C37

11β-Hydroxysteroid dehydrogenase type 2 heterozygote mice display blunted natriuresis, increased blood pressure and impaired ENaC regulation on a high Na^+ diet

E. Craigie and M. Bailey

Centre for Cardiovascular Sciences, The University of Edinburgh, Edinburgh, UK

The enzyme 11β-hydroxysteroid dehydrogenase type 2 (hsd11b2) confers mineralocorticoid specificity to the mineralocorticoid receptor (MR) by converting the potent agonist cortisol to inactive cortisone. Inactivating mutations in HSD11B2 cause Apparent Mineralocorticoid Excess (AME), resulting in severe hypertension and hypokalemia. AME is rare, but polymorphisms within the general population have been linked to Na^+ sensitive hypertension (1, 2). We assessed the effect of a high Na^+ diet on blood pressure (BP) and renal function in hsd11b2+/- mice, which have a 50% reduction in enzyme activity.

Two protocols were used to measure BP and renal Na^+ handling. In the first, male hsd11b2+/- (n=6) and hsd11b2+/+ (n=5) mice were housed in metabolic cages with daily Na^+ excretion measured. Mice were fed control diet (0.25% Na^+) for 3 days, followed by high Na^+ diet (2.5% Na^+) for 7 days. After this period, systolic BP (SBP) was recorded by tail plethysmography.

In the second protocol, hsd11b2+/- and hsd11b2+/+ mice (both n=7) were maintained on a high Na+ diet before being anaesthetized (Inactin, 100mg/kg IP) and prepared for renal function experiments as described (3). After a control collection of urine, the ENaC antagonist amiloride (2mg/kg IV) was administered, and a second collection made. Arterial BP was measured throughout. Data are mean ± SE; statistical comparisons were made using either t-test or ANOVA, as appropriate.

On control diet SBP was similar in hsd11b2+/- and hsd11b2+/+ groups (123±4mmHg vs 127±3mmHg), as was Na^+ excretion (4.9±6.7 vs 6.3±1.4 μmol/24h/g). Rapid adaptation to the high Na^+ diet in hsd11b+/+ mice contrasted with blunted natriuresis in hsd11b+/- mice (170.3±7.2 vs 81.7±10.2 μmol/24h/g within 24hrs). Indeed, hsd11b+/- mice did not match control levels of excretion during the study. As a result, after 7 days on high Na^+ diet, SBP was significantly higher in hsd11b2+/- mice than controls (146±3 vs 127±2 mm Hg; $P<0.05$). Similar data were obtained in anaesthetized mice on high Na^+ diet. BP was higher in hsd11b2+/- mice than controls (101±2 vs 90±2mmHg; $P<0.01$), and fractional Na^+ excretion was lower (0.65±0.16 vs 1.54±0.19 %; $P<0.01$). Na^+ loading prompted a down-regulation of ENaC in control mice so that amiloride did not increase Na^+ excretion. In contrast, a robust amiloride-sensitive Na^+ reabsorption was still observed in hsd11b2+/- mice ($P<0.01$).

In summary, reduced 11β-HSD2 activity impairs the ability of the kidney to down-regulate ENaC-mediated Na^+ reabsorption in response to Na+ loading, which has an adverse effect on BP. These data may be clinically significant: polymorphisms affecting the activity of 11β-HSD2 may contribute towards Na^+ sensitive hypertension with in the general population.

(1) Alikhani-Koupaei R et al. (2007). FASEB J. 13, 3618-28.

(2) Mariniello B et al. (2005). Am J Hypertens. 8, 1091-8.

(3) Bailey MA et al. (2008). J Am Soc Nephrol. 1, 47-58.

Authors have confirmed where relevant, that experiments on animals and man were conducted in accordance with national and/or local ethical requirements.

C38

High salt upregulates the Gamma-Melanocyte Stimulating hormone receptor 3(MC3R)in primary rat collecting duct cells

G. Cope, V. Healy and E. Johns

Physiology, University College Cork, Cork, Ireland

Gamma melanocyte hormone(γ-MSH) has been shown to induce natriuresis in the rat[1],is this affect augmented in rats exposed to a high salt diet?

Male Wistar rats were fed either a normal salt chow(0.4% Harlan Tecklad) or high salt diet(4% NaCl Lillico® Biotechnology) for 3 weeks ad libitum. For γ-MSH infusion studies (n=6) male Wistar rats were anaesthetised using urethane/chloralose 4ml/kg i.p. The right femoral vein and artery were cannulated for blood pressure and heart rate recordings and for saline and FITC-inulin infusion.Kidneys were exposed using flank incisions and the ureters cannulated. Right kidneys were cannulated into the medullary-cortex boundary and infused at 1ml/hour with either vehicle or γ-MSH at 500fmol/min.The left kidney acted as control for each rat. Renal blood flow to the infused kidney was monitored by an ultra sonic flow probe, attached to the right renal artery. A significant $p>0.05$ (t-test) increase in blood pressure and heart rate was noted during peptide infusion, which returned to baseline levels following cessation of the infusion. UNaV increased by 70% and %FEV increased by 46% in the infused kidney as compared to the contralateral control kidney $p<0.001$. There were no associated changes in GFR.

