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Expression of Water Channels in the Human Heart

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Myocardial blood flow and oxidative metabolism are heterogeneous. Both measures correlate significantly in the normoxic myocardium. We assume that in high flow areas with high metabolism, water channels (aquaporins; AQPs) secure transport of the respiratory water through membranes. Thus, high metabolism areas should express high AQP levels and *vice versa*.

We investigated AQP1 and AQP4 – that are not well defined in the human myocardium – in normoxic and postischaemic/reperfused rabbit hearts, in intact human atria, and in human hearts with dilatative cardiomyopathy (DCM). The existence of both AQPs was assessed on the mRNA level, the protein level, and by using immunohistochemistry.

AQP1 mRNA is heterogeneously expressed in the rabbit heart and the human heart. The expression and the heterogeneity are reduced in impaired hearts (postischaemic rabbit hearts and DCM hearts) compared with the expression and the heterogeneity in normoxic rabbit hearts and in intact human atrium. The AQP1 protein is also heterogeneously distributed in the myocardium. However, the protein expression in the left ventricles of the DCM hearts is not reduced in parallel with the mRNA expression. We attribute the low left ventricular values to a loss of heterogeneity in metabolism in these hearts. In addition, we provide the first direct evidence of AQP4 mRNA expression in normoxic rabbit hearts and in DCM hearts. Moreover, we show a different pattern between the AQP1 and the AQP4 proteins in the rabbit myocardium. AQP1 appeared as randomly distributed points and AQP4 had a garland-like appearance reminding one of the distribution of capillaries and the contour of cardiomyocytes, respectively. We suggest that AQP1 is responsible to transport water through the capillary endothelium, and that AQP4 facilitates the water transport across cardiomyocyte membranes. *J Clin Basic Cardiol 2003; 6: 77–9.*

Key words: heterogeneity, myocardial blood flow, oxidative metabolism, respiratory water, aquaporin, water channel

Myocardial blood flow (MBF) in different areas of the mammalian heart varies from 20 % up to 200 % of the average flow [1], corresponding to low-flow or high-flow areas. Because a MBF of only 20 % cannot be compensated by increasing the arteriovenous oxygen difference, low-flow areas would undergo necrosis. Consequently, metabolism in low-flow areas should be low, and *vice versa,* metabolism in high-flow areas should be high.

Our own investigations on blood-perfused, isolated rabbit hearts showed a significant correlation between regional MBF (coloured microspheres) and regional, oxidative metabolism (^{18}O-labeling of respiratory water [2]). In high-flow areas with high oxidative metabolism, oxidative phosphorylation in mitochondria should produce high amounts of respiratory water that needs to be transported across the outer membrane of mitochondria, and the membranes of cardiomyocytes and endothelial cells. In general, the passage of water through membranes can be maintained by either rather slow diffusion or rapid water channels required to transport high amounts of water. We postulated that water channels – so-called aquaporins – would be required for the respiratory water transport, and that in high-flow areas with high metabolism and high respiratory water production, the expression of aquaporins should be high. As a consequence, we suggest a heterogeneity of myocardial aquaporins corresponding to heterogeneities in flow and metabolism.

Ten different aquaporins specific for certain tissues have been identified so far [3–6]. AQP2 can be excluded for the myocardium, because it is located only in the renal collecting duct [7]. AQP1 is predominantly located on the erythrocyte membrane but also on renal proximal tubules, liver, colon and others [8], eg on the rat subendocardium [9]. Moreover, using immunostaining, AQP1 was shown on the capillary endothelium of the rat heart but not on cardiomyocytes [8], and more recently, these water channel proteins were described to be located in rat cardiomyocyte caveolae [10]. In contrast, the existence of aquaporins was completely challenged for ventricular myocytes after providing experimental evidence for a membrane hydraulic conductivity high enough to permit water flux penetrating the lipid layer itself rather than passing through pores [11]. We wanted (1) to demonstrate the existence of AQP1 in the human heart, (2) to describe the AQP1 distribution, and (3) to demonstrate the existence of another water channel (AQP4) that should be located on cardiomyocytes.

Material and Methods

RT-PCR

Total RNA of 37 tissue samples of explanted hearts (dilatative cardiomyopathy) was isolated with RNeasy kit (Qiagen, Hilden). Briefly, 30 mg tissue was shock-frozen in liquid nitrogen and pulverised in a pre-cooled metal mortar. After adding 700 μl lysis buffer (supplied by the manufacturer), the tissue was homogenised with a rotor-strator homogenisator (Miccra, Muelheim/Rhein). The samples were centrifuged at 13,000 min^{-1} in a microcentrifuge (Eppendorf, Hamburg). 700 μl 70 % ethanol were added to the supernatant, and the mixture processed as described by the manufacturer.

