Pressor and renal regional hemodynamic effects of urotensin II in neonatal pigs

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Abstract

Renal expression of the peptide hormone urotensin II (UII) and its receptor (UTR) are dependent on kidney maturation and anatomical regions. However, renal regional hemodynamic effects of UII in neonates are unclear. Here, we investigated regional hemodynamic responses to acute intrarenal arterial administration of UII in newborn pigs. Western immunoblotting and immunofluorescence confirmed UTR expression and membrane localization in newborn pig renal afferent arterioles and afferent arteriolar smooth muscle cells respectively. Intrarenal arterial bolus injections of human UII (hUII; 1–100 ng/kg) resulted in a dose-dependent decrease in total renal blood flow (RBF) and an increase in mean arterial pressure (MAP) and renal vascular resistance (RVR) in newborn pigs. Moreover, hUII dose dependently reduced cortical blood flow (CBF) but increased medullary blood flow (MBF) in the piglets. hUII-induced MAP elevation and hemodynamic changes were inhibited by urantide, a UTR antagonist, but not losartan, a type 1 angiotensin II receptor antagonist. U-73122, a phospholipase C (PLC) inhibitor, and 2-aminoethoxy-diphenyl borate, an inositol 1,4,5 trisphosphate (IP₃) receptor antagonist, attenuated hUII-induced MAP and RVR elevations, RBF and CBF reductions, but not MBF increase. These findings indicate that intrarenal arterial administration of hUII elevates blood pressure and induces region-selective renal hemodynamic changes in newborn pigs. Our data also suggest that the PLC/IP₃ signaling pathway contributes to hUII-induced alterations in MAP, RBF, RVR, and CBF but not MBF in newborn pigs.

Key Words

- ▶ Urotensin II
- ▶ neonates
- ▶ renal hemodynamics
- ▶ mean arterial pressure
- ▶ renal arterioles

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Introduction

The kidney is a major source of vasoactive mediators, including angiotensin II (AngII), prostaglandins (PGs), endothelin-1 (ET-1), bradykinin, thromboxane, and urotensin II (UII). These compounds can act in an endocrine, autocrine, or paracrine fashion to modulate cardiovascular and renal homeostasis. Thus, alterations in expression, function, and regulation of these mediators and their receptors underlie pathophysiological mechanisms of a

variety of cardiovascular and renal diseases (Douglas *et al.* 2004, Ponnuchamy & Khalil 2009).

UII, a cyclic peptide hormone, has been described as the most potent vasoconstrictor, constricting human coronary, mammary and radial arteries, rat aorta and pulmonary arteries, pig and monkey coronary arteries, and monkey basilar, renal, and mesenteric arteries with potency up to 109-fold more than ET-1 (Ames *et al.* 1999,

Douglas *et al.* 2000, MacLean *et al.* 2000, Maguire *et al.* 2000). However, other studies have shown that UII can also cause vasodilation. For example, UII-induced endothelium-dependent vasodilation in rat-isolated coronary and mesenteric arteries precontracted with 5-HT and methoxamine respectively (Bottrill *et al.* 2000). UII also dilated ET-1-precontracted human pulmonary and abdominal resistance-sized arteries and phenylephrine-precontracted rat renal arteries (Stirrat *et al.* 2001, Zhang *et al.* 2003). These studies suggest that UII-induced regulation of vascular tone may depend on species and blood vessel anatomical origin or size (Douglas *et al.* 2000, Ashton 2006, Zoccali & Mallamaci 2008).

Alterations in UII and UII receptor (UTR) expression and plasma and urinary UII concentrations have been associated with renal dysfunctions, including hypertensive renal disease, diabetic nephropathy, glomerulonephritis, and renal tubular diseases (Matsushita *et al.* 2001, Douglas *et al.* 2004, Langham *et al.* 2004, Ashton 2006, Balat *et al.* 2007, Ross *et al.* 2010). However, the physiological role of UII in the kidney remains controversial with studies reporting attenuation and elevation of important renal parameters, including total renal blood flow (RBF), glomerular filtration rate (GFR), urine flow rate (UV), and urinary sodium (U_{NaV}) and potassium (U_{KV}) excretion (Zhang *et al.* 2003, Ovcharenko *et al.* 2006, Song *et al.* 2006, Abdel-Razik *et al.* 2008a,b, Shi *et al.* 2008).