For isolated cell studies, 3 male rats were anaesthetised as for functional experiments and the kidneys extracted via flank incisions. Kidneys were maintained in ice cold media prior to dissection. Papillae and medulla were dissected with the aid of a chilled dissecting microscope. The tissue obtained, was incubated in 1mg/ml collagenase for 1 hour at 37°C, papilla and medullary cells were harvested by isotonic shock lysis to give pure collecting duct cells. Western blot/densitometric analysis on freshly isolated cells indicates that in rats fed a high salt diet for 14 days, there was a 20 fold increase in expression of MC3R in inner medullary/papillary collecting duct cells compared to inner medullary/papillary collecting duct cells from rats fed a normal salt diet($P>0.01$, t-test. n=3). Following *in vitro* treatment of inner medullary/papillary collecting duct cells from high salt and normal salt fed rats (14 days) with 2 mM NDP γ-MSH for 15 min there was a 6 fold increase in cAMP production in cells isolated from high salt fed rats compared to cells isolated from normal salt fed rats ($P>0.05$, t-test. n=3). The associated parallel increases in cAMP production concurrent with MC3R activation confirm salt responsive upregulation of MC3 receptor activity.

The natriuretic response can therefore be induced by the infusion of γ-MSH and is one method of regulating osmolality and plasma sodium concentration in rats chronically fed a high salt diet. This system may be an important target to modulate in those with salt-sensitive hypertension.

Chen XW *et al.*(1997).Am J Physiol Regulatory Integrative Comp Physiol **242**,R1946-1953.

Authors have confirmed where relevant, that experiments on animals and man were conducted in accordance with national and/or local ethical requirements.

C39

Renal sodium handling in a mouse model of Cushing's Syndrome

M.A. Bailey, N. Wrobel, J.J. Mullins and C.J. Kenyon

Centre for Cardiovascular Science, The University of Edinburgh, Edinburgh, UK

Cushing's Syndrome can be caused by ACTH secretion from pituitary adenomas or from ectopic tumours and is characterized by glucocorticoid excess, central obesity, altered metabolism of glucose and hypertension. Hypertension is an important predictor of outcome in Cushing's Syndrome and although the causes are not fully known, renal sodium retention may be a contributory factor (Ferrari, 2003). Cushing's has been modeled experimentally in rats but this is not ideal as ACTH excess causes a profound weight loss, rather than the central obesity seen in humans. Our previous studies indicate that ACTH infusion in the C57BL6 mouse causes hypertension without the catabolic effects seen in rats (Kenyon et al, 2007). In the present study, we have investigated the effect of ACTH infusion on renal sodium handling.

Male C57BL6 mice (~25g) were infused via osmotic minipump (under halothane anaesthesia (2% with oxygen by inhalation)) with ACTH (n=7. Synacthen; 3μg/day) or saline (n=7) for two weeks before being anaesthetized (Inactin; 100mg/kg IP) and prepared surgically for renal function experiments as described (Bailey et al, 2008). Mice were infused IV with a saline solution (0.2ml/h/10g) containing FITC-inulin for the measurement of glomerular filtration rate (GFR). After a control urine collection, amiloride (2mg/kg; IV) was injected and a second urine collection made. At the end of the experiments, mice were killed with an overdose of anaesthetic and the kidneys removed for extraction of RNA. The expression of mRNA encoding subunits of the epithelial sodium channel (ENaC) and the Na-K-2Cl cotransporter (NKCC2) were measured by quantitative RT-PCR. Data are mean± S.E. and statistical comparisions were made using Student's t test.

Mean arterial blood pressure was significantly elevated in mice receiving ACTH infusion (114±3 vs 90±3 mmHg; P<0.01). Sodium excretion was similar in both groups. Since plasma sodium (157±1 vs 148±1 mmol/l; P<0.01) and GFR (0.27±0.03 vs 0.16±0.02 ml/min; P<0.01) were elevated in the ACTH-treated group, fractional sodium excretion was significantly reduced compared to controls (0.76±0.1 vs 1.31±0.2%; P<0.01). The ENaC-inhibitor, amiloride, caused an increase in sodium excretion in both groups of animals but the natriuretic effect was significantly greater in mice receiving ACTH (0.63±0.13 vs 0.17±0.07 μmol/min; P<0.01). mRNA expression was expressed as a ratio to that of 18S RNA: expression of ENaCα was increased by ACTH treatment (0.65±0.09 vs 0.46±0.05; P<0.05), whereas that of NKCC2 was reduced (0.7±0.08 vs 1.24±0.11; p<0.01).

Our data show that ACTH treatment causes hypertension in the mouse and this is associated with inappropriately elevated tubular reabsorption of sodium via ENaC. After 14 days of treatment, absolute sodium excretion is normalized, possibly via down-regulation of sodium reabsorption in the loop of Henle.

Ferrari, P. (2003). Best Prac Res Clin Endocrinol Metab 17: 575-589.

Kenyon, C. et al (2007) Endocrine Abstracts 13: P191.

Bailey, M.A. et al (2008). J Am Soc Nephrol 19: 47-58.

Funded by The MRC and the Wellcome Trust.

Authors have confirmed where relevant, that experiments on animals and man were conducted in accordance with national and/or local ethical requirements.

C40

Role of the N-terminal in the targeting of the mouse urea transporter mUT-A3

V.M. Collins and G. Cooper

Biomedical Science, University of Sheffield, Sheffield, UK

The efficient handling of urea by the kidney is crucial for the development of maximally concentrated urine. The inner medullary collecting duct (IMCD) plays an important role in urea reabsorption. The transport of urea across the IMCD is governed by the UT-A family of urea transporters, with UT-A3 providing a pathway across the basolateral membrane. In the current study we have investigated the role of the N-terminal of UT-A3 on membrane targeting.