cDNA was synthesised with 50 μM random hexamers (Promega, Mannheim), 20 mM dNTPs each, 20 U RNasin (Promega, Mannheim) and 10 U MULV reverse transcriptase (Amersham-Pharmacia, Freiburg) in a 20 μl assay at 42 °C for 45 min. AQP1 primers of conserved sequences of mouse and man were used: sense 5'-GAAGAAGCTCTTCTGGAGGGC-3', antisense 5'-AGCCAGGTCATTGCGGCCAAG-3'. β-actin primers of human sequence were used: sense 5'-AGAGAT-GGCCACGGCTGCTT-3', antisense 5'-ATTTGCGGTGGA-

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CGATGGAG-3'. AQP4 primers of conserved sequences of human brain were used: sense 5'-TGGTGGGAGGCCTGGGAGT-3', antisense 5'-GTCCTCCACCTCCATGTAGC-3'. PCR amplification (annealing at 57.8 °C, 33 cycles) was performed with 2 U Taq-Polymerase (Amersham-Pharmacia, Freiburg). All mRNA data were normalised to the RNA signal of β-actin. We used this ubiquitous cytoskeleton protein because it is not primarily affected by any human disease [12], and it is widely used as housekeeping gene for both normoxic and ischaemic myocardium [13–15]. Samples were analysed on 2 % agarosegels stained with ethidiumbromide followed by semiquantitative analysis on a phosphoimager (Fuji, BAS 1500; TINA software).

Western Blots

Membranes from 100 tissue samples of DCM hearts were prepared with slight modifications as follows [16]: 70 mg tissue were washed in PBS buffer, minced with a scalpel and homogenised in 1 ml ice-cold buffer H (300 mM sucrose, 25 mM HEPES; pH 7 with Tris, 1 $\mu g/\mu l$ pepstatin A in DMSO, and 2 mM PMSF in isopropanol). Samples were centrifuged at 1000 × g for 20 min. The supernatant was centrifuged at 80,000 × g in a microcentrifuge (TL100.3 rotor; Beckman). After discarding the supernatant, the pellet was resuspended in 40 μl of buffer H. Protein was determined with BSA as standard according to Bradford [17], using a kit from BioRad (BioRad, Munic). 30 μg membrane protein extracts were loaded on 12 % SDS-PAGE [18]. Gels were silver-stained [19] or Western blotted on a Hybond N^+membrane (Amersham-Pharmacia, Freiburg) [20].

Blots were blocked with 3 % (w/v) dry milk powder in PBST and washed with PBST. Anti-AQP1 antibody (developed in rabbit) against residues 243–261 of human AQP1 (Alomone labs, Israel) was used in a 1:200 dilution, and the second antibody anti-rabbit-IgG alkaline phosphatase conjugate (developed in goat; Sigma, Deisenhofen) was diluted 1:2000. Color developing was performed with BCIP/NBT solution (Sigma, Deisenhofen). Other buffers were made according to Sarnbrook et al., 1989 [20].

Results

Aquaporin-1 mRNA Expression and Protein Expression

RT-PCR using highly conserved primers of aquaporin-1 (AQP1) resulted in products of 373 bp; this result was confirmed by dideoxysequencing. Thus, AQP1 mRNA is expressed in the failing human heart. In addition, AQP1 mRNA is also expressed in the healthy human heart (right atrial appendage). The ratio of AQP1 to β-actin expression revealed a relatively high AQP1 expression with much scatter in the right ventricle (1.5 ± 1.5; mean ± standard deviation, n = 37). The expression level (1.2 ± 1.2, n = 37) was decreased in the septum. Surprisingly, the left ventricular free wall showed a significantly lower AQP1 expression (0.7 ± 0.3, n = 37) with only low scatter compared with the right ventricle. The protein expression was high in the right ventricle and low in the left ventricle (Fig. 1). Again, the expression was intermediate in the ventricular septum. Thus, the mRNA expression and the protein expression correlated.

AQP4 mRNA Expression

RT-PCR with RNA of myocardial tissue of healthy human heart (right atrial appendage) resulted in a fragment of 424 bp corresponding in part to AQP4 (Fig. 2). Thus, we provide evidence of mRNA expression of a second water channel (AQP4) in the healthy human heart. The water channel AQP4 was also expressed in the failing human heart (dilatative cardiomyopathy), ie both in the subendocardial and in the subepicardial layer, and also in the isolated rabbit heart (not shown).

Discussion

Our previous, preliminary results showed water channel AQP1 mRNA expression in normoxic rabbit hearts [21]. AQP1 mRNA was also expressed in the healthy human heart (right atrial appendage) as well as in the murine myocardium. We conclude that AQP1 is generally expressed in the mammalian myocardium.

