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THE EFFECT OF LOW DOSE EIPA ON WATER FLUX ACROSS *RANA TEMPORARIA* BLADDER

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Exposure of the serosal face of anuran urinary bladder to hyperosmotic solutions (SH), results in a reversible and reproducible increase in water flux (J_w) which is similar in time course and magnitude to that elicited by AVP Bentley,1964. The hydro-osmotic response to SH and AVP in combination is additive. The SH response is absent in nominally chloride- free solutions, Hanna-Mitchell, & Gebruers, (2001), and is also inhibited by 100 μM ethylisopropylamiloride (EIPA) suggesting a role for a Na /H exchanger (NHE) in the response (Hanna-Mitchell, et al. 2003).

In this study male Rana Temporaria bladders were used in a series of experiments employing a modification of the gravimetric method of Bentley (1958) to study J_w. Frogs were stunned, decerebrated, pithed and bladders were dissected out. These were filled with hypotonic Ringer, R/2, (120 $mOsm.kg^{-1}$ H_2O) and immersed in a bath of either isotonic R, 230 $mOsm.kg^{-1}$ H_2O (Control) or hypertonic R3/2, 335 $mOsm.kg^{-1}$ H_2O (SH), pH was 7.9 and temperature 19oC. Changes in J_w following immersion in SH solution was determined in the presence and absence of 1μM EIPA on either apical or basolateral faces of the bladder. This concentration is reported to inhibit more sensitive isoforms of the exchanger such as NHE_1. Flux values represent cumulative values for the 30 minute period during control or following experimental stimulation. A pilot study on the effect of 5mM $BaCl_2$, an agent which blocks K channels involved in cell volume regulation, was also carried out. Results were analysed using Students paired and unpaired t test as appropriate, and expressed as Mean ± S.E.M.

A significant increase in flux from basal levels of 19±3 μl/30 min to 249±74 μl/30 min occurred following SH stimulation ($P < 0.01$, n=5). Exposure to SH in the presence of basolateral 1μM EIPA reduced Jw to 154±46 μl/30 min ($P < 0.05$, n=5). Exposure to SH the presence of apical EIPA reduced flux to 156±16 μl/30 min. Exposure of four bladders to basolateral 5mM $BaCl_2$ did not alter the SH response.

In a previous series of experiments 100μM EIPA reduced flux to baseline values. The partial inhibition seen here is consistent with activation of NHE isoforms other than NHE_1 in the SH stimulated increase in water flux. The absence of inhibition in the presence of $BaCl_2$ would suggest that more than a simple volume regulatory response is involved in the increase in J_w. The use of specific inhibitors for NHE_1 and NHE_3 would clarify these results.

Bentley, P.J. (1964). Comp. Biochem. Physiol. 12, 233-239

Bentley, P.J. 1958 J. Endocrin. 17: 201-209

Hanna-Mitchell, A.T. & Gebruers, E.M. (2001). J.Physiol. 533.P, 9P

Hanna-Mitchell, A.T., O'Donoghue, U. Hayes M. & Gebruers E.M. (2003). J Physiol. 551.P C12

Where applicable, the experiments described here conform with Physiological Society ethical requirements.

PC41

A MORPHOMETRIC AND FUNCTIONAL STUDY OF THE HYPERTONIC HYDRO-OSMOTIC RESPONSE IN RANA TEMPORIA URINARY BLADDER

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In hypertonic bath, anuran bladder exhibits a reversible increase in osmotic permeability. This response to serosal hypertonicity (the SH response) has been considered to be partly due to epithelial cell shrinkage with a consequent increase in intracellular cAMP concentration, a known mediator of the ADH-water response.

We have shown that the SH response in Rana temporaria bladder may be modulated (Hanna-Mitchell & Gebruers 2001, 2002). These findings indicate that specific biochemical events are involved in the SH response.

Results reported here are from a combined functional and morphometric study of paired hemibladders from humanely killed male Rana temporaria; one hemibladder was fixed following a five-minute exposure to SH (SH+5 group) and the corresponding hemibladder was fixed following a 30-minute exposure to SH (SH+30 group). Following fixation in hypertonic medium, bladders were processed both for TEM imaging and for light microscopy. Using stereological methods, granular cell volume was calculated for each of the groups; granular cells are functionally analogous to the principal cells of the mammalian nephron.

The SH+5 group exhibited a significant ($p<0.05$) mean water loss of 3.13 ±0.6 mg.min-1 compared with 0.5±0.4 mg.min-1 in control isotonic bath, over a similar time interval.

SH+30 group hemibladders showed a significant ($p<0.005$) hydro-osmotic response with a mean peak water loss of 14.2±1.0 mg.min-1 compared with control values of 1.3 ± 0.4 mg.min-1.

Mean granular cell volume in the SH+30 group was 2218.5±249.7 μm3 which was not significantly different to a value of 2432.9±249.7μm3 recorded in the SH+5 group; $p>0.05$.