All clones were N-terminally tagged with eGFP. N-terminal truncations were created by standard PCR protocols, verified by sequencing and subcloned into the eGFP expression plasmid pEGFP-C2. The MDCK II cells were grown on permeable filters and transiently transfected with 1μg of cDNA. Cells were fixed and the localisation of each construct determined by immunofluorescence and confocal microscopy. The apical membrane was labelled with TRITC-conjugated peanut agglutinin and the basolateral membrane was labelled using a primary antibody against β-catenin and a Cy-5 conjugated secondary antibody. Transfections were replicated 3 times for each construct.

When expressed in MDCK II cells, mUT-A3 is delivered to the basolateral membrane of the cell (Stewart *et al.*, 2004). We have shown previously (Cooper and Collins, 2006) that removing the initial 55 residues of UT-A3 (M55-start) does not affect urea-transporting ability. However when expressed in MDCK II cells, M55-start targets to the apical membrane. When we removed the initial 25 residues (S25-start) we found that targeting was once again basolateral. When expressed in LLC-PK1 cells mUT-A3 targeted to the apical membrane. LLC-PK1 cells lack the μ1B subunit of the adaptor protein 1 complex (AP1-μ1B). The μ1B subunit has been implicated in basolateral targeting of a number of proteins (Folsch *et al.*, 1999). We expressed mUT-A3 and M-55start in LLC-PK1 cells stably transfected with AP1-μ1B. Although mUT-A3 was now targeted to the basolateral membrane, the M55-start clone was still resident in the apical membrane. Our results indicate that a region between residues 25

and 55 in the N-terminal is involved in the basolateral targeting of mUT-A3, probably via an interaction with the AP1-μ1B subunit.

Stewart *et al*. (2004), Am.J.Physiol., **286**, F979-87

Cooper and Collins, (2006), FASEB J., **20**, A1219

Folsch *et al*. (1999), Cell **99**, 189-198.

The financial support of Kidney Research UK is gratefully acknowledged. The LLC-PK1-μ1B cell line was a generous gift from Prof Ira Mellman.

Authors have confirmed where relevant, that experiments on animals and man were conducted in accordance with national and/or local ethical requirements.

C41

TASK-3 potassium channels: gating at the cytoplasmic mouth of the channel

P.R. Stanfield[1], I. Ashmole[1], P.J. Stansfeld[2] and M.J. Sutcliffe[3]

[1]Biological Sciences, University of Warwick, Coventry, UK, [2]Biochemistry, University of Oxford, Oxford, UK and [3]Manchester Interdisciplinary Biocentre, University of Manchester, Manchester, UK

We have used patch- and two electrode voltage-clamp recording, site directed mutagenesis and molecular model building to ask whether the tandem pore potassium channel TASK-3 is gated at the 'helix bundle crossing'.

Although tandem pore channels are described as constitutively open, the P(open) of wild type TASK-3 channels, heterologously expressed in CHO cells, was only 0.023 ± 0.0004 (mean ± sem, n=6) at -80mV (140mM-K). P(open) increased with depolarisation, but with a low gating charge (z′=0.16). We have used this voltage dependence to examine effects of mutations on gating.

Replacement with threonine of an alanine residue, A237, close to the cytoplasmic end of membrane helix M4, raised P(open) to 0.205 ± 0.031 (n=3) in the same conditions. The voltage dependence of P(open) is shifted to more negative potentials without change in gating charge. The shift gives an energy difference of ~1.2kcal/mol, consistent with the mutant channel being held open by a H-bond (or bonds). Contrary to our previous report, we can find no change in the response of A237T to acidification (measured in channels expressed in Xenopus oocytes) (Ashmole et al., 2005). Gating in response to acidification occurs at the selectivity filter (Yuill et al., 2007).

Modelling TASK-1 (which has strong identity with TASK-3) using KvAP (Jiang et al, 2003) as template suggests a H-bond between Thr in position 237 and Asn133, at the cytoplasmic end of M2. However, N133A also has a raised P(open), as does the dual mutant N133A/A237T. Neither mutant form of the channel has an altered response to acidification.

Gating at the bundle crossing of helices M2 and M4 is expected to occur around hinge glycines. Such residues are conserved through the tandem pore family, in TASK-3 at positions 117 (M2) and 231 (M4). In G117A and G231A; P(open) is reduced to 0.0021 ± 0.0003 (n=3) and 0.0039 ± 0.0013 (n=4) (respectively). The voltage dependence is shifted positive, again without change in gating charge. In spite of a low P(open), macroscopic currents can be recorded from G117A and G231A (oocyte expression). The pH-sensitivity of these currents is again unaltered from wild type.

Voltage dependence is weak and its physiological importance is uncertain. But this voltage dependence appears to involve gating by movements of M2 and M4 similar to those found in other potassium channels. It is likely that modulation of TASK-3 by hormones, neurotransmitters and inhalational anaesthetics (Kim, 2005) occurs by gating at this part of the channel.

Ashmole et al (2005) J Physiol 565P PC90

Jiang et al (2003) Nature 423:33-41

Kim (2005) Curr Pharm Des 11:2717-2735

Yuill et al (2007) Pfluegers Arch 455:333-348

We thank the Wellcome Trust for support

Authors have confirmed where relevant, that experiments on animals and man were conducted in accordance with national and/or local ethical requirements.