The mRNA expression in the rabbit hearts exhibited a considerable heterogeneity that was reduced in the ischaemic/reperfused rabbit myocardium [21]. In the present study, both RT-PCR and Western blotting demonstrated high AQP1 levels in the right ventricle and low levels in the left ventricular free wall. The high levels were associated with remarkable scatter indicating a heterogeneous distribution of this water channel. Conversely, the AQP1 levels were low in the left ventricular myocardium with almost no scatter, indicating a loss of heterogeneity.

If this water channel permits rapid transport of oxidative water through membranes, then metabolism in areas with

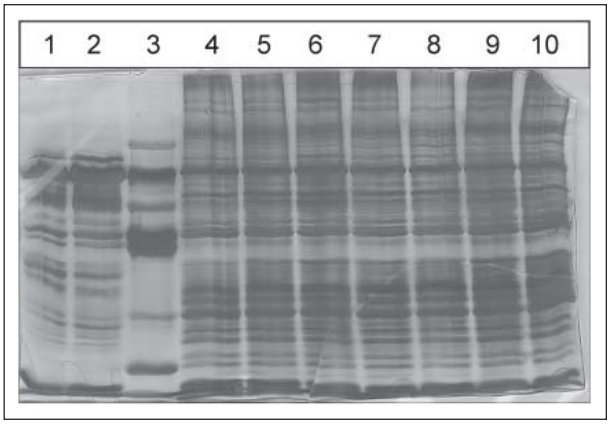

Figure 1. 12 % SDS-PAGE of membrane preparations. a) silver-stained b) immuno-stained with anti-AQP1 antibodies. 28 kDa AQP1 protein is marked. Lane (1) and (2) supernatant, lane (4)–(10) membrane proteins. Lane (1) right ventricular endocardium (2) septum, (3) midrange marker, (4) and (5) right ventricular endocardium, (6) to (8) septum, (9) and (10) left ventricle endocardium.

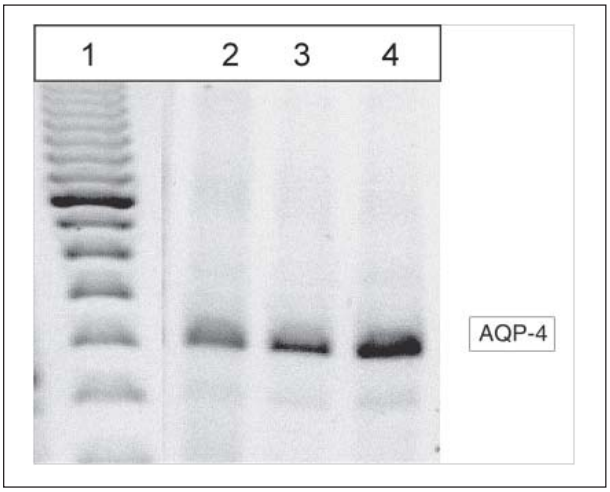

Figure 2. RT-PCR of total RNA resulting in a fragment of 424 bp shows an expression of water channel AQP4 mRNA in normal human myocardium (right atrial appendage)

high AQP1 expression should be high. If so, the metabolism in the left ventricular free wall would be greatly reduced. Moreover, whereas the scatter in the mRNA expression was high in the right ventricle indicating 'physiologic' heterogeneity in AQP1 expression, this measure was low in the left ventricle, indicating loss of heterogeneity.

Although no direct evidence exists, many lines of evidence suggest that the heterogeneity of metabolism is the result of a heterogeneity in function. We therefore support the notion that the substantial loss of function in the left ventricles of hearts with dilatative cardiomyopathy (DCM) is associated with a considerable loss of heterogeneity in the AQP1 pattern. Whether the latter loss is causal for the left ventricular dysfunction remains unclear.

We had shown AQP1 to be expressed in subendocardial and subepicardial layers of the normoxic rabbit heart and the failing human heart in whole tissue extracts, ie without having separated endothelial cells from cardiomyocytes. If AQP1 should be located only on the endothelium of the DCM-hearts, an additional water channel for cardiomyocytes, possibly AQP4 (MIWC) (personal communication, Nielsen, Aarhus, DK), would be required. AQP4 is mercury-insensitive and was so far mainly found in the brain-glia cells and the striatum, but had not yet been described for the heart. For the first time, we provide evidence of the mRNA expression of a second water channel in the healthy human heart, which might be responsible for water transport across the membranes of cardiomyocytes. Similar with AQP1, AQP4 seems to be expressed in general in mammalian myocardium, because we could identify this water channel also in the failing human heart (subendocardial and subepicardial layers of DCM hearts) and in the normoxic rabbit heart.

Recent evidence of a third water channel (AQP8) [4] suggests that the aquaporin story has not yet ended. In contrast, the concept that the vast majority of water flux in ventricular myocytes penetrates the lipid bilayer itself rather than passing through water-filled pores [11] has seemingly come to an end.

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