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

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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 superfusate of 1%BSA (in Ringer solution) at 37°C. Switching the superfusate 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 (Vi) and LpA was noted (p<0.005; Spearman r = 0.45; n=47); henceforth LpA values are corrected for Vi (LpA/Vi; min⁻¹.mmHg⁻¹). Control LpA/Vi values failed to display normal distribution. A linear relationship was demonstrated between the initial rate of volume change per unit Vi [(Δvol/Δt)/Vi] and the magnitude of the oncotic gradient applied (p<0.001; Spearman r = 0.59; n=28) (figure 1). LpA/Vi 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 LpA/Vi; baseline 1.04 (0.77-1.96) [median (range)] vs VEGF 0.87 (0.60-2.03) vs >0.6, Wilcoxon; n=5 pairs] nor 30 seconds [paired LpA/Vi; baseline 1.07±0.49 (median±IQR) vs VEGF 0.98±0.43; p>0.7, Wilcoxon; n=10 pairs] elicited a rise in LpA/Vi over baseline. These results show that prolonged exposure to exogenous VEGF can increase the ultrafiltration coefficient of renal glomeruli, ex vivo.

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

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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 within Bowman’s capsule.

Narrowing of the SPS exit pores and ‘clamping down’ and its supernormal filtration by increasing the anchorage area within Bowman’s capsule.

Altering the characteristics of two of the three urinary spaces global responses to changes in filtration pressure and/or flow, widening into the PUS suggesting podocytic control at the edge of the SPS.

Where applicable, the experiments described here conform with Physiological Society ethical requirements.
serine residue $S621$ of the $^{616}{\text{RSRYWS}}^{621}$ sequence in the C-terminus of the $\alpha$-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 $\alpha$-subunit. The activation of ENaC by PKB may be relevant for insulin induced stimulation of ENaC in vivo.


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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**

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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 B₂ receptors, coupled to G₁ or G₂, 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 waterbalance 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 hypertensive properties of ACE inhibitors.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Vasopressin</th>
<th>Bradykinin</th>
<th>VP + BK</th>
</tr>
</thead>
<tbody>
<tr>
<td>AQP2 Shuttling (PM:ICV ratio, % of control; n=5)</td>
<td>100</td>
<td>24 ± 16</td>
<td>61 ± 6</td>
<td>82 ± 14</td>
</tr>
<tr>
<td>cAMP (pM/ml; n=8)</td>
<td>25 ± 5</td>
<td>58 ± 9</td>
<td>23 ± 4</td>
<td>54 ± 9</td>
</tr>
<tr>
<td>phosphorylated AQP2 (% of control; n=8)</td>
<td>100</td>
<td>326 ± 39</td>
<td>105 ± 21</td>
<td>337 ± 30</td>
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</tbody>
</table>

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


This work was funded by the National Kidney Research Fund.

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**PC53**

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

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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). Duct diameter was measured using an optical system. CCDs were superfused with NaCl Ringer solution which contained (mM): 112 NaCl, 3 KCl, 2 CaCl₂, 1 MgCl₂, 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 Ba²⁺ (general K⁺ channel inhibitor), 100 nM apamin (small conductance Ca²⁺-activated K⁺ channel blocker), 100 nM iberiotoxin (large conductance Ca²⁺-activated K⁺ channel blocker), 20 nM tertiapin (inhibitor of Kir channels) or in the absence of extracellular Ca²⁺. 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, Ba²⁺, apamin, tertiapin and zero Ca²⁺ 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 Ba²⁺, apamin, tertiapin and zero Ca²⁺, 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 Ca²⁺ suggest that small conductance Ca²⁺-activated K⁺ channels are involved in this process. Strange K. 1988. RVD in principal and intercalated cells of rabbit cortical collecting tube. American Journal of Physiology, 255: C612-C621


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Expression of nephrogenic proteins during experimental acute tubular necrosis in rats

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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. We assessed 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 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.

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

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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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**PC57**

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

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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 CaCl2, 0.5 MgCl2, 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 Mg2+ levels (1-10mM; Mg2+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 [Mg2+]o are mediated by the CaR, we cotreated the cells with the novel CaR “antagonist” (calcilytic; negative allosteric modulator) NPS-89635 (1µM) and found that the cotreatment abolished the responses. This drug also blocked ERK activation in response to increased extracellular Ca2+ concentration (4mM; control, 1.0 ± 0.5 arbitrary densitometry units ± SEM; 4mM Ca2+, 34.5 ± 17.2, P<0.001 by ANOVA; 4mM Ca2+ plus calcilytic 2.5 ± 1.3, P<0.001 vs 4mM Ca2+ only; n=4), or to the CaR agonists Gd3+ (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.


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