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An investigation of water movement across pig bladder urothelium and the cellular localisation of aquaporin water channels

Manso M[1], Fry C[2], Drake M[2] , Vahabi B[1]

University of the West of England[1], University of Bristol[2]

Introduction

The bladder urothelium is generally considered to be poorly permeable blood-urine barrier. However, recent studies have shown that the urothelium expresses transmembrane water channels, aquaporins (AQPs). Currently, 13 AQPs (0-12) subtypes have been identified in mammalian tissues, and of these subtypes, AQP1, AQP2, AQP3, AQP4, AQP7, AQP9 and AQP11 have been found in the urothelium of various species (1-3) indicating that AQPs could regulate urothelial cell volume and osmolarity, and thus determine the final composition of urine.

However, the exact functional role of AQPs and their cellular regulation and distribution under different osmotic conditions in bladder urothelium remains to be elucidated. Therefore, this study aimed to investigate the functional role and cellular distribution of AQP3 in adult pig bladder urothelium.

Methods and Materials

Ussing chamber experiments: Bladders from ~6-months old female pigs (Sus scrofa domestica) were obtained from a local abattoir (University of Bristol, Bristol, UK). Bladder mucosa strips (urothelial & suburothelial layes) were dissected from the bladder dome and mounted in an Ussing chamber system.

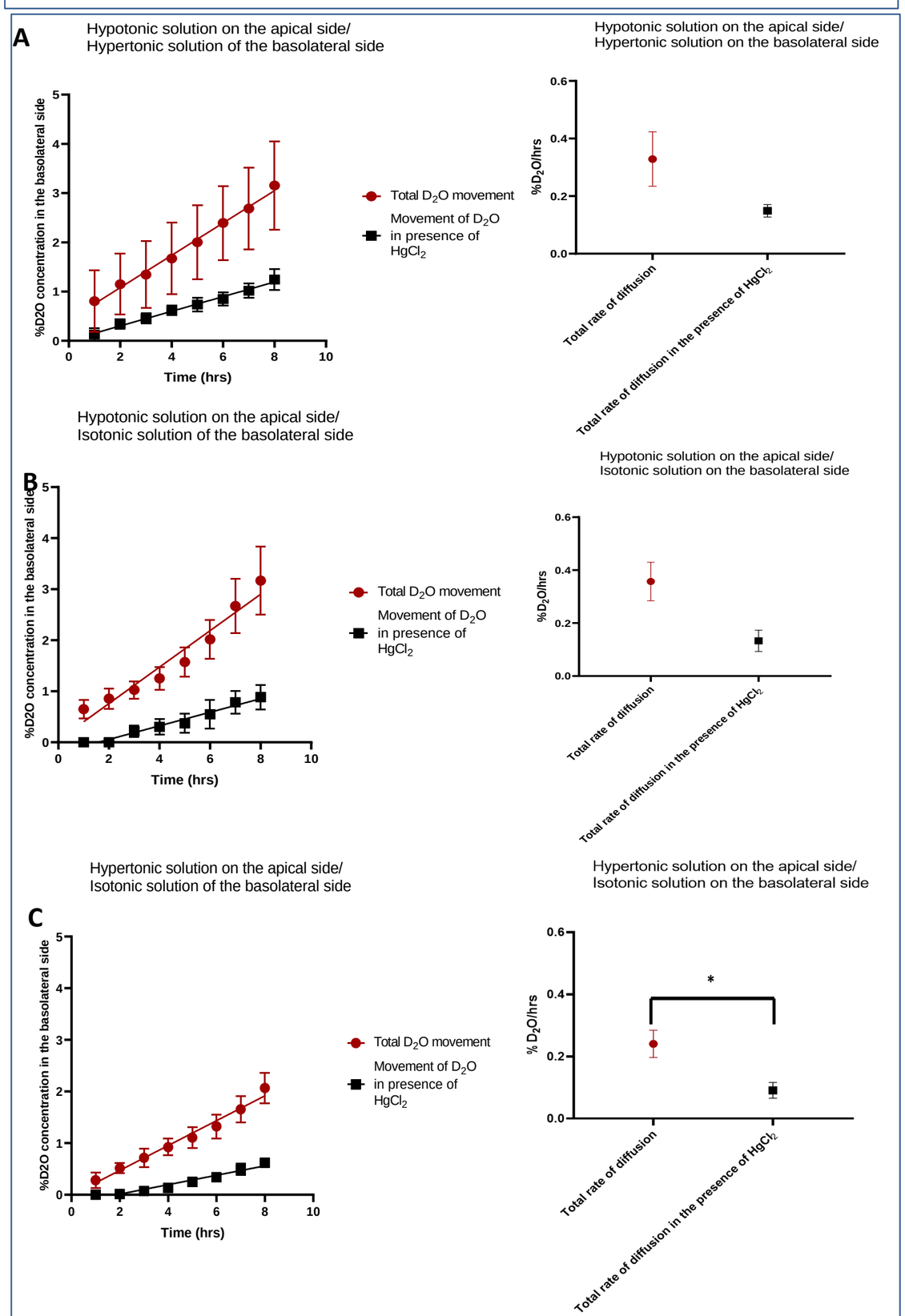
The orientation of the urothelium as either basolateral or apical (bladder lumen) face was noted. Each half chamber (20 ml) was a circulating reservoir of Krebs solution, gassed with 95% O2/5% CO2 and maintained at 37°C by means of a thermostatted water jacket. After an equilibration period of 60-min, the basolateral side of the mounted mucosal strips were exposed to an isotonic Krebs solution whilst the apical side was exposed to a hypertonic or hypotonic Krebs solution containing 40% D_2O in presence and absence of 300 µM mercuric chloride (HgCl2, a non-selective AQP inhibitor). The movement of D_2O across the mucosa barrier was assessed by taking 1 ml samples from the basolateral side every 1 hour for a period of 8 hours. The samples were then analysed using a PerkinElmer FT-IR spectrometer.

