C11

Vascular Endothelial Growth Factor increases the ultrafiltration coefficient of isolated rat glomeruli

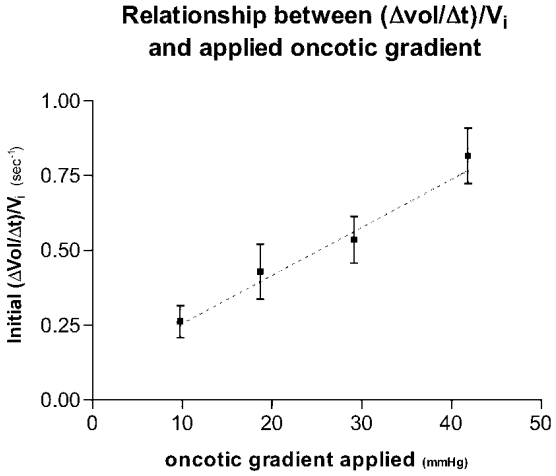
A.H. Salmon, D.O. Bates and S.J. Harper

Department of Physiology, University of Bristol, Bristol, UK

Vascular Endothelial Growth Factor (VEGF) potently increases systemic microvascular permeability to water (Bates & Curry, 1996), and is produced by glomerular cells (podocytes) (Bailey *et al.* 1999). We have refined the methods of Savin and colleagues (Savin & Terreros, 1981) to examine the effect of VEGF on the filtration characteristics of whole isolated glomeruli *ex vivo*.

Adult male Wistar rats were anaesthetised with 5% halothane, and killed by cervical dislocation. Glomeruli were isolated using a standard sieving technique with mammalian Ringer solution containing 1% bovine serum albumin (BSA), and incubated in either control or VEGF (1nM)-containing solution for up to 3 hours. Glomeruli were individually loaded onto a suction micropipette and exposed to a flowing superperfusate of 1%BSA (in Ringer solution) at 37°C. Switching the superperfusate to a solution containing a higher BSA concentration (4-8%; in Ringer solution; Fig. 1) created a transglomerular oncotic gradient. Consequent fluid efflux caused a reduction in glomerular volume, which was recorded on videotape and measured off-line. The initial rate of change of glomerular volume was used to calculate glomerular ultrafiltration coefficient (LpA).

A significant correlation between initial glomerular volume (V_i) and L_pA was noted ($p<0.005$; Spearman r = 0.45; n=47); henceforth L_pA values are corrected for V_i (L_pA/V_i; $min^{-1}.mmHg^{-1}$). Control L_pA/V_i values failed to display normal distribution. A linear relationship was demonstrated between the initial rate of volume change per unit V_i [$(\Delta vol/\Delta t)/V_i$] and the magnitude of the oncotic gradient applied ($p<0.001$; Spearman r = 0.59; n=28) (figure 1). L_pA/V_i values of glomeruli exposed to VEGF for 56 (mean) ±5 (S.E.M.) minutes were significantly higher [1.95±1.58; median±interquartile range (IQR); n=9] than those exposed to control solution (0.94±0.89; n=10) ($p<0.01$, Mann Whitney). Neither VEGF exposure for 15 minutes {paired L_pA/V_i: baseline 1.04 (0.77-1.96) [median (range)] vs VEGF 0.87 (0.60-2.03); $p>0.6$, Wilcoxon; n=5 pairs} nor 30 seconds [paired L_pA/V_i: baseline 1.07±0.49 (median±IQR) vs VEGF 0.98±0.43; $p>0.7$, Wilcoxon; n=10 pairs] elicited a rise in L_pA/V_i over baseline. These results show that prolonged exposure to exogenous VEGF can increase the ultrafiltration coefficient of renal glomeruli, ex vivo.



Bates & Curry, Am J Phys 271:H2520-2528, 1996.
Bailey et al., J Clin Pathol 52: 735-738, 1999.
Savin & Terreros, Kidney Int 20:188-97, 1981.