C42

cAMP-initiated but PKA-independent regulation of vascular ATP-sensitive K^+ channels: The role of exchange proteins directly activated by cAMP

G.I. Purves[1], T. Kamishima[2], L.M. Davies[1], J.M. Quayle[2] and C. Dart[1]

[1]Biological Sciences, University of Liverpool, Liverpool, UK and [2]Department of Human Anatomy and Cell Biology, University of Liverpool, Liverpool, UK

The exchange proteins directly activated by cyclic AMP (Epac or cAMP-GEF) are a family of cAMP-regulated guanine nucleotide exchange factors (GEF) [1]. Binding of cAMP to Epac activates the GEF activity thus stimulating the exchange of GTP for GDP on the monomeric G proteins Rap1/2 [1]. The discovery of the Epac proteins (Epac1/2) has raised the possibility of novel signalling pathways for cAMP that are independent of its traditional target, protein kinase A (PKA). It has previously been reported using yeast two-hybrid screening that Epac interacts with sulphonylurea receptors (SUR) [2], the regulatory subunits of ATP-sensitive potassium (K_{ATP}) channels. This suggests that Epac may play a role in the regulation of K_{ATP} channel activity. Indeed, we found that antibodies directed against Epac1 co-immunoprecipitate SUR2B, the dominant SUR subtype found in vascular smooth muscle, from rat aortic homogenates. Also, using the Epac-specific cAMP analogue 8-pCPT-2`-O-Me-cAMP at concentrations that activate Epac but not PKA, we show cAMP-mediated but PKA-independent modulation of vascular $K_{ATP}$ channels. Application of 8-pCPT-2`-O-Me-cAMP (5 μM) caused a 41.6 ± 4.7 % inhibition (mean ± SEM; n = 7) of pinacidil-evoked whole-cell K_{ATP} currents recorded in isolated rat aortic

(Simán *et al* 2001). Medium was collected daily for analysis of hCG (ELISA: mIU/h/mg protein) and lactate dehydrogenase (LDH), a marker of cellular integrity, (Cytotoxicity Detection Kit: absorbance units/h/mg protein), and explants treated daily with K^+ channel modulators, or medium alone, on days 3-6. Data are presented as mean±SE, with n=5 placentas and analysed by 2 way ANOVA with Bonferroni post tests.

hCG secretion increased from day 3 of culture, rising from 1.13±0.14 to 3.63±0.69 at day 6, while LDH release was stable at 0.071±0.02 and 0.066±0.01 respectively, coincident with syncytiotrophoblast regeneration previously reported (Simán *et al* 2001). Tetraethylammonium (TEA: blocker of voltage-gated (K_V) and calcium-activated (K_{Ca}) K^+ channels) prevented the increase in hCG secretion over days 4-6, significantly reducing hCG secretion at 5mM (1.61±0.24) and 10mM (1.11±0.28) on day 6 compared with control ($p<0.05$). 4-aminopyridine (4-AP: blocker of K_V channels) did not alter hCG secretion at 1mM but 5mM significantly inhibited secretion (1.03±0.23 at day 6: $p<0.05$ vs control). In contrast, the K^+ channel opener cromakalim (10μM) was without effect (3.41±0.63 at day 6, n=4). None of the K^+ channel modulators altered LDH release, indicating that the inhibition of hCG secretion by TEA and 4-AP was not caused by loss of tissue integrity.

We conclude that K_{Ca} and/or K_V channels, sensitive to TEA and 4-AP, modulate hCG secretion by placental trophoblast. Modulation may occur either directly, by altering the secretory mechanism, or indirectly, by influencing the cellular turnover required for syncytiotrophoblast renewal.

O'Grady SM and Lee SY (2005) Molecular diversity of voltage-gated (K_V) potassium channels in epithelial cells. Int J Biochem Cell Biol. 37(8): 1578-94.

Simán CM, Sibley CP, Jones CJ, Turner MA and Greenwood SL (2001) The functional regeneration of syncytiotrophoblast in cultured explants of term placenta. Am J Physiol Regul Integr Comp Physiol. 280(4): R1116-22.

Supported by Tommy's, the baby charity.

Authors have confirmed where relevant, that experiments on animals and man were conducted in accordance with national and/or local ethical requirements.

PC40

Placental materno-fetal transfer of amino acids by exchangers

J.K. Cleal, K.M. Godfrey, M.A. Hanson and R.M. Lewis

The University of Southampton, Southampton, UK

Objectives: The mechanisms mediating amino acid transport across the basal membrane of placental syncytiotrophoblast and into the fetal circulation are not well understood. Our previous data indicate that amino acid exchangers mediate serine (ser) and leucine (leu) transport into the fetoplacental circulation in exchange for specific amino acids (Cleal JK *et al.,* (2007) *J Physiol* 582, 871-882). This study characterises amino acid stimulation of alanine (ala), phenylalanine (phe), tyrosine (tyr), isoleucine (iso), lysine (lys), threonine (thr), and glutamine (gln) transfer into the fetoplacental circulation.

Methods: Human placentas ($n = 5$ per amino acid) were collected within 30 minutes of delivery and an intact cotyledon was perfused with a modified Earl's bicarbonate buffer. The maternal arterial circulation was perfused with 50 μmol/l of amino acid plus radio-labelled tracer amino acid and 1.8 mM creatinine to determine the rate of paracellular diffusion. Amino acid [12.5 μmol] boluses were administered to the fetal side inflow perfusate. ^{14}C- and ^{3}H-labelled amino acids were measured in maternal and fetal (indicating transport) venous samples by liquid scintillation counting. Data (mean ± SEM) were expressed as area under the curve and analysed by one-way ANOVA.

Results: Following fetal arterial boluses of specific amino acids transfer of ala, phe, tyr, iso, lys, thr, and gln increased, indicating that transport by exchange was taking place (Table 1).

