleted, resuspended at ~1 mg protein/mL in PBS and passed 10 times through a 27-gauge needle. Stopped-flow measurements of vesicle osmotic water permeability were carried out on a Hi-Tech Sf-51 instrument with measurements repeated 5–10 times per group and repeated in two independent experiments. Membrane vesicles were subjected to a 140 mosM osmotic gradient by mixing with an equal volume of 280 mM mannitol in PBS. The kinetics of decreasing vesicle volume was measured from the time course of 90° scattered light intensity at 530 nm. The data were fitted to a single exponential function. The osmotic water permeability coefficient ($P_f$) was calculated as described previously (48).

**Isolated heart perfusion and ischemia.** Rats were anesthetized with inhaled isoflurane-oxygen mixture and injected with 0.5 ml of heparin into the renal vein. A thoracotomy was performed, and the hearts were isolated heart perfusion and ischemia and 70 min of reperfusion. Individual hearts were only accepted if the intrinsic heart rate was above 200 beats/min and achieved >100 mmHg systolic pressure within 20 min of perfusion. The 2-h control animals demonstrate stability of the model; however, meaningful analysis after this point is limited by the inevitable development of cardiac edema.

**Results**

**Species variation in AQP expression.** A systematic screen was conducted to determine which AQPs are expressed in the hearts of mice, rats, and humans. Comparison of expression patterns revealed AQP-1, -4, -7, and -11 transcript in the hearts of all three species. RT-PCR of total RNA from mouse heart revealed transcript for AQP-1, -4, -6, -7, -8, and -11 (Fig. 1). The expression profile for the rat heart showed overlap with the min of stabilization perfusion, followed by 20 min of zero-flow ischemia and 70 min of reperfusion. Individual hearts were only accepted if the intrinsic heart rate was above 200 beats/min and achieved >100 mmHg systolic pressure within 20 min of perfusion. The 2-h control animals demonstrate stability of the model; however, meaningful analysis after this point is limited by the inevitable development of cardiac edema.

**Statistics.** Analysis was performed with SPSS (version 1.5) for Windows. A paired t-test was used to compare the difference between the mean values for the control and experimental groups. Statistical significance was determined by a two-tailed t-test. A value of $p < 0.05$ was considered significant. 

**Results and Discussion**

**Heart function.** The expression profile for the rat heart showed overlap with the min of stabilization perfusion, followed by 20 min of zero-flow ischemia and 70 min of reperfusion. Individual hearts were only accepted if the intrinsic heart rate was above 200 beats/min and achieved >100 mmHg systolic pressure within 20 min of perfusion. The 2-h control animals demonstrate stability of the model; however, meaningful analysis after this point is limited by the inevitable development of cardiac edema.

**Figure 1.** Expression of aquaporin (AQP) transcript demonstrated by RT-PCR. RNA was extracted from mouse, rat, and human heart specimens and purified rat cardiomyocytes (CM). It was then subjected to RT-PCR amplification for the AQP-1, AQP-4, AQP-6, AQP-7, AQP-11, and AQP-10 (lanes are empty, because AQP-10 has been shown to be a pseudogene in this species).
mouse heart, with transcript detectable for AQP-1, -6, -7, -9, and -11 (Fig. 1). AQP-4 mRNA was detectable in some rat heart samples but only at low levels compared with the positive controls of skeletal muscle and brain (data not shown). To assess the contribution of nonmyocyte sources of AQP expression, cultured rat neonatal cardiomyocytes (“CM” in Fig. 1) were also screened, demonstrating mRNA for AQP-1, -3, -4, -5, -7, -9, -10, and -11. Human hearts contained transcript for AQP-1, -3, -4, -5, -7, -9, -10, and -11 (Fig. 1).

AQP-1 and AQP-4 protein is detected in whole cell lysates of cardiac muscle. Immunoblot analysis was done to determine whether the AQP mRNAs detected produce measurable protein. AQP-1 expression was seen as a 28-kDa nonglycosylated band and a diffuse ~40-kDa glycosylated band in the heart (“H” in Fig. 2A) and skeletal muscle (“M” in Fig. 2A) of all species. Specificity of the AQP-1 antibody was demonstrated by the absence of specific immunostaining in the heart and muscle of AQP-1 knockout mice (Fig. 2A). Western blot analysis of AQP-4 revealed a nonspecific protein band at ~34 kDa that was in all heart (“H” in Fig. 2B) and muscle (“M” in Fig. 2B) samples, including AQP-4 knockout mice (Fig. 2B). Brain (“B” in Fig. 2B) acted as a positive control for AQP-4 because it contains abundant AQP-4 protein. Two micrograms of brain whole cell lysate contained readily detectable AQP-4 protein for both rats and wild-type mice with no signal in brain homogenate of AQP-4 knockout mice (Fig. 2B). With the use of this band as a size reference to demonstrate the specific detection of AQP-4, protein was demonstrated in skeletal muscle of humans, rats, and mice but only in the heart in mice (Fig. 2B). When large amounts (>40 μg) of protein were loaded, AQP-4 protein could be detected in some human heart samples but not in rat hearts (data not shown).