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

C12

Urinary space reconstruction shows three subdivisions in which increased perfusion pressure induces a complex rapid podocytic response

C.R. Neal, E. Bell, H. Crook, D. Bates and S. Harper

Department of Physiology, University of Bristol, Bristol, UK

Recently, we discovered that some of the fluid which filters across the glomerular filtration barrier (GFB) must enter restrictive spaces under the podocyte (SPS) (Neal et al). Fluid then enters interpodocyte spaces (IPS) draining fluid to the capillary tuft edge and into the peripheral urinary space (PUS) and hence the proximal convoluted tubule (PCT). We have now investigated the effects of increased perfusion pressure on these divisions of urinary space.

Kidneys from rats humanely killed in accordance with UK guidelines were either immersion fixed (n=3, renal arterial pressure of 0mmHg) or perfusion fixed immediately after cervical dislocation with glutaraldehyde after a flush through with Ringer solution (n=5, renal arterial pressure of 100mmHg throughout). Kidney pieces were postfixed, dehydrated, embedded and serial ultrathin sections of glomeruli were cut. Regions of the capillary tuft were reconstructed from electron micrographs. In glomerular sections the SPS and IPS were measured, reconstructed and compared between groups (mean±SEMs compared by unpaired Student's t-tests)

In immersion and perfusion fixed glomeruli the coverage of the GFB by the SPS was not significantly different (60±3%. v 57±5%). The SPS height frequency distribution showed that perfusion fixed podocytes were more closely opposed to the GFB in some regions and raised in others compared with immersion fixed podocytes. However, the SPS exit pores became narrower with perfusion fixation (0.21±0.02µm, n=7 v. 0.33±0.04µm, n=6;

p<0.05) and the area of attachment of processes anchoring the podocyte onto the GFB more than doubled with perfusion fixation (11±6%, n=3; 26±2%, n=5; p<0.05). The IPS doubles in width with perfusion fixation (0.74±0.06μm, n=29; 1.38±0.19μm, n=12; p<0.001) however the IPS pores at the edge of the glomerular tuft do not change (0.61±0.08μm,n=29; 0.77±0.15μm,n=12; p>0.05).

The podocytes that cover the SPS respond to perfusion fixation and its supernormal filtration by increasing the anchorage area to the GFB, narrowing the SPS exit pores and 'clamping down' onto the GFB. Although the IPS gets wider with increased filtration, at the capillary tuft edge, the IPS stays narrow before widening into the PUS suggesting podocytic control at the edge of the tuft. The podocytes appear capable of rapid individual and global responses to changes in filtration pressure and/or flow, altering the characteristics of two of the three urinary spaces within Bowman's capsule.

Neal CR et al (2003). J Physiol 552P: C26

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

C13

Effect of renal sympathetic nerve activity (RSNA) on NHE3 in the proximal tubule of the kidney

C. Thompson, V. Healy and E.J. Johns

Physiology, University College, Cork, Ireland

Renal Sodium Hydrogen Exchanger Isoform 3 (NHE3) is an apical membrane protein which mediates the reabsorption of Na^+ from, and the extrusion of H^+ into, the lumen of the proximal tubule. Previous studies have demonstrated that inhibitory agents such as PTH cause a retraction of NHE3 from the top of the microvilli to inter-microvillar cleft regions (Yang et al. 2004). Activation of the renal sympathetic nerves directly increases proximal tubular fluid reabsorption and one mechanism may be a stimulation of NHE3. The aim of this study was to evaluate whether changes in renal nerve activity could also modulate movement of NHE3 into the inter-microvillar clefts.

In vivo studies were performed on chloralose/ urethane (1 ml, 16.5:250 mg/ml I.P.) anaesthetised Wistar rats. Cannulae were placed in a femoral artery to measure blood pressure and in the femoral vein to infuse saline and inulin. Using flank incisions, the right ureter was cannulated; the left kidney was exposed, it's ureter cannulated and it was subjected to surgical denervation. After 2h stabilisation, two 15 min clearances were undertaken, the kidneys were excised and placed on ice. The cortices were dissected free and brush border membranes were obtained using Mg^{2+} precipitation and differential centrifugation (Weinman et al. 1987). The membranes were subjected to SDS-PAGE and after Western blotting, NHE3 was quantified and identified.