Conclusion: This study demonstrates that in the perfused human placenta amino acids are transported into the fetal circulation by exchange mechanisms. The data indicates activity of the exchangers ASC, LAT1 and y^+LAT between the placenta and fetal circulation. This provides an important mechanism by which maternal amino acids can be transported to the fetus and thus be available for fetal growth and development.

Table 1: **Amino acid exchange between the placenta and feto-placental circulation**

	Maternal amino acid (nmol)						
Fetal bolus	ala	phe	iso	tyr	gln	lys	thr
glu	0.1 ± 0.7	-14 ± 3	-8 ± 4	-7 ± 6	-7 ± 6	3 ± 3	-7 ± 3
ala	174 ± 56*	82 ± 45	68 ± 37	61 ± 21	88 ± 10*		76 ± 17*
thr	83 ± 13*	108 ± 25	131 ± 39*	72 ± 14	86 ± 13*	11 ± 3	89 ± 13*
ser	86 ± 25*	32 ± 14	53 ± 15	57 ± 27	79 ± 4*	25 ± 6	65 ± 9*
gln	69 ± 15	74 ± 29	17 ± 6	39 ± 18	81 ± 17*	66 ± 8*	40 ± 6*
lys	7 ± 4	0.6 ± 10			10 ± 5	62± 10*	
leu	20 ± 3	277 ± 69*	181 ± 32*	172 ± 36*	78.8 ± 16*	81 ± 15	31± 7*
trp		197 ± 30*	164 ± 29*	191 ± 24*	12 ± 5	-38 ± 4*	36 ± 19*
bch	5 ± 3	140 ± 50*	115 ± 19*	144 ± 25*	14 ± 5	15 ± 7	6 ± 7

*$P < 0.05$, significantly different to glutamate (glu). 2-aminobicyclo- (2,2,1)-heptane-2-carboxylic acid (bch), glycine (gly), tryptophan (trp).

This work was supported by The Henry Smiths Charity.

Authors have confirmed where relevant, that experiments on animals and man were conducted in accordance with national and/or local ethical requirements.

PC41

Renal actions of urotensin II in anaesthetised young rats

E.J. Forty and N. Ashton

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

Urotensin II (UII) has been implicated in transepithelial sodium transport in fish [1] and in mammalian renal function [2]. We have shown that infusion of exogenous rat UII in the adult Sprague-Dawley (SD) rat, at a dose with minimal effect on the renal vasculature, decreased renal tubular sodium reabsorption [3]. As urinary concentrating ability develops over the first few post-natal weeks in the rat, the aim of this study was to

determine the renal actions of UII in young SD rats. Inactin anaesthetised (10mg.100g bwt^{-1}, i.p.) 4-week-old male SD rats (n= 5 per group) were infused i.v. with 0.154M NaCl containing ^{3}H inulin (0.3μCi h^{-1}) and para-aminohippuric acid (3mg h^{-1}) as markers of glomerular filtration rate (GFR) and effective renal blood flow (ERBF), respectively, at a rate of 40μl min^{-1} for 2.5h. Animals then received either vehicle or rat UII at 0.6 or 6 pmol.min^{-1}.100g bwt^{-1} for 1h. Urine was collected from a bladder catheter every 15 mins, two blood samples (0.4ml) were taken via a carotid artery catheter. Animals were killed humanely at the end of the experiment. Rat UII at either dose had no effect on mean arterial blood pressure (vehicle 92±5 vs rUII at 6pmol 108±7mmHg) or ERBF (Table 1). Compared with the vehicle-infused group, rUII at 6pmol induced an initial 30% reduction in GFR after 15 mins (vehicle 0.6±0.1 vs rUII 0.4±0.1 ml.min^{-1}.100g bwt^{-1}) which returned to control levels after 45 mins. Rat UII also induced a 50% reduction in urine flow (UV) and sodium excretion rates ($U_{Na}V$) which reached a nadir after 1h. This was accompanied by a trend towards lower fractional excretion of sodium (FE_{Na}, Table 1). No changes were observed at the lower rUII dose. These data show that rUII influenced renal excretion indirectly through a reduction in filtered load, and are suggestive of a direct effect on tubular sodium reabsorption by the nephron. Results are consistent with reported expression of the UII receptor (UT) in the thin ascending limb of the loop of Henle and the inner medullary collecting ducts [2]. Young SD rats appear to be less sensitive to rUII than adults, which respond to 0.6pmol rUII [3], suggesting that UT expression is not yet fully mature at 4 weeks.

Table 1. Renal variables after 1h i.v. infusion of vehicle or rUII at 6pmol.min^{-1}.100g bwt^{-1}. Data presented as mean±S.E.M. * p<0.05, repeated measures ANOVA

Parameter	Vehicle	rUII 6 pmol.min^{-1}.100g bwt^{-1}
ERBF ml.min^{-1}.100g bwt^{-1}	6.2±0.8	5.2±1.4
UV μl.min^{-1}.100g bwt^{-1}	29.2±6.1	13.8±3.5*
$U_{Na}V$ μmol.min^{-1}.100g bwt^{-1}	5.2±1.2	2.3±0.5
FENa %	7.6±1.8	2.9±0.3

Winter MJ *et al.* (1999). *Gen Comp Endocrinol* **114**,249-256.

Song W *et al.* (2006). *Kidney Int* **69**,1360-1368.

Abdel-Razik AE *et al.* (2006). *Proc Physiol Soc* **3**, PC13.

Authors have confirmed where relevant, that experiments on animals and man were conducted in accordance with national and/or local ethical requirements.

