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

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Introduction

The bladder urothelium is generally considered to be poorly permeable bistrone-barrier. However, recent studies have shown that the urothelium expresses transmembrane water channels, aquaporins (AQPs). Currently, 13 AQPs (AQP 0–12) have been identified in human urothelium. In the present study, the expression and localization of aquaporins in pig urothelium were investigated. Of the 13 AQPs, 9 AQP11 have been found in the urothelium of various species [1-3], indicating that AQPs can regulate urothelial cell volume and osmolality, 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

Using chip experiments: Bladders from 6-month-old female pigs (Sus scrofa domesticus) were obtained from a local abattoir (University of Bristol, Bristol, UK). Bladder mucosa strips (urothelium & suburothelial layers) 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, passed with 95% O2/5% CO2 and maintained at 37°C by means of a thermostated water jacket. After an equilibration period of 60 min, the basolateral side of the mounted mucosal strips was exposed to an isotonic Krebs solution whilst the apical side was exposed to a hypertonic or hypotonic Krebs solution containing 40% D2O in presence and absence of 300 mM mercuric chloride (HgCl2), a non-selective AQP inhibitor. The movement of D2O 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 IT-95 spectrometer.

The change in concentration of D2O on the basolateral side of the mucosa over time, in the presence and absence of HgCl2, was used to estimate the diffusion rate. A pattern Student’s t-test was used to compare the rate of D2O diffusion in the presence and absence of HgCl2. Comparisons between the diffusion rate of different Krebs toxicity 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% H2O2, 5 mM EGTA and 10 mM HEPES for approximately 1 hour. Urothelial cells were released by gentle titration. Isolated cells were then incubated in hypotonic and hypertonic Krebs solutions for 4 hours. Cells were then fixed with 100% methanol followed by incubation with primary antibodies for AQP5 and cytokeratin 7 (dilutions of 1:1000 and 1:2000, 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 a Nikon eclipse TE300.

Results

Using chip experiments

Movement of D2O 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 mucosal strip was exposed to HgCl2.

Discussion

Water movement was detected across pig bladder urothelium which was significantly inhibited by HgCl2, demonstrating a potential role of AQPs in mediating transcellular movement of water across bladder urothelium. Immunofluorescent staining revealed cryptoplastic localization of AQP5 in urothelial cells not exposed to osmotic gradients and its transmission to the cell membrane under osmotic stress.

Conclusions

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

References


Figure 1. The diffusion rate of D2O in the presence and absence of HgCl2 in pig bladder mucosa strip.

Figure 2. The diffusion rate of D2O under different osmotic conditions.

Figure 3. Immunofluorescence labelling of isolated primary urothelial cells from pig urinary bladder shows AQPs localised around the nucleus. A) Primary cells exposed to hypertonic Krebs solution for 4 hours resulted in AQPs trafficking to the cell membrane. B) Primary cells exposed to hypertonic Krebs solution for 4 hours caused AQPs trafficking to the cell membrane. C) Cells were incubated with HgCl2 and the expression and localization of AQPs was detected using immunofluorescent staining.

Figure 4. The diffusion rate of D2O between treatments.

Figure 5. Immunofluorescence labelling of isolated primary urothelial cells from pig urinary bladder shows AQPs localised around the nucleus. A) Primary cells exposed to hypertonic Krebs solution for 4 hours resulted in AQPs trafficking to the cell membrane. B) Primary cells exposed to hypertonic Krebs solution for 4 hours caused AQPs trafficking to the cell membrane. C) Cells were incubated with HgCl2 and the expression and localization of AQPs was detected using immunofluorescent staining.

Figure 6. The diffusion rate of D2O between treatments.