A recent study demonstrated that UII plasma level was increased after birth in lambs (Simpson *et al.* 2010). An examination of the ontogenetic pattern of UII and UTR expression in rat kidney has also revealed regional variability in UII and UTR expression from embryonic day 19 to 4 weeks after birth (Forty & Ashton 2012). However, unlike in adult, rat UII (rUII) did not alter renal hemodynamics in young (4-week-old) rats (Forty & Ashton 2012).

The physiological characteristics of newborn renal circulation differ significantly from those of young and old adults (Hook & Bailie 1979, Toth-Heyn *et al.* 2000). Newborns are unable to concentrate urine and exhibit higher renal vascular resistance (RVR) and lower RBF and GFR compared with adults (Hook & Bailie 1979, Toth-Heyn *et al.* 2000). Accumulating evidence also suggests that alterations in the physiological functions of endogenous vasoactive mediators contribute to maturation-dependent changes in renal hemodynamics (Hook & Bailie 1979, Toth-Heyn *et al.* 2000). Here, we examined two important, yet largely unexplored, renal actions of UII. First, we studied the effects of UII on neonatal renal circulation. Second, we tested the hypothesis that UII differentially regulate neonatal regional renal perfusion.

Materials and methods

Animals

All animal protocols were reviewed and approved by the Animal Care and Use Committee of the University of Tennessee Health Science Center (UTHSC). Newborn pigs (1–3 day old; 1.5–2 kg) were purchased from Nichols Hog Farm (Olive Branch, MS, USA) and maintained at the UTHSC Comparative Medicine Department animal core facility. Animals were used within the first week of life.

Renal vascular smooth muscle cell isolation

Afferent arterioles were manually dissected from decapsulated newborn pig kidneys using a Zeiss SteREO Discovery.V12 stereomicroscope (Carl Zeiss, Thornwood, NY, USA). Smooth muscle cells were dissociated from the microvessels using a HEPES-buffered isolation solution containing (in mM) 55 NaCl, 80 sodium glutamate, 5.6 KCl, 2 $MgCl_2$, 10 HEPES, and 10 glucose (pH 7.3). Afferent arterioles were placed into isolation solution containing 1 mg/ml papain, 500 μ g/ml elastase, 1 mg/ml dithioerythritol, and 1 mg/ml BSA for ~45 min at 37 °C. Arterioles were then washed and incubated in isolation solution containing 1 mg/ml collagenase F and H (2:1), 100 μ M $CaCl_2$ and 1 mg/ml BSA for ~20 min at 37 °C. Digested vessels were subsequently washed in isolation solution and triturated using a fire-polished glass Pasteur pipette to yield single smooth muscle cells.

Immunofluorescence

Renal vascular smooth muscle cells were allowed to adhere to collagen-coated coverslips. The cells were then fixed with 4% paraformaldehyde in PBS for ~15 min and permeabilized with 0.2% Triton X-100 for ~20 min at room temperature. Following a 1 h incubation in PBS containing 5% BSA to block nonspecific immunoreactive sites, cells were treated overnight at 4 °C with rabbit polyclonal anti-UTR (Alomone Lab, Jerusalem, Israel) or normal rabbit IgG (GenScript, Piscataway, NJ, USA). After a wash, cells were incubated for 1 h at room temperature with Alexa 555-conjugated Donkey Anti-Rabbit (Life Technologies). Following wash and mount, fluorescence images were acquired using a Zeiss LSM Pascal laser-scanning confocal microscope.