Analysis of human, mouse, and rat heart samples did not detect AQP-3, with kidney as positive control (Fig. 2C). Other organs known to lack AQP-3 expression and included as negative controls were testis, liver, and lung (Fig. 2C). No signal was seen in human or mouse hearts for AQP-5, with lung homogenate as positive control (Fig. 2D). The AQP-5 antibody cross-reacted with a minor, nonspecific band in the mouse heart and rat kidney that was larger than the 29-kDa AQP-5 (Fig. 2D). AQP-6 mRNA was seen in mouse and rat hearts in our screen but not in purified rat cardiac myocytes. AQP-6 protein has been demonstrated in intracellular vesicles in kidneys (56). However, its water permeability is low and its function is presently unclear, so AQP-6 was excluded from further analysis. AQP-7 is highly expressed in adipose tissue so the presence of transcript may be a contaminant. Western and immunohistochemical analysis to test this were attempted but were not informative as the commercially available AQP-7 antibodies are poor (data not shown). Similarly, the available AQP-8 and AQP-9 antibodies did not produce specific banding on Western blots, precluding analysis of protein expression (data not shown). No antibodies are commercially available for AQP-11.

![Fig. 2. Analysis of AQP-1 and AQP-4 protein expression. A: AQP-1 is expressed in hearts (H) and skeletal muscle (M) of all species. No signal is detected in tissues derived from an AQP-1 knockout (-/-) mouse, and mouse brain (B) contains levels of AQP-1 that are undetectable under these conditions. B: AQP-4 is expressed in muscle of all three species. AQP-4 protein is easily detectable in wild-type (WT) mouse heart but not human or rat heart. No AQP-4 signal is present in AQP-4 knockout mouse tissue, but nonspecific bands are notated. Brain (B) acts as a positive control for AQP-4, demonstrating high levels of expression. Twenty micrograms protein (heart and muscle) or 2 μg protein (brain) were loaded per lane. C: AQP-3 protein is known to be expressed in kidney and is seen as a positive band on Western blot. No AQP-3 protein is detectable in other organs, including heart. D: AQP-5 expression is not apparent in human or mouse heart or the negative control rat kidney. Whole rat eye does not contain detectable AQP-5 protein, whereas rat lung acts as the positive control to demonstrate AQP-5 expression. Analysis is representative of 3 individuals of each species for all Western blots shown and are representative of 3 independent sets of experiments. E: cardiac myocytes were isolated from whole hearts and cultured for 0–4 days. Analysis was performed on all culture days with similar results, and the results are representative of 2 independent sets of experiments. Shown is a 3-day culture. Enrichment of the culture is demonstrated by high levels of cardiac actin relative to vascular endothelial (VE)-cadherin. In contrast, heart and muscle contain a greater proportion of VE-cadherin relative to cardiac or skeletal actin. Liver (L) contains vascular tissue but not cardiac or skeletal actin. AQP-1 is expressed by cardiac myocytes as well as being shown in whole organ preparations.](http://ajpheart.physiology.org/)
**AQP-1 protein is expressed by cardiac myocytes.** RT-PCR screening revealed the presence of AQP-1 transcript in rat cardiac myocytes. Endothelial AQP-1 likely contributes to AQP-1 protein, which is evident in whole heart samples. Although we (1) and others (38) have reported myocyte AQP-1 expression, we sought to confirm this using purified cardiomyocytes.

Cardiac myocytes were purified and cultured from neonatal rat hearts to reduce the signal derived from other cells present in whole heart, in particular, endothelial cells. Myocyte enrichment was confirmed by comparative Western analysis for the endothelium-specific VE-cadherin and the myocyte-specific cardiac/skeletal actin (Fig. 2E). High levels of cardiac actin and barely detectable levels of VE-cadherin (Fig. 2E) demonstrated a predominance of cardiac myocytes in the purified sample. By contrast, whole hearts contained a much greater proportion of VE-cadherin to cardiac actin (Fig. 2E). AQP-1 transcript was present in cardiac myocyte cultures, providing evidence for myocyte-derived expression in addition to the known endothelial expression.

