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Transient hyperosmolality modulates expression of cardiac aquaporins

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ABSTRACT

Purpose: Hyperosmolarity is a common complication in intensive care patients, dysregulating water balance in many organs including brain and heart. The aquaporin (AQP) water channels, in particular AQP1 and −4, have been suggested to play an important role in fluid homeostasis of the myocardium. In many organs AQP expression is regulated by osmolarity, drastically altering water permeability of the cell membranes. The aim of our study was to investigate if plasma hyperosmolality may regulate cardiac expression of AQP1 and −4, and if so, at which magnitude and time frame such regulation takes place.
Methods: C57Bl6 mice were injected intraperitoneally with either 1.5 ml 0.154 Mol (isoosmotic), 0.5 ml 1 Mol (mild hyperosmotic) or 0.5 ml 2 Mol (strong hyperosmotic) NaCl. Plasma, hearts, and forebrains were harvested before injection ("time 0"), and after 1, 4, 8 and 24 h. AQP1 and −4 expression were analyzed using qPCR and Western blot.
Results: Isoosmotic and mild hyperosmotic injections caused no important changes in cardiac AQP expression. Strong hyperosmotic NaCl injections induced an upregulation of AQP1 mRNA and glycosylated fraction of AQP1 protein in the heart without changes of the total protein. AQP4 mRNA and protein decreased in the heart and increased in the brain after hyperosmotic NaCl. The change in AQP4 protein content in the brain preceded the increase of mRNA.
Conclusion: As in the brain, expression of AQP1 and −4 in the heart is influenced by changes in plasma osmolality. Changes in AQP expression may alter cardiac function in hyperosmotic states.

1. Introduction

Osmolality is concentration of particles in a solution expressed in mM/kg [1]. In humans the physiological blood osmolality is 285–290 mOsm/kg [2]. Together with the hydrostatic and oncotic pressures it determines water fluxes across membranes, i.e. osmosis. Several clinical conditions are associated with both transient and long-lasting increases of plasma osmolality [3–5]. Hyperosmotic solutions are infused in patients to alleviate swelling in the post-ischemic brain and heart. Osmolality may also increase in the tissue following ischemia secondary to an elevated concentration of lactate [6]. The resulting edema promotes cell death and aggravates functional impairment caused by ischemia alone, as even a small increase in myocardial water content causes a reduction of cardiac function [7].

Aquaporins (AQPs) transport water across cell membranes in many organs and tissues [8]. AQP4 is abundantly expressed in the brain, and it mediates edema caused by ischemia and trauma [9,10]. However, due to a bidirectional nature of water transport through AQPs, its expression influences both the outcome of stroke [10] and the effect of osmotherapy used to alleviate the potentially fatal brain swelling [11]. Expression of AQP4 in the brain is osmodependent. Both hyperosmotic mannitol and saline increase AQP4 protein expression in the rat cerebral cortex [12,13]. Such an effect may facilitate edema resolution and encourage the effect of osmotherapy.

AQP1 and AQP4 are expressed in human and mouse hearts [14,15], (Rutkovskiy et al., submitted). According to our recent data, AQP1 is expressed in cardiac endothelial cells, while AQP4 is found on cardiomyocytes, and deletion of the AQP4 gene reduces infarct size and osmotic resistance of cardiomyocytes in the presence of

Abbreviations: AQP, aquaporins; ECL, enhanced chemiluminescence; GAPDH, glyceraldehyde 3-phosphate dehydrogenase.

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hypercontracture (Rutkovskiy et al., submitted). Consequently, a regulatory decrease in myocardial AQP4 expression may be cardioprotective. Hyperosmotic reperfusion of ischemic hearts alleviates cardiac muscle swelling [16]. The aim of the current study was to investigate if an acute transient increase of plasma osmolality influences expression of of AQP1 and −4 in the mouse heart. As a control, we measured AQP4 osmoregulation in the brain.