Data (means±SEM) were subjected to Student's t-test and significance taken when P<0.05. Western blot analysis showed that NHE3 protein abundance was reduced 3-fold after denervation in relation to the innervated kidney (n=6). There was a significantly higher (P<0.01) urinary Na^+ excretion in the denervated kidney (4.1±1.2μmol/min/kg) as opposed to the innervated kidney (1.0±0.2μmol/min/kg). Fractional sodium excretion was also significantly elevated (p<.01) in the denervated kidney (0.7±.02% vs 0.2±.05%). GFR was similar in both the innervated and denervated kidneys at 3.3±0.3 and 3.2±0.3ml/min/kg, respectively. The data show that renal denervation increased sodium excretion and decreased apical membrane NHE3 abundance, independently of renal haemodynamics. This suggests that changes in RSNA may affect trafficking of NHE3 between microvilli and other subcellular domain.

Weinman EJ et al. (1987). Am J Physiol 252, F19-25.

Yang LE, Maunsbach AB, Leong PKK, Mc Donough AA. (2004). Am J Physiol Renal Physiol 287, F896-906.

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

C14

Protein kinase B (PKB) stimulates the epithelial sodium channel (ENaC) in outside-out membrane patches of *Xenopus laevis* oocytes

A. Diakov and C. Korbmacher

Institute of Cellular and Molecular Physiology, University of Erlangen-Nurmberg, Erlangen, Germany

The regulation of ENaC in the aldosterone sensitive distal nephron is critically important for the body sodium homeostasis and hence for the control of arterial blood pressure. The molecular mechanisms of ENaC regulation are not yet fully understood but probably involve several kinases. In particular aldosterone induced SGK (serum and glucocorticoid inducible kinase) is thought to enhance ENaC surface expression by phosphorylating Nedd4-2 and thereby preventing ENaC retrieval and degradation (Debonneville et al. 2001; Snyder et al. 2002). Recently, we identified an additional regulatory pathway which involves an SGK consensus motif (^{616}**RSR**YW**S**^{621}) in the C-terminus of the channel's α-subunit (Diakov & Korbmacher, 2004). However ^{616}**RSR**YW**S**^{621} sequence is not an exclusive consensus motif for SGK1 but may also be relevant for PKB. PKB is a kinase that is involved in receptor tyrosine kinase regulatory pathways which are activated by insulin and several growth factors. In the present study we investigated the regulation of ENaC activity in outside-out macro patches of *Xenopus laevis* oocytes heterologously expressing the three subunits (α, β, γ) of rat ENaC. To prevent Na^+ feedback inhibition the pipette solution had a low Na^+ concentration containing (mM): 5 NaCl, 90 K-gluconate, 2 EGTA, 10 HEPES, 2 MgATP adjusted to pH 7.3 with Tris. At a holding potential of -70 mV the activity of ENaC was monitored by repeatedly assessing the amiloride (2 μM) sensitive current (ΔI_{ami}). Current values are given as means ± S.E.M. and significance was evaluated using Student's paired t test. In control experiments (n=5) ΔI_{ami} averaged 328 ± 152 pA 5 min after patch excision ($\Delta I_{ami\text{-}initial}$) and remained 24 min after excision at 288 ± 99 pA ($\Delta I_{ami\text{-}late}$). Recombinant constitutively active PKB (T308D, S473D) included in the pipette solution caused a significant ($p < 0.05$) increase of ENaC currents from 242 ± 82 pA ($\Delta I_{ami\text{-}initial}$) to 905 ± 249 pA ($\Delta I_{ami\text{-}late}$) (n=7). Thus, on average PKB increased ENaC current by about 4-fold. Replacing the

serine residue S621 of the ^{616}**RSR**YW**S**^{621} sequence in the C-terminus of the α-subunit by an alanine abolished the stimulatory effect of PKB (n = 6). We conclude that PKB can stimulate ENaC activity, and that this stimulation requires a specific kinase consensus motif in the C-terminus of the channel's α-subunit. The activation of ENaC by PKB may be relevant for insulin induced stimulation of ENaC *in vivo*.

Debonneville, C et al. (2001). *EMBO J.* **20**, 7052-7059.

Snyder, P. M., Olson, D. R., and Thomas, B. C. (2002). *J. Biol. Chem.* **277** 5-8.

Diakov A & Korbmacher C (2004). *J Biol Chem.* **279(37)** 38134-38142.

The expert technical assistance of Ralf Rinke is gratefully acknowledged. This work was supported by a grant of the Deutsche Forschungsgemeinschaft (SFB 423).