**Distribution of AQP-1 and AQP-4 in the heart.** AQP expression was visualized by immunohistochemical staining (IHC). AQP-1 was highly expressed in vascular endothelial cells in skeletal (Fig. 3A) and cardiac (Fig. 3C) muscle. Specificity of staining was demonstrated by absence of signal in tissues from AQP-1−/− mice (Fig. 3, B and D). AQP-1 expression was confirmed to be endothelial by colocalization with isolectin B1, an endothelium-specific marker (Fig. 3I). Nonspecific staining of skeletal myofibers (Fig. 3B) is noted in knockout tissue when viewed at high exposures, but this is not evident in the cardiac sections (Fig. 3D).

AQP-4 IHC revealed myocyte plasma membrane localization in skeletal muscle (Fig. 3E) that is consistent with previous reports (6). Mouse hearts also contained AQP-4 protein with a cardiomyocyte membrane distribution (Fig. 3G). Staining of skeletal muscle (Fig. 3F) and heart (Fig. 3H) from AQP-4−/− mice was negative, confirming that the immunoreactivity seen in wild-type tissue was specific for AQP-4. Cardiac myocytes were highlighted by staining with the marker α-actinin to demonstrate that the AQP-4 signal was present in the myocyte membrane and not that of other cells in the heart (Fig. 3J).

IHC staining of heart and muscle with the use of the AQP-4 antibody sc-9888 (Santa Cruz Biotechnology) revealed the expected membrane localization and an additional intracellular signal in the myocytes (data not shown). We investigated this further by preparing subcellular fractions of mouse heart and skeletal muscle containing different organelles and performing Western analysis of the protein obtained from these fractions. The fraction enriched for plasma membrane was demonstrated with the marker β-dystroglycan, with both AQP-1 and AQP-4 proteins seen in this fraction (Fig. 4). Neither AQP was localized to fractions containing intracellular organelles, including the mitochondrial fraction marked by the presence of mitochondrial porin (Fig. 4). Thus AQP-1 and AQP-4 protein is present at the plasma membrane and not in appreciable quantity in intracellular organelles.

**AQP-1 is the dominant functional AQP in mouse heart.** AQP-1 and -4 were detectable at the transcript level in the hearts of humans, rats, and mice and at the protein level in the mouse heart. We tested the functional relevance of AQP-1 and -4 using membrane vesicle preparations from the hearts of

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**Fig. 3. Immunolocalization of AQP-1 and AQP-4 protein.** AQP-1 protein is pronounced in skeletal muscle (A) and heart (C) of WT mouse. Signal is endothelial, shown by colocalization of AQP-1 signal (red) with isolectin B1 (green) in semithin sections of WT heart (I). Endothelial staining is specific, as shown by absence of signal in AQP-1−/− muscle (B) and heart (D). AQP-4 protein shows typical sarcolemmal distribution in skeletal muscle fibers (E) and a similar distribution in heart (G) of WT mice. Colabeling with AQP-4 (red) and α-actinin (green) demonstrates that AQP-4-positive membranes are cardiac myocytes (J). Membrane labeling is not seen in skeletal muscle (F) or heart (H) of AQP-4−/− animals. Scale bars represent 20 μm.
HgCl₂ reduced the permeability to that of AQP-1 (B). Pretreatment of vesicle preparations with the AQP-inhibitor P-channel (P) bramate (P) reduced vesicle movement (Fig. 5, A and B). Pretreatment of vesicle preparations with the AQP-inhibitor HgCl₂ reduced the permeability to that of AQP-1⁻/⁻ samples (Fig. 5, A and B), further supporting AQP-1-mediated water movement.

Ischemia-reperfusion injury does not induce acute regulation of AQP-1 or AQP-4 expression. Acute regulation of AQP expression has been demonstrated in other organ systems, including the brain (29). This was tested in isolated rat hearts by using a Langendorff-based model of ischemia and reperfusion. Four treatment groups were compared with six animals in each group; baseline (hearts excised and not perfused), 20-min perfusion controls, 120-min perfusion controls (without ischemia), and 20-min perfusion/20-min ischemia/70-min reperfusion. Heart function was assessed as the LVDP measured at systole (Fig. 6A) and diastole (Fig. 6B). Ischemic insult did not affect the systolic LVDP (Fig. 6A) but resulted in a significant increase in diastolic LVDP during the reperfusion phase; P = 0.002 (Fig. 6B). This increase in diastolic pressure confirms that the hearts tested suffered a significant ischemic insult. Other indexes of heart function, including heart rate and contractility, recovered to control levels in ischemic hearts during the reperfusion phase (data not shown). AQP-1 and AQP-4 mRNA and protein levels were measured by quantitative RT-PCR and Western blotting, respectively. AQP-1 transcript (Fig. 6D) and protein (Fig. 6C) were not significantly different across all treatment groups. No AQP-4 protein was evident in the hearts, regardless of treatment (data not shown). Although AQP-4 transcript was detectable, it did not reach the amounts measured in skeletal muscle and so was considered not to be physiologically relevant (data not shown).