2. Materials and methods

All experiments conform to the guidelines for use and care of the laboratory animals (“Principle of laboratory animal care”, NIH publication No. 86–23. revised 1996) and the study was approved by the Norwegian Animal Health Authority. All animals had conventional microbiological status. They had free access to food

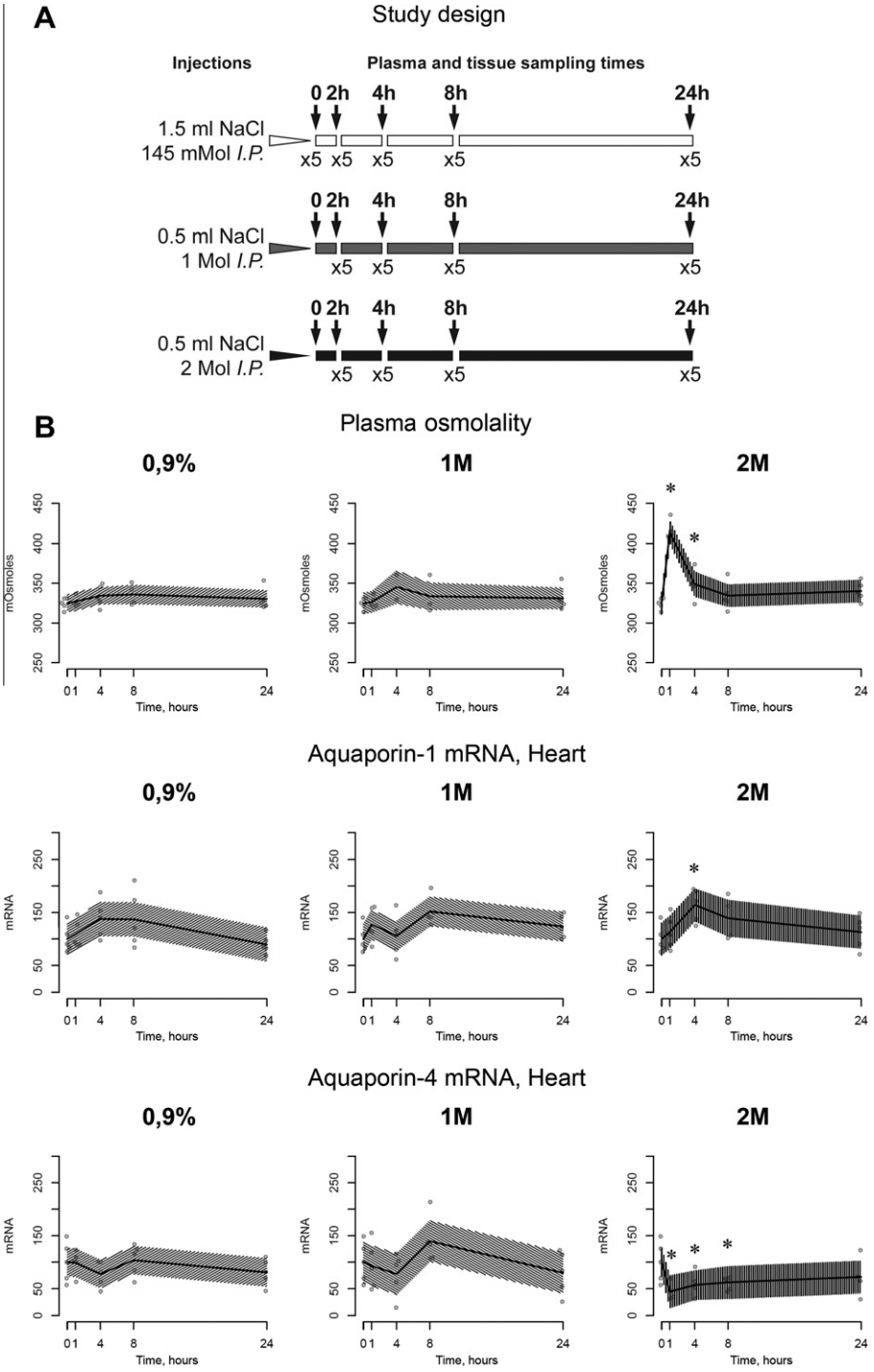

Fig. 1. Study design, plasma osmolality and RNA expression of AQP1 in mouse hearts (A). Study design. Mice were blindly assigned to one of three groups receiving single intraperitoneal injections of 0.154 mM, 1 and 2 M NaCl (horizontal arrow). Tissue and blood plasma were sampled at time “0”, at 1, 4, 8 and 24 h. $N = 5$ for each timepoint in each of the groups. (B). Plasma osmolality and mRNA expression of AQP1 and AQP4 in hearts of mice subjected to hypervolemia (intraperitoneal injection of 1.5 ml 0.9% NaCl), moderate or severe plasma hyperosmality (intraperitoneal injection of 0.5 ml 1 M NaCl or 2 M NaCl respectively) collected after different time intervals (1–24 h) in vivo. $N = 5$ at each timepoint. Values are expressed as mean and 95% confidence bands of 5 samples at each time point. * denotes $p < 0.05$ compared to time 0.