The change in concentration of D_2O on the basolateral side of the mucosa over time, in the presence and absence of $HgCl_2$, was used to estimate the diffusion rate. A paired Student's *t*-test was used to compare the rate of D_2O diffusion in the presence and absence of $HgCl_2$. Comparisons between the diffusion rate of different Krebs tonicity solutions were performed using analysis of variance (one-way ANOVA) with $p<0.05$ considered significant. Data are presented as mean±SEM.

Immunocytochemistry: Pig bladder mucosa, removed by blunt dissection, was incubated at 37°C with 1% HBSS, 5 mM EDTA and 10 mM HEPES for approximately 1 hour. Urothelial cells were released by gentle titration. Isolated cells were then were incubated in hypotonic and hypertonic Krebs solutions for 4 hours. Cells were then fixed with 100% methanol followed by incubation with primary antibodies for AQP3 and cytokeratin 7 (dilutions of 1:1000 and 1:500 respectively) for 1 hour followed by incubation with secondary antibodies (Alexa-Fluor 488 and 647, dilution of 1:1000) for 45-min. Cells were then mounted on slides and viewed using Nikon eclipse TE300.

Results

Ussing chamber experiments Movement of D_2O from the apical to the basolateral side of the mucosa was detected in pig bladder. A reduction in the diffusion rate was observed when the mucosa strip was exposed to $HgCl_2$.



•**Figure 1. The diffusion rate of D_2O in the presence and absence of $HgCl_2$ in pig bladder mucosa strip.**

•D_2O diffusion rate in mucosa strip constructs are shown in the presence and absence of 300µM $HgCl_2$. hypotonic Krebs solution, hypertonic Krebs solution and isotonic Krebs solution was added as indicated above. Samples were collected from the basolateral side over 8 hours every hour for each independent repeat. **A,B&C)** Reduction in the rate of D_2O diffusion in the presence and absence of $HgCl_2$ was observed. **C)** Significant ($p=0.0243$) reduction in the rate of diffusion was observed under hypertonic solution apical and isotonic solution basolateral. The values were compared using Student's paired *t*-test (n=3) *$p < 0.05$

Movement of D_2O from the apical to the basolateral side of the mucosa was detect in pig mucosa bladder under different osmotic conditions. The diffusion rate between the different osmotic conditions was compared, no significant differences in the diffusion rate was measured