Western immunoblotting

Afferent arterioles were homogenized in ice-cold RIPA buffer (Thermo Scientific, Rockford, IL, USA) followed by

centrifugation to extract protein. Protein lysates were mixed with SDS sample buffer containing 5% β -mercaptoethanol and boiled at $\sim 100^\circ\text{C}$ for 5 min. Protein samples were thereafter resolved on a 7% NuPAGE Tris-Acetate Gel (Life Technologies) and then transferred to polyvinylidene difluoride membranes using a Pierce Fast Semi-Dry Blotter (Thermo Scientific). Nonspecific binding sites on the membranes were blocked by Tris buffered saline supplemented with 0.1% Tween 20 (TBS-T) and 5% nonfat milk (Research Products International Corp., Mount Prospect, IL, USA) for 1 h at room temperature. The membranes were probed with rabbit polyclonal anti-UTR (GPR14; Alpha Diagnostic International, Inc., San Antonio, TX, USA) overnight at 4°C . After washing with TBS-T, the membranes were incubated in a HRP-conjugated secondary antibody for ~ 1 h at room temperature. Membranes were then processed with a Pierce Chemiluminescence kit (Thermo Scientific) and immunoreactive proteins were visualized on a Kodak Imaging system (Carestream Molecular Imaging, Rochester, NY, USA).

Renal hemodynamic measurements

Newborn pigs were anesthetized with ketamine hydrochloride (20 mg/kg) and xylazine (2.2 mg/kg) intramuscularly and maintained on α -chloralose (50 mg/kg, i.v.) at 37°C . The animals were intubated via tracheostomy and mechanically ventilated. Animals were continually monitored during experiments for anesthesia depth and re-dosed if necessary. The right femoral artery was catheterized for continuous measurement of mean arterial pressure (MAP) using a MLT1199 physiological pressure transducer (ADInstrument, Colorado Spring, CO, USA). Another catheter was inserted in the left femoral artery and advanced through the abdominal aorta until its tip was positioned at the junction of the aorta and left renal artery for intrarenal administration of drugs. The position of the catheter tip was confirmed at the end of each experiment. A femoral vein was also catheterized for anesthetic and fluid administration. Arterial blood partial pressures of CO_2 and O_2 , pH, and hematocrit were measured periodically with a Blood Gas Analyzer (Instrumentation Laboratory, Lexington, MA, USA). Ventilation was adjusted to maintain PCO_2 , PO_2 , and pH at physiological ~ 30 mmHg, >85 mmHg, and 7.4 respectively.

The left kidneys of newborn pigs were exposed retroperitoneally through flank incisions to allow clear access to the renal pedicles. To determine total RBF, a left renal artery was carefully separated from the vein and connective tissues. A flow probe (Transonic Systems, Inc.,

Ithaca, NY, USA) was then placed around the main renal artery and connected to a T206 dual channel small animal flow meter (Transonic Systems). Regional RBF in the pigs was measured using laser Doppler flowmetry (LDF). A laser Doppler probe (PF 407) connected to a holder (Perimed, Jarfalla, Sweden) was placed on the kidney surface to measure cortical blood flow (CBF). Medullary blood flow (MBF) was measured using a needle probe (Perimed) inserted into the kidney to a depth of ~ 8 mm. The laser Doppler probes were connected to a flow meter (Periflux 4001, Perimed). After each experiment, the position of the intrarenal probe tip was confirmed by dissecting the kidneys. CBF and MBF measurements were obtained as laser Doppler perfusion unit (PU). Laser Doppler probes were calibrated with a standard calibration device using a motility standard (Perimed) such that 1 PU corresponds to an analog output of 10 mV. Data were acquired and analyzed using a PowerLab data acquisition system and LabChart software (ADInstrument).

Figure 1 Newborn pig renal afferent arterioles and arteriolar smooth muscle cells express UTR protein. (A) Western immunoblotting detected unglycosylated (~ 40 kDa) and glycosylated (~ 60 kDa) forms of UTR in newborn pig renal afferent arterioles. (B) Immunofluorescence staining demonstrating plasma membrane localization of UTR in smooth muscle cells isolated from newborn pig renal afferent arterioles. Negative controls prepared using normal rabbit (Rb) IgG did not show fluorescence. Bar = $10\ \mu\text{m}$.

Table 1 Basal values of MAP and renal hemodynamic parameters measured in newborn pigs

	Mean $\pm$ S.E.M.	n
MAP (mmHg)	80.2 $\pm$ 2.5	26
RBF (ml/min)	12.0 $\pm$ 0.8	22
RVR (mmHg/ml per min)	7.1 $\pm$ 0.4	22
CBF (LDF PU)	*312.8 $\pm$ 12.2	26
MBF (LDF PU)	140.4 $\pm$ 10.6	26

* $P < 0.05$, compared with MBF.