(RM3 from Scanbur BK AS, Norway) and water, and were kept at a 12 h light/12 h darkness cycle in rooms, where the temperature was set to 23 °C and humidity to 55–60%. All animals were acclimatized for at least 4 days before experiments.

2.1. Hypervolemia and hyperosmolarity

The study design is shown on Fig. 1a. Sixty-five wild type male age-matched C57Bl6 mice (B&K Universal, Sollentuna, Sweden) were randomized to be injected intraperitoneally with 1.5 ml isoosmotic NaCl (154 mM, 0.9%), 0.5 ml 1 M, or 0.5 ml 2 M NaCl solutions. Blood, hearts, and forebrains were sampled at time 0 (control, no injection) and 1, 4, 8 or 24 h after injection ($n = 5$ at each time point in each group). The forebrain and the heart were excised and immediately freeze-clamped in liquid nitrogen for extraction of RNA and protein. For details on the procedure, please see Supplementary methods.

2.2. Osmometry

Blood was pipetted into the separation columns (BD Vacutainer® PST™ Plasma Separation Tubes), which were gently inverted 10× and centrifuged at 1400*g* for 10 min. The supernatant was transferred to Eppendorf tubes and used for measurements of osmolality. Fiske 110 freeze point osmometer (Fiske associates, Norwood, Massachusets, 02062 USA) was used.

2.3. Immunoblots

The protocol used for Western blot was similar to our previous investigations [17]. The primary antibodies we used were # AQP11-1 (Alpha Diagnostic, Inc., San Antonio, USA, AQP1) and AB3594 (Millipore Inc., Billerica, USA, AQP4). The signal was detected using secondary goat-anti-rabbit antibodies conjugated with horseradish peroxidase, and visualized using enhanced chemiluminescence (ECL). The ECL signal was related to that of general protein stain Coomassie Blue. For detailed procedure, please see Supplementary methods. The analysis was blinded.

2.4. Real-time qPCR

The primers were designed to span exon–exon junction areas. For primer sequences and detailed procedure please see Supplementary methods. The test gene was related to the expression of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) using commercially available primers (PE Applied Biosystems). The analysis was blinded.

2.5. Statistical analysis

Both PCR and Western blot data were analyzed using the statistical programming language R (R Development Core Team, 2008 [18]). In the figures the curves, with associated 95% confidence bands, are natural cubic splines [19] with degrees of freedom equal to the number of time points. Data in text are presented as mean ± SD.

3. Results

3.1. Plasma osmolality

Mice injected with hypervolemic isoosmotic solution (0.9% NaCl) or mildly hyperosmotic (1 M) NaCl had no important changes in plasma osmolality. However, there was a large transient increase of osmolality in mice injected with 2 M NaCl 1 and 4 h following the injection (414 ± 13 mOsm after 1 h vs. 325 ± 7 mOsm at baseline) (Fig. 1b).

3.2. mRNA of AQP1 and AQP4 in the heart

Hypervolemic isoosmotic 150 mM (0.9%) or mildly hyperosmotic (1 M) NaCl injections did not induce significant changes in mRNA of either AQP. Following 2 M NaCl injections, AQP1 mRNA was upregulated after 4 h by 64 ± 31%. One hour after injection of 2 M NaCl, AQP4 mRNA decreased to 45 ± 18% of the control value. It remained decreased after 4 and 8 h and returned to baseline levels after 24 h (Fig. 1b).

3.3. AQP1 and AQP4 proteins in the heart

Strong hyperosmotic (2 M) injections did not influence the total protein expression of AQP1. However, the glycosylated fraction of AQP1 increased by 40 ± 27% and 29 ± 12% after 1 and 4 h, respectively. AQP4 protein decreased by 36 ± 24%, 50 ± 20%, 67 ± 15%, and 51 ± 19% of the control value after 1, 4, 8, and 24 h, respectively (Fig. 2a).