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

PC52

Effects of Bradykinin on AQP2 Water Channel Shuttling in Rat Inner Medullary Collecting Ducts

S. Chipperfield and D. Marples

School of Biomedical Sciences, University of Leeds, Leeds, UK

Body fluid balance is controlled by alteration of urine concentration, under the influence of vasopressin (VP). VP, via cAMP and protein kinase A (PKA), causes exocytosis of AQP2 water channels in the collecting duct (Nielsen et al., 1995). Local factors such as bradykinin (BK) may modulate this response. BK probably acts via B_2 receptors, coupled to G_i or G_q, either inactivating adenylate cyclase, or activating protein kinase C (PKC) respectively. This study aims to determine the effects of BK on AQP2 shuttling, and to elucidate the second messengers involved. An understanding of these mechanisms may improve management of water balance disorders.

Male Wistar rats were humanely killed by terminal anaesthesia with pentobarbitone sodium (240mg/kg I.P.) and cervical dislocation. The kidneys were removed and inner medullary tubules prepared as previously described (Shaw & Marples, 2002). The tubule suspension was divided into four aliquots treated as follows: control, VP (1nM), BK (10nM), VP + BK. The tubules then either underwent fractionation into plasma membrane (PM) and intracellular vesicle (ICV)-enriched fractions to determine the cellular AQP2 distribution by western blotting (expressed as a PM:ICV ratio normalised to the control), or were lysed in 0.1M HCl to release cytosolic cAMP, which was quantified using a standard kit (Sigma). Total protein from these samples was western blotted using a phospho-specific AQP2 antibody. Data are presented as means ± S.E. and compared using the false discovery rate procedure (Curran-Everett, 2000).

VP stimulation caused a shift of AQP2 from ICV to the PM. In contrast, BK caused a significant decrease in the PM:ICV ratio, indicating either a decrease in constitutive AQP2 shuttling, or an increase in AQP2 endocytosis. When VP and BK were added simultaneously, the increase in PM:ICV ratio previously seen with VP was no longer evident, demonstrating that BK completely inhibits the VP induced shuttling of AQP2. As expected, VP caused an increase in cAMP and AQP2 phosphorylation, but neither effect was inhibited by BK. BK also had no effect on basal cAMP and AQP2 phosphorylation levels.

In summary, BK reduces basal AQP2 in the plasma membrane, and inhibits AQP2 shuttling distal to AQP2 phosphorylation. These effects are consistent with the known diuretic effect of BK and may contribute to the hypotensive properties of ACE inhibitors.

	Control	Vasopressin	Bradykinin	VP + BK
AQP2 Shuttling (PM:ICV ratio, % of control) (n=5)	100	244 ± 16*	61 ± 6*	82 ± 14
[cAMP] (pmol/ml, n=8)	25 ± 5	58 ± 9*	23 ± 4	54 ± 9*
phosphorylated AQP2 (% of control, n=8)	100	326 ± 39 *	105 ± 21	337 ± 30*

***p<0.05 w.r.t. control.**

Curran-Everett D (2000). Am. J. Physiol. 279, R1-8.

Nielsen S. et al. (1995). Proc. Natl. Sci. USA, **92**, 1013-1017.

Shaw S & Marples D (2002). Am. J. Physiol. **283**, F1160-1166.

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

PC53

The effect of K^+ channel blockers on volume regulation in mouse cortical collecting duct

H.C. Taylor and L. Robson

Biomedical Science, University of Sheffield, Sheffield, UK

One of the mechanisms by which the renal cortical collecting duct (CCD) maintains cellular composition and volume, in the face of changes in transepithelial transport, is by the activation of volume regulatory pathways. A previous study has demonstrated that rabbit isolated CCDs regulate their volume on exposure to a hypotonic shock (Strange, 1988), with ducts demonstrating regulatory volume decrease (RVD) in response to hypotonic shock induced cell swelling. The aim of the following study was to examine the effect of K^+ channel blockers on hypotonic shock induced volume regulation in mouse CCDs.