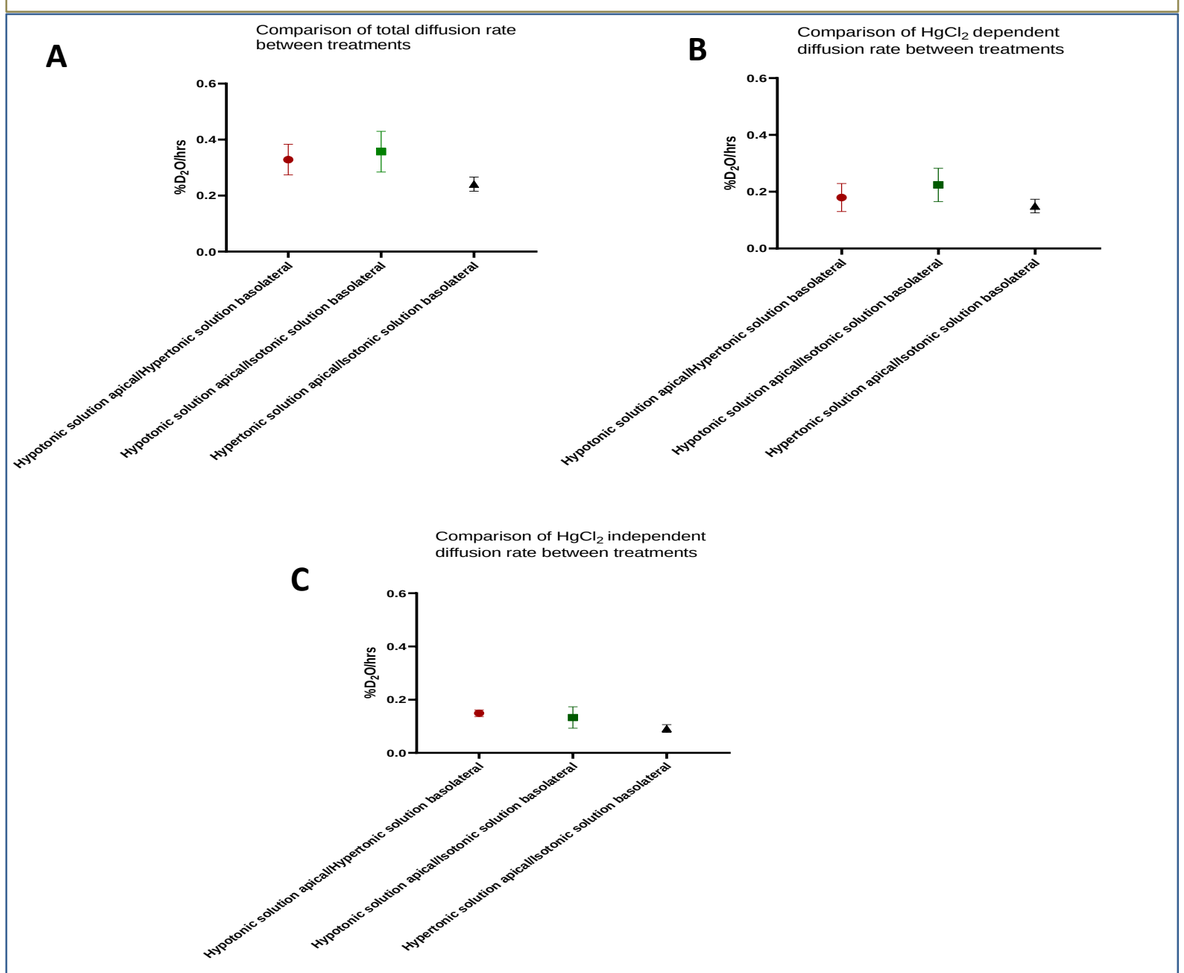


Figure 2. The diffusion rate of D_2O under different osmotic conditions.
D_2O diffusion rate in mucosa strip constructs are shown. **A)** Total D_2O duffusion rate under 3 different osmotic conditions. **B)** AQP independent D_2O diffusion rate under 3 different osmotic conditions. **C)** AQP independent D_2O diffusion rate under 3 different osmotic conditions. Comparisons between the effect of different Krebs tonicity solutions were analysed using analysis of variance (one-way ANOVA) (n=3)

Immunocytochemistry AQP3 localisation was detected around the nucleus of urothelial cells. Exposure to different osmotic solutions resulted in AQP3 translocation to the membrane.