3.4. Expression of AQP4 in the brain

After hypervolemic isoosmotic injections of NaCl, AQP4 mRNA level in forebrain homogenates varied during the next 24 h, but decreased to 53 ± 6% and 57 ± 18% of the control value after 1 and 24 h, respectively. No effect was observed after injection of 1 M NaCl (data not shown), but injection of 2 M NaCl induced an insignificant decline followed by a gradual increase to 75 ± 94% above baseline after 24 h. AQP4 protein expression was not affected by hypervolemia, while 2 M NaCl injections increased AQP4 protein content by 68 ± 93% after 8 h (Fig. 2b).

4. Discussion

The current study shows that, as in the brain [12,13], expression levels of AQPs in the heart are influenced by blood osmolality. Plasma osmolality is a key physiological parameter. Pronounced plasma hyperosmolality is observed in patients with diabetes insipidus, diarrhea, and diabetic ketoacidosis [3,5]. Hyperosmolality may develop locally in ischemic tissues due to production of lactic acid and impaired ion transport. In addition, artificial hyperosmolality (osmotherapy) is widely used for the treatment of brain tumors, trauma, and ischemic strokes. Both ischemia and osmotherapy influence expression of AQP4, the major aqueduct in the blood–brain barrier [9,20–22]. Osmotherapy following ischemia induces AQP4 protein expression in the ischemic part of the rat brain [13] and reduces it in the peri-ischemic area [23]. Changes in AQP4 expression have dramatic effects on cell death in the brain [9,10], therefore such regulation potentially has a major clinical significance. AQP4 expression changes may partly explain the effects of osmotherapy in patients.

The data on regulation of AQP4 in the heart is largely missing. Several studies including our recent paper (Rutkovskiy A, submitted) suggest that ischemia may influence expression of AQP1 and −4 [24,25]. Hyperosmotic reperfusion may reduce postischemic swelling and improve cell survival [16]. The only other study to address osmotic influence on AQP expression in the heart was carried out by Page et al. [26]. They showed that hyperosmolarity led to intracellular translocation of AQP1 in rat cardiomyocytes [26]. However, their finding has a limited translational value, since humans, like mice, do not express AQP1 in cardiac muscle cells [14].

In our experiments hyperosmolality increased AQP1 mRNA and protein glycosylation. Total AQP1 protein was not influenced. Gly-