C57/B6 mice were humanely killed by cervical dislocation and CCDs isolated by enzyme digestion (Schafer et al. 1997). Tubule diameter was measured using an optical system. CCDs were superfused with NaCl Ringer solution which contained (mM): 112 NaCl, 3 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 10 HEPES and 60 mannitol, and then exposed to a hypotonic solution (removal of 40 mM mannitol). This was carried out in the presence of either 5 mM Ba^{2+} (general K^+ channel inhibitor), 100 nM apamin (small conductance Ca^{2+}-activated K^+ channel blocker), 100 nM iberiotoxin (large conductance Ca^{2+}-activated K^+ channel blocker), 20 nM tertiapin (inhibitor of Kir channels) or in the absence of extracellular Ca^{2+}. All values are expressed as means ±SEM. Statistical significance was tested using ANOVAS and assumed at the 5% level.

The control diameter of CCDs was 25.6 ± 0.55 μm (n=35). Hypotonic shock increased diameter by 0.94 ± 0.07 μm and this was followed by RVD. At steady-state after RVD diameter was 0.27 ± 0.12 μm above the pre-shock diameter. Iberiotoxin had no effect on volume regulation, steady-state diameter was 0.23 ± 0.25 μm above the pre-shock level (n=9). In contrast, Ba^{2+}, apamin, tertiapin and zero Ca^{2+} were all associated with inhibition of RVD. Steady-state diameters were 1.23 ± 0.34 μm (n=7), 1.21 ± 0.29 μm (n=8), 0.84 ± 0.20 μm (n=10) and 1.72 ± 0.18 μm (n=7) above the pre-shock level with Ba^{2+}, apamin, tertiapin and zero Ca^{2+}, respectively.

In conclusion, these data indicate that K^+ channels play an important role in RVD in mouse CCDs. The inhibitory actions of apamin and zero Ca^{2+} suggest that small conductance Ca^{2+}-activated K^+ channels are involved in this process.

Strange K. 1988. RVD in principal and intercalated cells of rabbit cortical collecting tubule. American Journal of Physiology; 255: C612-C621

Schafer J.A., Watkins L., Li L., Herter P., Haxelmans S. & Schaller E., 1997. A simplified method for isolation of large numbers of defined nephron segments. American Journal of Physiology, 273, F650-F657

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

PC54

Expression of nephrogenic proteins during experimental acute tubular necrosis in rats

S.P. Villanueva[1], C. Cerspedes[1] and C. Vio[1]

[1]Department of Physiology, P. Catholic University of Chile, Santiago, Santiago, Chile, [2]Department of Physiology, P. Catholic University of Chile, Santiago, Santiago, Chile and [3]Department of Physiology, P. Catholic University of Chile, Santiago, Santiago, Chile

Recovery from acute renal failure (ARF) requires the replacement of injured cells with new cells that restore tubule epithelial integrity. The new cells, which are seen to grow from the edges of the injured tubule, are actively engaged in DNA synthesis. The morphologic characteristics of the damage have been described, but not the cellular events that regulate this process.

Pax-2 is a transcription factor that is normally expressed in the metanephric mesenchyme in the embryo and is limited to the collecting duct in adult kidney; however, immature regenerating cells express the Pax-2 damage (Imgrud et al. 1999). Other changes in gene expression have been reported in ARF but these studies have not investigated genes of nephrogenesis (Yoshida et al. 2002). We sougth to test the hyphotesis that tissue regeneration is characterized by re-expression of important developmental regulatory proteins.

We used male Sprague-Dawley rats (n=5) with 30 min of bilateral ischaemic injury, followed by reperfusion. Animals were anaesthetised I.P. with a combination of ketamine:xylazine (25:2.5 mg kg^{-1}) and maintained at a temperature of 37°C. The rats were killed 24, 48, 72 and 96 h after ischaemia reperfusion and both kidneys were recovered. The expression of Lim 1/2, Noggin, Pax 2/5/8, BMP-7, Smads 2/3, p-Smad, Vimentin, NCam, ZO-1, VEGF, Tie-2, HIF-1 αand Engrailed protein was analysed by immunohistochemistry and Western blotting as previously done for other proteins (Rodriguez et al. 2004). The expression of CD34, haematopoietic stem cells were analysed by immunohistochemistry (replicates=5). All proteins were analysed 24, 48, 72 and 96h after I/R.