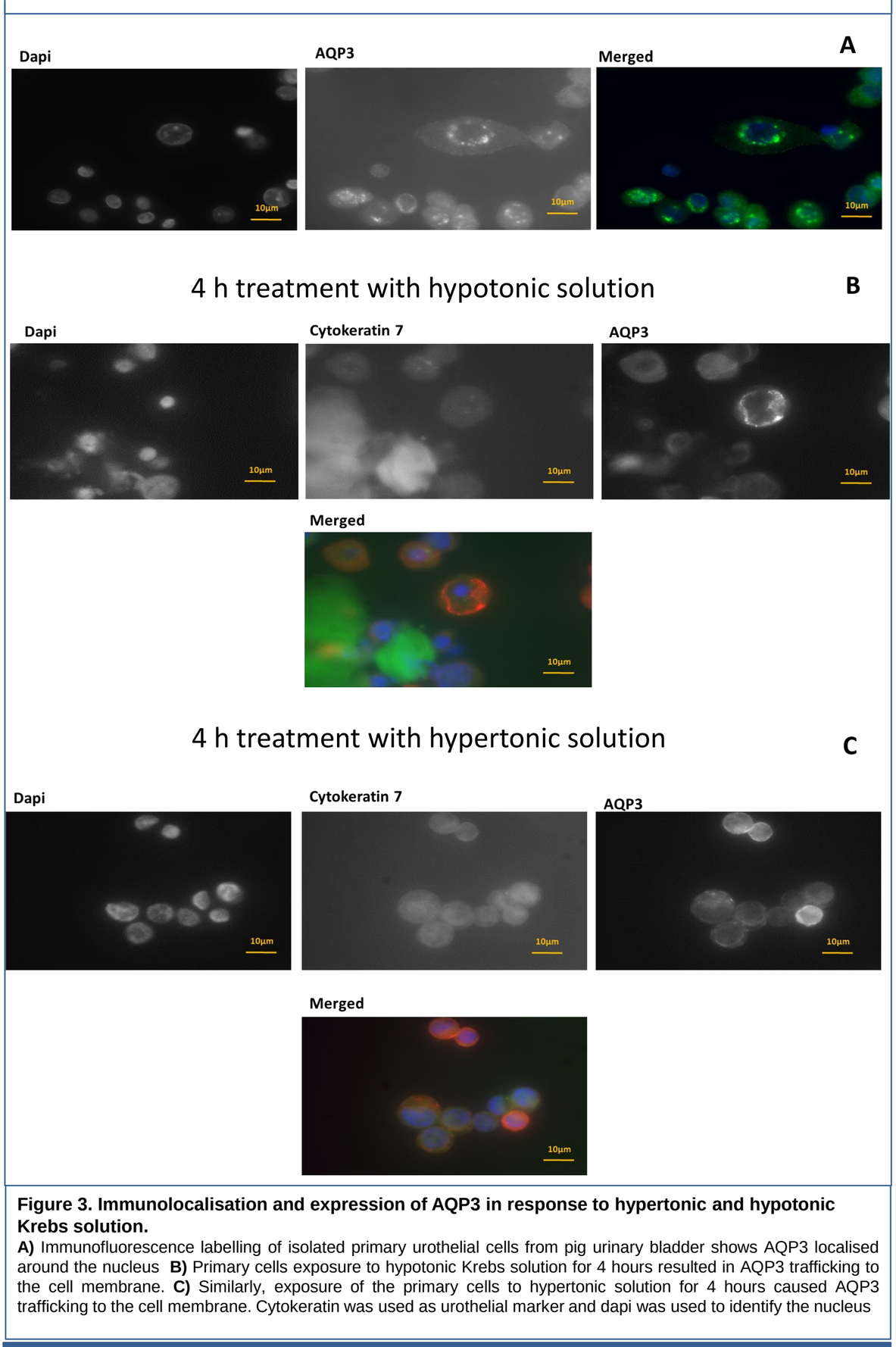


Figure 3. Immunolocalisation and expression of AQP3 in response to hypertonic and hypotonic Krebs solution.
A) Immunofluorescence labelling of isolated primary urothelial cells from pig urinary bladder shows AQP3 localised around the nucleus **B)** Primary cells exposure to hypotonic Krebs solution for 4 hours resulted in AQP3 trafficking to the cell membrane. **C)** Similarly, exposure of the primary cells to hypertonic solution for 4 hours caused AQP3 trafficking to the cell membrane. Cytokeratin was used as urothelial marker and dapi was used to identify the nucleus

Discussion

Water movement was detected across pig bladder urothelium which was significantly inhibited by $HgCl_2$, demonstrating a potential role of AQPs in mediating transcellular movement of water across bladder urothelium. Immunofluorescent staining revealed cytoplasmic localisation of AQP3 in urothelial cells not exposed to osmotic gradients and its translocation to the cell membrane under osmotic stress.

Conclusions

AQPs in the urinary bladder urothelium may play a regulatory role in urothelial cell volume and modifying final urine composition depending on the requirements of fluid homeostasis.

References

1. Manso, M., Drake, M. J., Fry, C. H., Conway, M., Hancock, J. T., & Vahabi, B. (2019). Expression and localization of aquaporin water channels in adult pig urinary bladder. *Journal of cellular and molecular medicine*, *23*(5), 3772.
2. Rubenwolf, P. C., Georgopoulos, N. T., Kirkwood, L. A., Baker, S. C., & Southgate, J. (2012). Aquaporin expression contributes to human transurothelial permeability in vitro and is modulated by NaCl. *PloS one*, *7*(9), e45339.
3. Spector, D. A., Wade, J. B., Dillow, R., Steplock, D. A., & Weinman, E. J. (2002). Expression, localization, and regulation of aquaporin-1 to-3 in rat urothelia. *American Journal of Physiology-Renal Physiology*, *282*(6), F1034-F1042.