DISCUSSION

Maintenance of a normal osmotic environment in the heart involves water and solute movements among cellular, interstitial, and blood compartments. Under normal conditions, this is mostly affected by changes in blood osmolality, whereas under pathological conditions, dysfunction of myocardial cell solute transporters and increased capillary leakiness contribute to myocardial edema. Myocardial edema impairs the contractile function of the heart and is a major contributor to the morbidity and mortality associated with myocardial ischemia and cardiac surgery. The heart is remarkable for its high capillary density and regulation of nutrient flow to avoid impairment in cellular metabolism. The purpose of this study was to define the expression pattern and water transport functions of AQPs in the hearts of mice, rats, and humans. Because AQP-4 has been shown in the brain to play a major role in the development and resolution of cerebral edema caused by cellular (cytotoxic) and vascular leak (vasogenic) mechanisms (28), we postulated that AQP(s) in myocardial cells may be involved in clinically relevant cardiac edema.

![Fig. 4. Subcellular fractionation followed by Western analysis to demonstrate AQP localization. Plasma membrane (PM) and mitochondrial (Mit) fractions were prepared from WT mouse skeletal muscle and heart. Mitochondrial fractions contained mitochondrial porin, unique to this organelle. Mitochondrial membranes were also present as a contaminant in the plasma membrane fraction. Membrane protein β-dystroglycan was only seen in membrane fractions and did not copurify with the mitochondrial fraction. Both AQP-1 and AQP-4 were highly enriched in the plasma membrane fraction.](http://ajpheart.physiology.org/)

![Fig. 5. Osmotic permeability of membrane vesicles. Rate of osmotic equilibration and calculated permeability were compared among vesicles prepared from WT, AQP-1⁻/⁻, AQP-4⁻/⁻, and AQP-8⁻/⁻ mouse hearts. A minimum of five replicates was performed for each experimental group, and studies were performed on two separate membrane preparations from three hearts. AQP-1⁻/⁻ plasma membrane vesicles were significantly slower to equilibrate (P < 0.01; A) and displayed a lower permeability (P) compared with WT, AQP-4⁻/⁻, and AQP-8⁻/⁻ preparations. Addition of HgCl₂ inhibited AQP function and reduced the rate of osmotic equilibration (A) and permeability (B) of all vesicle preparations to that of AQP-1⁻/⁻ values.](http://ajpheart.physiology.org/)
Southern blot data consistent with the cardiac AQP expression was not performed in this study, however, this cannot be reasonably achieved with the use of an isolated perfused heart system and will require whole animal models.

Species differences were revealed in cardiac AQP-4 expression. Mouse cardiac and skeletal muscle contains AQP-4 protein, whereas human and rat hearts do not express appreciable protein. A question raised is the physiological relevance, if any, of AQP-4 expression that is specific to the murine heart.

Despite the expression of transcripts for multiple AQPs in the heart by RT-PCR analysis, appreciable protein expression was demonstrated only for AQP-1 and AQP-4. This finding parallels that of several other tissues, such as cholangiocytes and hepatocytes in the liver (30), where transcripts for many AQPs have been identified, although their physiological relevance is questionable. Although Northern blot analysis of cardiac AQP expression was not performed in this study, others have reported Northern blot data consistent with the RT-PCR data here. This includes the presence of AQP-1 in human and rat hearts (9), AQP-4 in human (51) and mouse (27) hearts, AQP-7 in both human and rat hearts (11, 13, 20), and AQP-11 in mouse hearts (33). The species difference in AQP-8 transcript expression described here is supported by separate studies that show the presence of mRNA in mouse hearts (26) and the absence in human (17) and rat (12, 18) hearts. AQPs shown to be absent in the heart by Northern blot analysis include AQP-2 (52) and AQP-5 (19) in mice and AQP-6 in humans (25).