PC42

Vasopressin regulation of the rat urea transporter rUT-A1 is calmodulin-dependent

P. Tickle, G. Stewart and C. Smith

University of Manchester, Manchester, UK

The antidiuretic hormone arginine vasopressin (AVP) mediates the calmodulin-dependent increase in urea permeability in the rat renal inner medullary collecting duct (IMCD; [1]). The urea transporter (UT) proteins UT-A1 and UT-A3 are located in the IMCD [2, 3]. It is therefore likely that one, or both, of these proteins is involved in the calmodulin-dependent increase in urea permeability in this portion of the renal tubule.

In this study, the effect of the calmodulin inhibitor W-7 on transepithelial urea flux by monolayers of Madin-Darby canine kidney (MDCK) cells stably expressing rat UT-A1 was investigated. In the absence of AVP, transepithelial urea flux was 1.64 ± 0.34 (mean ± S.D.) nmoles/cm^2/min (n = 4). Under these control conditions DMU-sensitive urea flux was 0.80 ± 0.22 nmoles/cm^2/min (n = 4). In the presence of 100nM AVP transepithelial urea flux increased to 4.41 ± 0.66 nmoles/cm^2/min (P<0.01; ANOVA; n = 4) and DMU-sensitive flux was 3.01 ± 0.25 nmoles/cm^2/min (n = 4). In the presence of the calmodulin inhibitor W-7, AVP-stimulated DMU-sensitive urea flux was reduced significantly to 1.14 ± 1.00 nmoles/cm^2/min (P<0.01; ANOVA; n = 4). In contrast, the presence of Calphostin-C (PKC inhibitor), CKII (Casein kinase II inhibitor) or H-89 (PKA inhibitor) had no effect on AVP-stimulated, DMU-sensitive urea flux (P>0.05, ANOVA; n = 4).

These data suggest AVP regulation of the UT-A1 urea transporter is dependent on calmodulin, supporting a role for UT-A1 in the calmodulin-dependent regulation of urea permeability in the rat inner medullary collecting duct. However, further studies are required to confirm this and to elucidate the molecular mechanisms involved.

Hoffert JD, Chou C-L, Fenton RA and Knepper MA (2005). J.Biol.Chem. 280, 13624-13630.

Terris J, Ecelbarger CA, Sands JM and Knepper MA (1998). J.Am.Soc.Nephrol. 9,729-736.

Stewart GS, Fenton RA, Wang W, Kwon T-H, White SJ, Collins VM, Cooper GJ, Nielsen S and Smith CP (2004) Am.J.Physiol. Renal Physiol. 286, F979-F987.

This work was funded by Kidney Research UK.

Authors have confirmed where relevant, that experiments on animals and man were conducted in accordance with national and/or local ethical requirements.

PC43

Increased receptor stimulation elicits differential calcium-sensing receptor$_{T888}$ dephosphorylation

W.D. McCormick[1], K.L. Bailey[1], A.D. Conigrave[2] and D.T. Ward[1]

[1]Faculty of Life Sciences, The University of Manchester, Manchester, UK and [2]School of Molecular and Microbial Biosciences, University of Sydney, Sydney, NSW, Australia

The calcium-sensing receptor (CaR) regulates parathyroid hormone secretion and renal Ca^{2+} reabsorption and thus controls Ca^{2+}_o homeostasis. CaR_{T888} is a key intracellular signalling determinant whose phosphorylation by protein kinase C inhibits CaR-elicited Ca^{2+}_i mobilisation [1,2].

Here, we examined the concentration-dependence of Ca^{2+}_o on CaR_{T888} phosphorylation by semi-quantitative immunoblotting [2]. HEK-293 cells stably expressing human CaR (CaR-HEK) were incubated for 10mins at 37°C in Hepes-containing buffer. Raising $[Ca^{2+}]_o$ from 0.5 to 2.5mM caused a 5.5-fold increase

in CaR_{T888} phosphorylation (160kDa mature, membrane-localised receptor; P<0.001 by ANOVA, N=6). However, following 10min treatment with higher Ca^{2+}_o concentrations (3-5mM), CaR_{T888} phosphorylation was not elevated. The effect of 2mM Ca^{2+}_o treatment (which also elicited oscillatory Ca^{2+}_i mobilisation) on CaR_{T888} phosphorylation was sustained for at least 20mins. However, 5mM Ca^{2+}_o (associated with sustained Ca^{2+}_i mobilisation) did increase CaR_{T888} phosphorylation briefly, but with levels returning to baseline within 2mins suggesting that increased CaR activation might induce subsequent CaR_{T888} dephosphorylation. To test this, CaR_{T888} phosphorylation was first induced by phorbol ester treatment (1μM PMA, 10 mins) with the cells then incubated for 30secs in buffer containing either 1.2, 2.2 or 5mM Ca^{2+}_o or 1.2mM Ca^{2+}_o plus the CaR positive allosteric modulator L-Phe (10mM) [3]. Increasing $[Ca^{2+}]_o$ stimulated subsequent CaR_{T888} dephosphorylation (1.2mM,33±14%; 2.2mM, 52±9%; 5mM, 78±5%, P<0.05 vs 1.2 by ANOVA; N=8) whereas L-Phe was without additional effect (31±9%). Therefore, orthosteric CaR activation elicits CaR_{T888} dephosphorylation perhaps contributing to the high frequency oscillatory and sustained Ca^{2+}_i mobilisation responses to Ca^{2+}_o. In contrast, the failure of L-Phe to stimulate CaR_{T888} dephosphorylation may explain why this CaR modulator elicits low frequency Ca^{2+}_i oscillations. Therefore, CaR_{T888} is subject to agonist-specific phosphorylation and dephosphorylation in CaR-HEK cells. Such receptor-elicited dephosphorylation of this protein kinase C consensus site could help explain the control of signal oscillation frequency observed in this and other type III G protein-coupled receptors.