Granular cells in both groups exhibited non-dilated junctional complexes, microvilli and a greatly expanded intercellular space, consistent with transepithelial water movement. Further examination of a possible role for granular cell volume in the hydro-osmotic response is warranted.

Paired Student t-test was used for statistical analysis and values are expressed as mean?SEM.

Hanna-Mitchell, A.T. & Gebruers, E.M. (2001). J.Physiol. 533.P, 9P.

Hanna-Mitchell, A.T. and Gebruers, E.M. (2002) J.Physiol.544P S084

Where applicable, the experiments described here conform with Physiological Society ethical requirements.

PC42

HETEROGENEOUS EXPRESSION OF AQP1 IN HIGH DENSITY HUMAN TUMOUR TISSUE MICROARRAYS

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Aquaporin 1 (AQP1) water channels are membrane proteins that control the permeability of endothelial and epithelial barriers by facilitating water movement across cell membranes. AQP1 is present in microvessel endothelia of many normal human tissues and is highly expressed in active water transporting epithelia such as the choroid plexus and the kidney. The potential importance of AQP1 in oncology and carcinogenesis has only recently been recognized. It has been suggested that AQP1 may be responsible for the high vascular permeability and interstitial fluid pressure in tumours of the brain, colon, breast and pancreas (Endo et al., 1999). AQP1 may also play a role in tumour angiogenesis and may be involved in development of effusions or oedema fluid (Vacca et al., 2001). The aim of the present study was to use immunohistochemistry to compare the distribution and relative abundance of AQP1 on National Cancer Institute TARP (Tissue Array Research Program) human multiple tumour Tissue MicroArrays (TMAs) with normal tissues represented on the National Institutes of Health CHTN (Cooperative Human Tissue Network) TMAs.

Immunohistochemistry and semi-quantitative histomorphometric analysis were used to compare the distribution of AQP1 in tumours of the prostate, colon, lung, breast and ovary represented on TARP TMAs with their normal counterparts on CHTN TMAs. AQP1 was expressed in capillary endothelia of all normal tissues. In most tumours AQP1 was confined to endothelial barriers. AQP1 expression was marginally higher in microvascular structures in prostate and ovarian tumours and was higher in a small number of advanced mammary and colorectal carcinomas where AQP1 immunoreactivity was also seen in some neoplastic tumour cells.

We therefore conclude that AQP1 is heterogeneously expressed in different human tumours and is rarely present in neoplastic cells. Increased AQP1 expression in some human adenocarcinomas may be a consequence of angiogenesis and important for the formation or clearance of tumour oedema. These preliminary data will form the basis of future studies to investigate the significance of AQP1 in solid tumours of humans and veterinary species.

Endo, M. et al., (1999) Microvasc. Res. 58, 89-98

Vacca, A. et al., (2001) Semin. Oncol. 28, 543-550

Where applicable, the experiments described here conform with Physiological Society ethical requirements.

PC44

AQP1 IS UPREGULATED IN HUMAN SYNOVITIS

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Rheumatoid arthritis (RA), is an autoimmune disorder of unknown aetiology characterised by synovial proliferation (synovitis), cartilage degradation, bone erosion and joint swelling. RA affects 1% of the adult population and exhibits a chronic fluctuating course which may result in progressive cartilage destruction, joint deformity and disability. The earliest abnormalities in RA consist of synovitis, soft tissue swelling, and osteoporosis. In later stages, the inflamed synovial tissue ('pannus') extends across the cartilage surface, leading to massive chondral and small bone erosions. The chronic inflammation is often accompanied by joint swelling and oedema. Previous reports have suggested that human chondrocytes and synoviocytes express aquaporin 1 (AQP1) water channels (Mobasheri et al., 2002; Mobasheri and Marples, 2004) and that AQP1 is upregulated in RA cartilage (Trujillo et al., 2004). In the present study, we have used immunohistochemistry to investigate the expression of AQP1 water channels in human synovitis Tissue MicroArrays (TMAs) and to determine if AQP1 expression is altered in synovitis.

Immunohistochemical staining of custom designed "normal" and "synovitis" TMAs revealed that AQP1 is expressed in synovial micro-vessels and synoviocytes from normal joints (n = 20 normal subjects). AQP1 was significantly upregulated in synovium derived from RA (n = 10) and psoriatic arthritis (n = 8) patients.

These results indicate a potential role for AQP1 in the joint swelling associated with synovial inflammation. It remains to be determined whether other aquaporins are involved in joint swelling in synovitis.

Mobasheri, A. et al., (2002) J. Physiol. 539P, S067.

Mobasheri, A. & Marples, D. (2004) Am. J. Physiol. Cell Physiol. 286, C529-C537.

Trujillo, E. et al., (2004) Histol. Histopathol. 19, 435-444.

Where applicable, the experiments described here conform with Physiological Society ethical requirements.