Chemicals

Unless otherwise stated, all reagents were purchased from Sigma Chemical. Human UII (hUII), urantide, U-73122, 2-APB, and ketamine/xylazine were purchased from California Peptide, Inc. (Napa, CA, USA), Peptide International (Louisville, KY, USA), Santa Cruz (Santa Cruz Biotechnology, Inc.), Cayman Chemicals (Ann Arbor, MI, USA), and Butler Schein Animal Health Supply (North Dublin, OH, USA) respectively.

Statistical analysis

All data are expressed as mean $\pm$ S.E.M. Statistical significance was determined using Student's *t*-tests for paired or unpaired data and ANOVA with Student–Newman–Keuls

for multiple comparisons tests. A *P* value < 0.05 was considered significant.

Results

Newborn pig renal afferent arterioles and arteriolar smooth muscle cells express UTR protein

Western blot analysis of neonatal afferent arterioles using a polyclonal rabbit anti-UTR antibody detected ~ 40 and ~ 60 kDa immunoreactive bands corresponding to the approximate molecular weights of unglycosylated and glycosylated UTR respectively (Fig. 1A). Immunofluorescence staining of smooth muscle cells isolated from neonatal pig afferent arterioles also revealed membrane localization of UTR in cells probed with rabbit anti-UTR antibody but not normal rabbit IgG (Fig. 1B). These data confirm that UTR protein is expressed in neonatal renal afferent arterioles and localized in the plasma membrane of afferent arteriolar smooth muscle cells.

hUII elevates MAP and RVR but attenuates RBF in newborn pigs

Basal MAP, RBF, and RVR in anesthetized newborn pigs are shown in Table 1. Cumulative intrarenal arterial bolus

Figure 2

hUII elevates MAP and RVR but attenuates RBF in newborn pigs. (A and B) Exemplar tracings illustrating the effects of hUII on newborn pig MAP and RBF respectively. (C, D and E) Mean ($\pm$ S.E.M.) data showing that hUII dose dependently increased MAP ($n=6$), attenuated RBF ($n=4$), and elevated RVR ($n=4$) in newborn pigs and that urantide (20 μ g/kg)

inhibited these effects. hUII and urantide were co-administered to the piglets ~ 10 min after intrarenal arterial bolus injection of urantide. # $P < 0.05$ when compared with basal values; * $P < 0.05$ when compared with hUII-induced responses.

injection of hUII dose dependently increased MAP in newborn pigs (Fig. 2A and C). To determine the effect of hUII on newborn pig RVR, we first measured RBF. As illustrated in Fig. 2B and D, hUII dose dependently reduced RBF in newborn pigs. RVR, calculated as a ratio of MAP:RBF was increased by hUII in the piglets (Fig. 2E). These findings indicate that intrarenal arterial administration of hUII elevates MAP and RVR and reduces RBF in newborn pigs.

hUII reduces cortical but increases medullary perfusion in newborn pigs

To examine whether there is a regional variation in hUII-induced renal hemodynamic changes in newborn pigs, we measured blood perfusion in cortical and medullary regions of newborn pig kidneys. Basal CBF was ~55% more than MBF in newborn pigs (Table 1). hUII dose dependently decreased CBF in newborn pigs (Fig. 3A and C). In contrast, hUII elevated MBF in the pigs (Fig. 3B and D). These data indicate that hUII induces region-selective regulation of renal perfusion in newborn pigs.

UTR mediates renal hemodynamic effects of hUII in newborn pigs

To test the hypothesis that UTR mediates renal hemodynamic effects of hUII in newborn pigs, we studied the effect of urantide, a selective UTR antagonist on hUII-induced renal hemodynamic changes in piglets. Urantide did not alter RBF, RVR, CBF, or MBF in newborn pigs (Table 2). However, urantide significantly reduced MAP by ~4 mmHg in the piglets (Table 2). Furthermore, urantide attenuated hUII-induced pressor effect and renal hemodynamic changes in newborn pigs (Figs 2 and 3). These findings suggest that inhibition of UTR reduces MAP in newborn pigs. Our data also indicate that UTR mediates hUII-induced pressor effect and renal hemodynamic changes in the piglets.