AQP-1 protein is expressed in the hearts of all three species, and with the use of established methodologies for measuring membrane permeability, AQP-1 was shown to be functional in facilitating osmotic water transport in membranes isolated from wild type mice compared with knockout mice. The AQP-1-dependent percentage of myocardial water permeability of between 20 and 30%, seen in membranes from knockout mice as well as from wild-type mice treated with HgCl₂, is consistent with the estimated contribution of AQPs to total myocardial water flux determined by Kellen et al. (14, 15) in whole heart experiments, although less than that estimated from isolated cardiomyocytes in studies by Ogura et al. (36, 37). Importantly, water permeability assessed by Kellen et al. (14, 15) is the net effect of endothelium- and cardiomyocyte- derived AQPs, whereas that in isolated cells used by Ogura et al. (36, 37) would include only cardiomyocyte-derived AQPs. It is acknowledged that the three different models discussed above are not strictly comparable.

The data here support AQP-1 expression in cardiomyocytes, although at a level considerably lower than that in the vasculature of the heart. AQP-1 expression was found at the sarcolemma in highly enriched rat cardiomyocyte cultures. Although IHC of the mouse heart (Fig. 3, B and C) shows little or no myocyte localization of AQP-1, we (1) and others (38) have previously reported cardiomyocyte expression of AQP-1 in the rat, and the Western analysis shown in Fig. 2E performed by using rat tissue supports this finding. We showed immunolocalization of AQP-1 with t-tubular proteins in the rat heart (1), and Page et al. (38) demonstrated AQP-1 in association with caveolae. Both structures can be considered as sarcolemmal, either the invaginations of the cell membrane into the Z line in the case of the t-tubules or the pinched-off vesicles in the case of caveolae. AQPs appear to be present in isolated cardiomyocytes as demonstrated by measurement of sarcolemmal permeability (37).

The apparent insensitivity of AQP-1 expression to osmotic stress provided by both isolated perfusion and global ischemia is consistent with our understanding of AQP-1 as a constitutively expressed element of the vascular system. Analyses of in vitro cultured fibroblasts (22) have demonstrated altered AQP-1 expression that occurred over several hours in response to osmotic stress. It is possible that over a longer time frame (>2 h), changes in mRNA or protein expression may occur; however, this cannot be reasonably achieved with the use of an isolated perfused heart system and will require whole animal models.

Fig. 6. Ischemic treatment of rat heart and AQP-1 expression. A and B: rat hearts were perfused via the ascending aorta for 20 min and then either processed as controls (○), made ischemic through cessation of perfusate flow (●) for 20 min before reperfusion, or continuously perfused without ischemia (□). Left ventricular systolic pressure (LVP Sys) recovers well after ischemia (A), whereas diastolic pressure (LVP Dias) is significantly increased (P = 0.002) in the reperfusion phase, as expected after a significant ischemic episode. AQP-1 protein expression, measured by Western blotting and normalized to the expression of cardiac actin (C), was not affected by osmotic or ischemic stress. AQP-1 transcript levels were unchanged (D), and AQP-4 transcript was not induced during recovery period.
AQP-4 increases water permeability by a much greater amount than does AQP-1 (55): the faster heart rate and metabolic rate of the mouse may mandate AQP-4 expression. However, it is noted that the permeability of cardiac membrane vesicles was not reduced by AQP-4 deletion. Furthermore, there was no evidence of upregulation of AQP-4 expression in the rat over the observation period after ischemia. Physiologically relevant AQP function can be difficult to distinguish in an in vitro analysis in some cases (43). On the other hand, the presence alone of an AQP does not provide evidence for physiological relevance, borne out by investigations of AQP-4 in skeletal muscle function (54). Cardiac and skeletal muscles exhibit many similarities, including the expression of AQP-1 (1) and AQP-4 (54) proteins, but also important differences in resistance to fatigue and choice of metabolic substrate. These differences are relevant to the way each muscle handles water.

We report the presence of AQP-3 and AQP-5 mRNA but the absence of detectable protein in the human heart. The same may be true of AQP-8 in the mouse heart, in that the AQP-8 transcript was easily detected; however, we were unable to satisfactorily assess AQP-8 protein expression using commercial antibodies. At this point, it is not possible to accurately determine the localization or functional significance of AQP-7, -8, -9, or -11 in the heart with antibody methods, and this situation highlights many contemporary issues in AQP research in which antibodies validated with positive and negative controls are not available. The creation of double knockout animals and better-controlled models may be required.

In conclusion, we demonstrate the expression and functional relevance of AQP-1 in the heart. We also show evidence for AQP-1 expression by rat and human cardiac myocytes and AQP-4 by mouse cardiac myocytes. Because only small changes in myocardial water content are associated with significant impairment of cardiac function (21, 31, 41), detailed examination of myocardial water handling and the role of AQP5s may reveal potential avenues for therapeutic intervention.

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