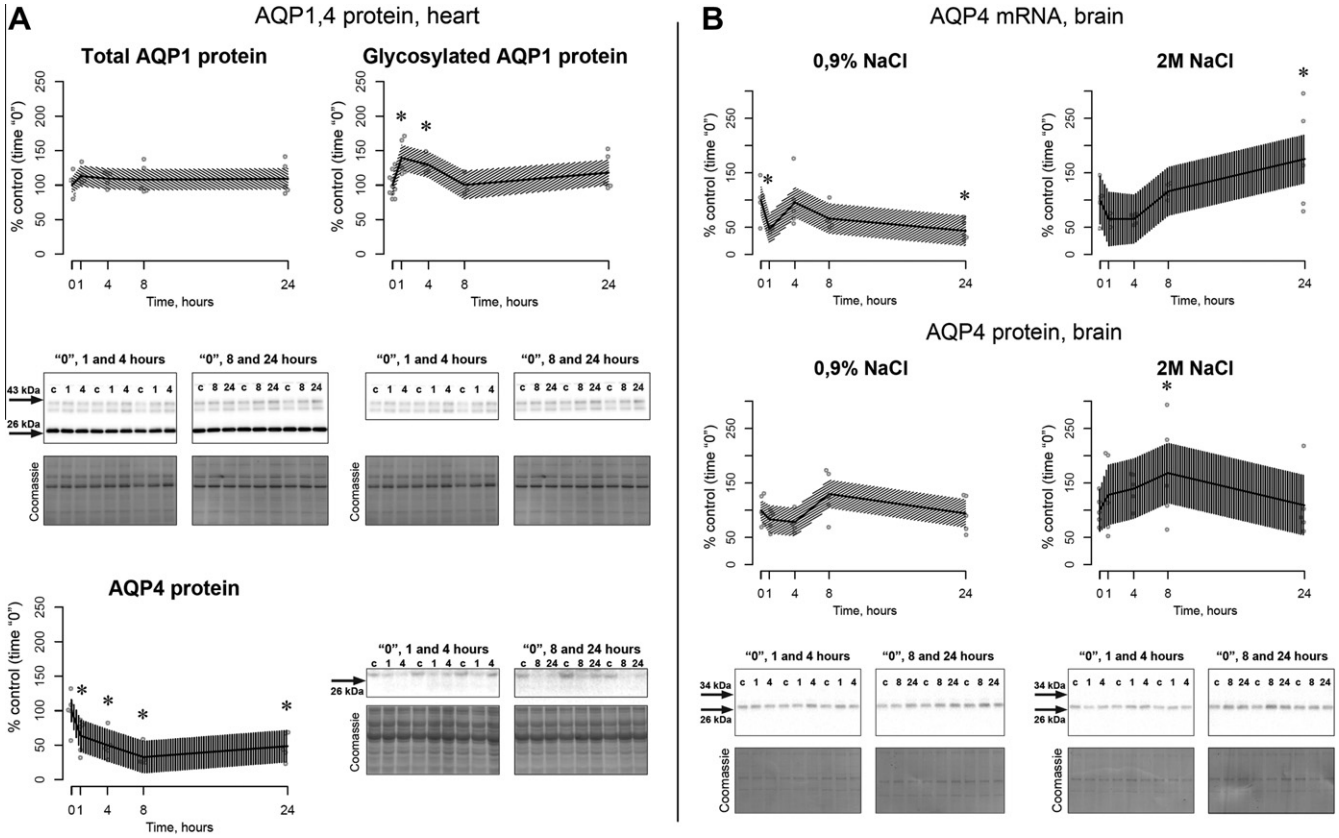

Fig. 2. Protein expression of AQP1 and 4 in hearts and brains of saline-injected mice (A). Protein expression of AQP1 and AQP4 in hearts of mice subjected to severe plasma hyperosmolality 1–24 h before tissue sampling (intraperitoneal injections of 0.5 ml 2 M NaCl). Representative blots are shown. $N = 5$ at each timepoint. Values are expressed as mean and 95% confidence bands of 5 samples at each time point. * denotes $p < 0.05$ compared to time 0. Coomassie staining to evaluate protein loading is shown. (B). mRNA and protein expression of AQP4 in forebrains of mice subjected to hypervolemia (intraperitoneal injection of 1.5 ml 0.9% NaCl) or severe plasma hyperosmolality (intraperitoneal injections of 0.5 ml 2 M NaCl), sampled 1–24 h after injections. $N = 5$ at each timepoint. Data are expressed as 95% confidence bands. Individual values shown as grey circles * denotes $p < 0.05$ compared to time 0. Coomassie staining to evaluate protein loading is shown.