Jiang YF, Zhang Z, Kifor O, Lane CR, Quinn SJ, Bai M. (2002) *J Biol Chem* **277**, 50543-50549.

Davies SL, Ozawa A, McCormick WD, Dvorak MM, Ward DT. (2007) *J Biol Chem* **282**, 15048-15056.

Conigrave AD, Quinn SJ, Brown EM. (2000) *Proc Natl Acad Sci USA* **97**, 4814-4819.

Authors have confirmed where relevant, that experiments on animals and man were conducted in accordance with national and/or local ethical requirements.

PC44

Renal haemodynamics in healthy volunteers and type 2 diabetic persons after endotoxin injection

A. Andreasen[1,2], T. Pedersen-Skovsgaard[1,2], H. Gutte[3], R.M. Berg[1,2], A. Kjær[3], B. Feldt-Rasmussen[4] and K. Møller[1,2]

[1]Centre of Inflammation and Metabolism M7641, Rigshospitalet, Copenhagen, Denmark, [2]Intensive Care Unit 4131, Rigshospitalet, Copenhagen, Denmark, [3]Department of Clinical Physiology, Rigshospitalet, Copenhagen, Denmark and [4]Department of Nephrology, Rigshospitalet, Copenhagen, Denmark

BACKGROUND: Acute kidney injury develops in 20-50% of patients with sepsis and septic shock (1), the risk being increased in patients with type 2 diabetes (2). To which extent, if any, changes in renal haemodynamics during sepsis contribute to the pathogenesis of AKI, remains controversial. The aim of this study was to measure renal plasma flow (RPF) and glomerular filtration rate (GFR) in healthy volunteers and persons with type 2 diabetes, respectively, using an intravenous injection of Escherichia coli endotoxin as a model of systemic inflammation during early sepsis.

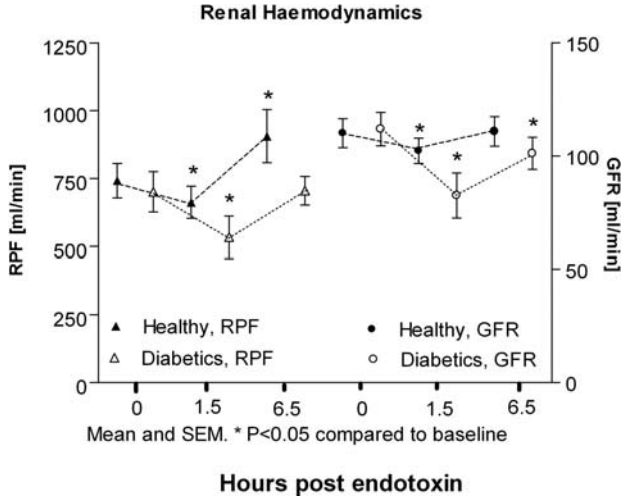
METHODS: After ethical approval, a thorough physical examination and informed consent, RPF and GFR were measured with DTPA renography in 16 healthy men and 16 men with type 2 diabetes at baseline as well as 1.5 hours and 6.5 hours after endotoxin injection (0.3 ng/kg). Noninvasive blood pressure and heart rate were measured every 15 minutes. Filtration fraction (FF) was calculated as GFR / RPF. Repeated measurements analysis of RPF, GFR and FF were performed using SAS Mixed Models with the model TIME GROUP TIME*GROUP and SUBJECT as a random factor. Significant changes from baseline were analysed post hoc with paired t-tests.

RESULTS: Data are presented in the Figure "Renal Haemodynamics" and Table 1. A mixed model analysis showed no interaction between time and group (healthy vs. diabetics). Time, but not group, had a significant effect on RPF (P<0.001), GFR (P<0.01) and FF (P<0.05). Mean arterial pressure and heart rate were unchanged. A systemic inflammatory response with leucocytosis, temperature rise and malaise was seen but resolved within 6 hours.

CONCLUSION: RPF, GFR and FF are reversible reduced during early systemic inflammation triggered by an injection of Escherichia coli endotoxin. The presence of type 2 diabetes did not affect these changes. The results suggest a role for renal haemodynamic alterations in sepsis-induced acute kidney injury. In contrast, the significance of these changes for the increased risk in diabetic patients of developing acute kidney injury during sepsis is less clear.

Table 1. Renal Haemodynamics - Healthy / Diabetics

Hours post Endotoxin	0 (Baseline)	1.5	6.5
Number of Observations	16 / 16	16 / 16	16 / 15
RPF [ml/min] - Mean (SEM)	742 (64) / 701 (74)	662 (58) / 534 (78)	905 (97) / 704 (52)
RPF [ml/min] - Mean change from baseline (SEM)		-79 (36) / -167 (66)	164 (68) / 24 (70)
GFR [ml/min] - Mean (SEM)	110 (6) / 111 (7)	102 (6) / 82 (10)	110 (7) / 110 (7)
GFR [ml/min] - Mean change from baseline (SEM)		-8 (5) / -29 (12)	0.8 (6) / -14 (7)
FF - Mean (SEM)	0.17 (0.002) / 0.18 (0.002)	0.17 (0.001) / 0.19 (0.003)	0.14 (0.001) / 0.15 (0.003)
FF - Mean change from baseline (SEM)		0 (0.01) / 0.02 (0.03)	-0.03 (0.01) / -0.03 (0.02)



Brivet F.G. et al (1996) Crit Care Med 24, 192-198.