To assess hypoxia the presence of HIF-1α protein and endothelial markers induced by hypoxia VEGF and TIE-2 were evaluated. We observed the expression of these markers in the kidney after bilateral ischaemia. To assess renal damage and regeneration the presence of ED-1, collagen III and α-actin were evaluated.

Immunohistochemistry revealed the expression of the mesenchyme proteins Vimentin and Ncam; the latter is essential for kidney development. In addition this kidney also expressed Pax-2, Noggin, Lim1/2, BMP7 and Engrailed, all proteins with important roles during nephrogenesis. We observed a transient, temporally and locally restricted re-expression of these proteins that was not observed in control animals ($p>0.05$).

We analysed the expression of transcription factors Smad and p-Smad, expressed by mesenchymal cells during kidney development. We observed the presence of these factors in regeneration of the kidney and not in control animals ($p>0.05$). We speculate that Smad may play specific roles in cell fate determination during kidney regeneration.

To assess the presence of haematopoietic stem cells the marker CD-34 was analysed. We did not observe haematopoietic stem cells.

These results indicate that the kidney after damage produced by bilateral ischemic injury can differentiate in a cellular type similar to metanephric mesenchymal cells and express morphogenic proteins before that differentiate into epithelia. This is a similar process to that seen in normal kidney development.

Imgrund M, Grone E, Grone HJ, Kretzler M, Holzman L, Schlondorff D, Rothenpieler UW. (1999). Kidney Int 56, 1423-1431.

Rodriguez JA, Vio CP, Pedraza PL, McGiff JC, Ferreri NR. (2004). Hypertension 44, 230-235.

Yoshida T, Kurella M, Beato F, Min H, Ingelfinger JR, Stears RL, Swinford RD, Gullans SR, Tang SS. (2002). Kidney Int 61, 1646-1654.

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

PC55

Quantitative and qualitative differences in albumin endocytosis in human renal cell models compared to the commonly used opossum kidney cell model

R. Diwakar[2], D. Baines[1], P. Colville-Nash[2] and M. Dockrell[2]

[1]Biomedical Sciences, St George's Hospital Medical School, London, UK and [2]S. W. Thames Inst. of Renal Research, St. Helier Hospital, Carshalton, UK

The effect of albumin on proximal tubule epithelial cells (PTEC) is believed to play a significant part in the development of interstitial fibrosis by various mechanisms including secretion of profibrotic and proinflammatory cytokines and epithelial mesenchymal transdifferentiation. Albumin has been shown to activate various signalling pathways and also result in secretion of TGF-β, endothelin-1, MCP-1 and RANTES by PTEC. These effects are thought to be mediated by the cell surface receptor megalin. Whether these effects of albumin requires its internalisation by megalin or can occur upon its binding followed by subsequent signalling is unclear. Opossum kidney (OK) cells have been extensively used to study albumin endocytosis. However, not much data exists in human PTEC. We investigated albumin endocytosis determined by fluoremetric analysis of Rhodamine Red-X labelled albumin up-take in human primary and transformed (HKC-8) PTEC cells and compared them with OK cells. Experiments were performed in cell culture medium, cells were lysed in 20mM TrisHCl containing 1% Triton and 0.5 % SDS, 2mM EDTA. Statistical analysis was by ANOVA followed by a Dunnett test; n=3-5.

A dose-dependent increase in albumin endocytosis was observed in all the 3 cell types at 30 min at 37°C (0.025 mg/ml to 0.2 mg/ml human serum albumin). The level of endocytosis in OK cells tended to plateau at 0.05 mg/ml. However, in human PTEC no plateau was observed and endocytosis continued to increase up to 0.2 mg/ml. The OK cells endocytosed significantly more albumin than both Primary PTEC and HKC cells (4690 fluorescent units/mg protein (FU/mg) Vs 831 FU/mg Vs 220 FU/mg, respectively, at 0.2 mg/ml, $P<0.01$).