Type 1 AngII receptors are not involved in hUII-induced pressor effect and renal hemodynamic changes in newborn pigs

Next, we investigated whether AngII system contributes to renal hemodynamic responses of newborn pigs to hUII. Losartan, a selective type 1 AngII (AT₁) receptor antagonist, did not alter basal RBF, RVR, CBF, or MBF but significantly reduced MAP by ~6 mmHg in newborn pigs (Table 2). Losartan essentially abolished AngII-induced

Figure 3

hUII reduces cortical but increases medullary perfusion in newborn pigs. (A and B) Representative tracings showing the effect of hUII on newborn pig CBF and MBF respectively. (C and D) Mean ($\pm$ S.E.M.) data illustrating that hUII dose dependently reduced CBF but increased MBF in newborn pigs and that urantide (20 μ g/kg) attenuated these effects. hUII and urantide were co-administered to the piglets ~10 min after intrarenal arterial bolus injection of urantide. $n=6$ each; $\#P<0.05$ when compared with basal values; $*P<0.05$ when compared with hUII-induced responses.

pressor effect and renal hemodynamic changes in newborn pigs (Table 3). In contrast, losartan did not alter pressor effect and renal hemodynamic responses to hUII in the piglets (Fig. 4A, B, C, D and E). Collectively, our data indicate that hUII-induced pressor effect and renal hemodynamic changes in newborn pigs are independent of AT₁ receptors.

Activation of the phospholipase C/inositol 1,4,5 trisphosphate pathway contributes to hUII-induced pressor effect and renal hemodynamic changes in newborn pigs

To test the hypothesis that phospholipase C (PLC)/inositol 1,4,5 trisphosphate (IP₃)-mediated renal vasoconstriction contributes to hUII-induced pressor effect and renal hemodynamic changes in newborn pigs, we studied the effect of U-73122, a PLC inhibitor and 2-APB, an IP₃ receptor (IP₃R) antagonist on hUII-induced MAP elevation

Table 2 MAP and renal hemodynamic responses to urantide (20 µg/kg), losartan (1 mg/kg), U-73122 (10 µg/kg), and 2-APB (2 mg/kg) in newborn pigs. Data are expressed as mean ± S.E.M.

	ΔMAP (mmHg)	ΔRBF (ml/min)	ΔRVR (mmHg/ml per min)	ΔCBF (LDF PU)	ΔMBF (LDF PU)
Urantide	*−3.5 ± 0.9 (n=6)	−0.1 ± 0.1 (n=4)	0.2 ± 0.2 (n=4)	−5.3 ± 5.0 (n=6)	1.5 ± 1.2 (n=6)
Losartan	*−5.5 ± 1.2 (n=13)	0.1 ± 0.1 (n=12)	−0.8 ± 0.4 (n=12)	6.0 ± 3.5 (n=13)	−1.9 ± 1.5 (n=13)
U-73122	−0.6 ± 0.6 (n=5)	0.1 ± 0.1 (n=5)	−0.3 ± 0.2 (n=5)	−2.3 ± 1.9 (n=5)	2.9 ± 1.9 (n=5)
2-APB	−1.4 ± 1.4 (n=6)	0.2 ± 0.2 (n=4)	−0.5 ± 0.4 (n=4)	−7.4 ± 4.0 (n=6)	1.6 ± 1.1 (n=5)

**P* < 0.05, compared with basal values.

and renal hemodynamic changes in piglets. U-73122 did not alter basal MAP, RBF, RVR, CBF, and MBF in newborn pigs (Table 2). However, U-73122 inhibited hUII-induced elevation in MAP and RVR (Fig. 5A and B). U-73122 also attenuated hUII-induced decrease in RBF and CBF but not hUII-induced increase in MBF (Fig. 5C, D and E). These data suggest that PLC activation is involved in hUII-induced pressor effect and renal vasoconstriction in newborn pigs.