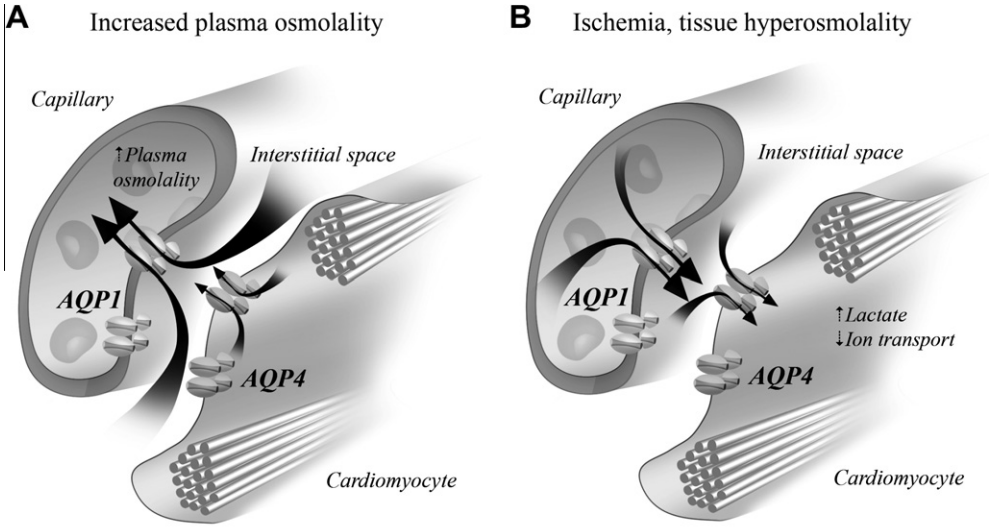

Fig. 3. Hypothetical roles of AQP1 and 4 in osmotic disturbances in the heart A cartoon shows the potential role of AQP1 and −4 in water fluxes caused by plasma hyperosmolality (A) or local tissue hyperosmolality caused by ischemia (B). Plasma hyperosmolality causes water flux from the tissue into the capillary lumen, while ischemia causes the extravasation of water and swelling of the cardiac myocytes. In both cases, a decrease in AQP4 expression would prevent water homeostasis disturbances in myocytes, and potentially improve cardiac function.

cosylation is a type of post-translational protein modification, likely independent of the mRNA content. An isolated change in AQP1 glycosylation has recently been reported in the kidney [27], but its functional significance is unclear as neither water transport, nor the ability to form tetramers are influenced by removal of sugars from the AQP1 molecule [28].

Our AQP4 data are more intriguing. Hyperosmolality downregulated AQP4 mRNA and subsequently the protein, indicating negative transcriptional regulation. mRNA was decreased for the initial 8 h but the protein remained low till the end of a 24 h observation period. Thus, even a transient hyperosmolar state will cause a prolonged depression of AQP4 in the heart.

What physiological implications do our results have? We suggest Fig. 3 as a simplified diagram of water fluxes in the myocardium. In short, AQP1 mediates transendothelial water flux, while AQP4 likely transports water across the cardiomyocyte membrane [14,29,30]. Reduction of AQP4 in ischemia might limit the osmotic water influx in the cardiomyocyte, while in a global hyperosmolar state it would reduce the water loss by these cells. Both effects are presumably beneficial for the function and survival of cardiomyocytes, which are highly dependent on the constant cell volume. Under these conditions, it seems logical to increase the water transport between the endothelium and interstitium by AQP1 and mobilize the water of the interstitial compartment and not the intracellular water of the cardiomyocytes. However, our data do not provide evidence for increased AQP1 protein.

As a control we investigated brain AQP4 regulation in the same animals. Hypervolemic saline injections decreased AQP4 mRNA expression without any effect on the protein. Injections of 2 M NaCl gradually increased both AQP4 mRNA and protein, and the protein peak preceded the peak of mRNA, suggesting post-transcriptional regulation, as previously shown by Arima et al. [12]. The findings in the brain also show that AQP4 is regulated in two organs in opposite directions, which is difficult to interpret.

4.1. Criteria and limitations of the study design

To emphasize short-term transcriptional regulation by osmolality, our study was designed to adhere to certain criteria, which led to a number of limitations.

First, hyperosmolality in plasma had to be powerful but short-lasting, to exclude the long-term compensatory mechanisms. Therefore we chose 1 and 2 Mol in single injections followed by free access to water. Assuming that a mouse weighing 25 g has 5.3 ml water in the extracellular domain [31] with an osmolality of 325 mOsm, our 0.5 ml injections of 2 M NaCl should theoretically yield a peak osmolality of 650 mOsm. To compensate, mice ingest water and excrete sodium, leaving behind chloride ions that will promote acidosis.

Second, water ingestion will cause a rapid increase in circulatory volume and pressure, and neither was measured in our study. However, to separate the effect of hypervolemia from osmolarity *per se*, we added a group with 1, 5 ml injection of isoosmotic saline, assuming that 0.5 ml injections of isoosmotic saline will not have any effects on the mouse at all.

Third, we chose intraperitoneal route of administration over intravenous in order to prevent excessive stress to the blood cells, but at the same time causing less controllable distribution of osmoles within the body.

Finally, to be able to influence cardiac AQP expression, we had to increase plasma osmolality to over 400 mOsm, which may seen non-physiological both in such a magnitude over such a short time (though not unseen in patients when developed gradually). With all this in mind, our study represents a proof of principle, rather than a clinically relevant model of a disease state.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2012.07.052.

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