Leblanc M et al (2005) Curr. Opin. Crit. Care. 11, 533-536.

The Centre of Inflammation and Metabolism is supported by a grant from the Danish National Research Foundation (#02-512-55). This study was further supported by grants from private funding organisations.

Authors have confirmed where relevant, that experiments on animals and man were conducted in accordance with national and/or local ethical requirements.

PC45

Using a human patient simulator to demonstrate the autonomic control of the cardiovascular system

P.D. Maskell, K. Brandom, E. Lloyd and E.J. Robinson

Department of Physiology and Pharmacology, University of Bristol, Bristol, UK

The METI Human Patient Simulator (HPS) is a state-of-the-art, life-sized, high fidelity mannequin linked to a computer through a host of electro-mechanical equipment. This integration of software-driven electro-mechanics allows the modelling of a wide range of physiological and pharmacological states. The data from the HPS is projected in the format of a clinical monitor and includes heart rate, systolic and diastolic arterial pressures and cardiac output. Drugs can 'interact' with the underlying HPS model via predefined pharmacokinetic and pharmacodynamic parameters.
In this study we describe the development of an HPS session integrating pharmacology and physiology in order to demonstrate sympathetic and parasympathetic control of the cardiovascular system.
A 30 minute HPS scenario for around 20 students is integrated into the existing first year MBChB and second year BSc practical classes in cardiovascular pharmacology. The students, using their knowledge of the cardiovascular system have to predict the effects of various drugs (phenylephrine, propranolol, atropine and dobutamine) that act to influence the parasympathetic and sympathetic nervous system on heart rate, blood pressure and systemic vascular resistance. The students then give i.v. administrations by 'injecting' the drugs into the HPS using a drug recognition system that detects different drugs using a barcode reader. Under the guidance of a member of staff the students discuss the responses obtained from the HPS. The students also learn about the tonic control of the heart by the autonomic nervous system via chemical denervation using atropine and propranolol.
The effects of the drug dosages 'given' to the HPS on the cardiovascular parameters correlate well with published human data (Atropine and propranolol for intrinsic heart rate[1], phenylephrine[2], atropine[3], propranolol[4], and dobutamine[5].)
The session was rated highly by students with 85% (n= 164) scoring the value at 4 or above (scale of 1-5, 5 being very well), when responding to the question how well did the HPS session help you understand the control of blood pressure? The students also thought the session helped improve their understanding of the clinical relevance of drugs acting on the CVS with 78% of students (n= 164) scoring the value of this session at 4 or above (scale of 1-5, 5 being very well).
We conclude that this HPS session enhances the current physiology and pharmacology student learning experience and gives accurate qualitative physiological changes to the drugs administered.

Jose AD (1966) *Am J Cardiol* **18**, 476-478.

Madden KM *et al.* (2006) *J Cardiovasc Pharmacol* **48**, 153-159.

Farham JV & Kennedy BR (1968) *Brit J Anaesth* **40**, 876-884.

Epstein SE *et al.* (1965) *J Clin Investigation* **44**, 1745-1753.

Jewitt D *et al.*(1974). *The Lancet* **7877**, 363-367.

HEFCE funding established and supports the Applied and Integrated Medical Sciences Centre for Excellence in Teaching and Learning (AIMS CETL).

Authors have confirmed where relevant, that experiments on animals and man were conducted in accordance with national and/or local ethical requirements.

PC46

The response to hypoxia: a comparison of the Human Patient Simulator (HPS) with human data

R. Helyer[1], A. Coombs[1,2], A. Cousins[1,2], H. Dee[1,2], E. Kermode[1], C. Rogers[1,2] and E. Lloyd[1]

[1]Department of Physiology & Pharmacology and AIMS Centre For Excellence in Teaching & Learning, University of Bristol, Bristol, UK and [2]Intercalating MB ChB students, University of Bristol, Bristol, UK

The Human Patient Simulator (HPS 337; METI, Sarasota, Florida) has a computer driven mechanical lung and gas exchange mechanism, designed to model the human respiratory system. We have previously demonstrated that the HPS is a useful tool for illustrating physiological principles but requires adjustments to the modeling software in order to improve the fidelity of the quantitative response to perturbations such as simulated hypovolaemia [1].
The aim of this study was to compare the response of the HPS to hypoxia with available human data in order to determine the accuracy of the HPS response and the utility of the HPS for teaching high altitude human physiology. Data can be obtained from the HPS for a range of respiratory variables including breathing rate, tidal volume, simulated alveolar and arterial partial pressures of oxygen and carbon dioxide, and arterial oxygen saturation.
The simulator was intubated and baseline measurements were made of the respiratory variables including the partial pressures of oxygen and carbon dioxide in alveolar (PAO_2 and $PACO_2$) gas whilst "breathing" atmospheric air at ambient pressure. The responses were then determined to breathing hypoxic gas mixtures (range = 19–5% O_2) applied using Douglas bags and a three-way valve system to separate inspired and expired gases. The data were used to construct an Oxygen-Carbon Dioxide diagram for comparison with published human data [2,3,4].
In response to hypoxia the HPS showed a linear relationship between PAO_2 and $PACO_2$ over the entire range investigated (PAO_2 = 25-110 mmHg; n = 6). Published human data for unacclimatised individuals shows a linear relationship between PAO_2 and $PACO_2$ over a PAO_2 range of 60-100 mmHg but below this