In keeping with current literature 225 μM 5-(N-ethyl-N-isopropyl) amiloride (EIPA) reduced endocytosis by 70% in OK cells. No reduction was seen in HKC-8 by EIPA. Endocytosis in

OK vs HKC-8 cells was significantly different as analysed by 2-way ANOVA where the 2 factors were cell type and concentration of EIPA ($P<0.01$). Primary PTEC responded to EIPA in a similar fashion to HKC-8 cells.
Statins are believed to inhibit albumin endocytosis via inhibition of Rho GTPases. Simvastatin dose dependently reduced albumin endocytosis in OK cells (0.1μM-50μM; 42-88%). However, endocytosis was not inhibitable with simvastatin in human cells.
Receptor Associated Protein (RAP) has a greater affinity for megalin than albumin, and consequently blocks albumin binding. A dose-dependent inhibition of albumin endocytosis by RAP was achieved in OK cells (59 ± 2.9% inhibition at 0.25μM, mean ± SEM). A similar trend was observed in HKC-8 cells. However, this did not reach statistical significance (31 ± 14.7% inhibition at 0.25μM).
These experiments show a differences in albumin endocytosis in OK cells compared to human PTECs.

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

PC57

Allosteric Modulation of the Calcium-Sensing Receptor Selectively Alters Cell Signalling and Morphology

S.L. Davies, A. Ozawa and D. Ward

Faculty of Life Sciences, The University of Manchester, Manchester, UK

The extracellular calcium-sensing receptor (CaR) inhibits parathyroid hormone secretion and renal calcium reabsorption to prevent hypercalcaemia however, the precise nature of the intracellular signals controlling this remain to be elucidated (Ward, 2004). Previous studies report an association of the CaR with the cytoskeletal protein filamin (Hjalm et al. 2001) and therefore here we examined the consequence of CaR activation on the cytoskeletal structure and cell morphology of human embryonic kidney-293 cells stably transfected with CaR (CaR-HEK; NPS Pharmaceuticals, UT, USA). For each treatment, 4 dishes of cells were incubated in serum-free DMEM medium for up to 3 h and then for each dish, 3 regions of cells were imaged digitally for morphological assessment. Alternatively cells were exposed to various CaR agonists at 37°C for 5 mins, in HEPES buffer ((mM): 20 HEPES (pH 7.4), 125 NaCl, 4 KCl, 0.5 $CaCl_2$, 0.5 $MgCl_2$ and 5.5 glucose), lysed in RIPA buffer and then phosho-ERK content was determined by semi-quantitative immunoblotting and densitometry (Ward et al. 2002).
Incubation of the cells in serum-free medium induced cell stellation, whereas cotreatment with the calcimimetic (CaR positive allosteric modulator) NPS-467R (0.1-1μM) or exposure to elevated extracellular Mg^{2+} levels (1-10mM; $Mg^{2+}{}_o$) elicited dose-dependent cell rounding with process retraction (>90% reduction in process number). This effect was detectable within 1 h and was sustained for at least 3 h following the removal of agonist. These treatments were without effect in vector-transfected HEK cells, and, in CaR-HEK cells, the calcimimetic effect was stereoselective since NPS-467S (1μM) failed to alter CaR-HEK cell morphology. To confirm that the responses to NPS-467R and high $[Mg^{2+}]_o$ are mediated by the CaR, we cotreated the cells with the novel CaR "antagonist" (calcilytic; negative allosteric modulator) NPS-89636 (1μM) and found that the cotreatment abolished the responses. This drug also blocked ERK activation in response to increased extracellular Ca^{2+} concentration (4mM; control, 1.0 ± 0.5 arbitrary densitometry units ± SEM; 4mM Ca^{2+}, 34.5 ± 17.2, $P<0.001$ by ANOVA; 4mM Ca^{2+} plus calcilytic 2.5 ± 1.3, $P<0.001$ vs 4mM Ca^{2+} only; n=4), or to the CaR agonists Gd^{3+} (60μM) and neomycin (100μM) confirming its inhibitory action on the CaR. Cotreatment with the rho-kinase inhibitor Y27632 (10μM) also attenuated CaR-induced cell rounding indicating that the response is most likely mediated via the small G protein rho. Together, these data demonstrate that the filamin-coupled CaR can elicit rho kinase-mediated morphological changes, raising the possibility that cytoskeletal changes may contribute, at least in part, to CaR function.

Ward DT (2004). Cell Calcium 35, 217-228.

Hjalm G, MacLeod RJ, Kifor O, Chattopadhyay N & Brown EM (2001). J Biol Chem 276, 34880-34887.

Ward DT, McLarnon SJ, Riccardi D (2002). J Am Soc Nephrol. 13, 1481-1489.

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