Similarly, 2-APB did not alter basal MAP, RBF, RVR, CBF, and MBF in newborn pigs (Table 2). However, 2-APB attenuated hUII-induced MAP and RVR elevations, RBF and CBF reductions, but not MBF increase (Fig. 6A, B, C, D and E). These findings signify that IP₃Rs play a role in pressor and renal vascular effects of hUII in the piglets. Taken together, our data suggest that the PLC/IP₃ pathway contributes to hUII-induced pressor effect and alterations in RBF, RVR, and CBF but not MBF in newborn pigs.

Discussion

We present new findings in this study indicating that UII-induced activation of renal vascular UTR regulates blood pressure and regional RBF in neonatal pigs. hUII elevates MAP and RVR and attenuates total RBF in newborn pigs. hUII also reduces CBF but elevates MBF in the piglets. Furthermore, our data indicate that hUII-induced MAP elevation and renal hemodynamic changes in newborn pigs are independent of AT₁ receptors but involve the PLC/IP₃ signaling pathway.

Table 3 MAP and renal hemodynamic responses of newborn pigs to AngII in the absence and presence of losartan (1 mg/kg). Data are expressed as mean ± S.E.M. AngII and losartan were co-administered to the piglets ~10 min after intrarenal arterial bolus injection of losartan

	ΔMAP (mmHg)	ΔRBF (ml/min)	ΔRVR (mmHg/ml per min)	ΔCBF (LDF PU)	ΔMBF (LDF PU)
AngII	22.4 ± 3.0	−5.0 ± 0.8	18.8 ± 4.8	−90.4 ± 17.4	−15.5 ± 7.2
Losartan + AngII	*1.6 ± 0.8	*−0.2 ± 0.2	*0.3 ± 0.2	*−1.2 ± 3.5	*2.3 ± 1.1

**P* < 0.05, compared with AngII; n=7, each.

UII activity is mediated by UTR, a G protein-coupled receptor (GPCR; Ames *et al.* 1999, Liu *et al.* 1999). Both unglycosylated (~42 kDa) and glycosylated (~60 kDa) forms of UTR protein have been identified in human adrenocortical tissues and COS-7 cells overexpressing UTR (Boucard *et al.* 2003, Giuliani *et al.* 2009). Ventricular samples from rats express the unglycosylated form of UTR, whereas the glycosylated form was the only immunoreactive UTR protein identified in rat kidneys, coronary arteries, and cultured coronary artery smooth muscle cells (Gong *et al.* 2004, Abdel-Razik *et al.* 2008b, Dominguez-Rodriguez *et al.* 2012). In this study, we found that newborn pig renal afferent arterioles express both unglycosylated and glycosylated forms of UTR. Thus, the expression pattern of UTR protein isoforms may vary between species and tissues. Our findings also confirm that similar to previous reports in adult rats (Ashton 2006, Song *et al.* 2006), UTR are expressed in neonatal pig renal vessels.

Previous studies examining renal actions of UII in mature animals have yielded inconsistent findings. For example, intrarenal arterial infusion of hUII did not alter MAP but increased RBF, GFR, UV, and U_{Na}V in rats (Zhang *et al.* 2003). In contrast, i.v. injection of rUII reduced blood pressure, RBF, GFR, UV, U_{Na}V, and U_KV in rats (Song *et al.* 2006). I.v. injections of hUII also decreased blood pressure, RVR, and GFR but did not alter RBF and U_{Na}V in rats (Ovcharenko *et al.* 2006). These conflicting reports have been attributed to several factors, including route of administration, dosage, and source of UII (Ashton 2006,

Figure 4

hUII-induced pressor effect and renal hemodynamic changes in newborn pigs are independent of AT₁ receptors. (A, B, C, D and E) Mean (±S.E.M.) data illustrating that losartan (1 mg/kg) did not alter hUII (100 ng/kg)-induced MAP elevation and renal hemodynamic changes in newborn pigs. hUII and losartan were co-administered to the piglets ~10 min after intrarenal arterial bolus injection of losartan. *n* = 7 each; #*P* < 0.05 when compared with basal values; NS, not significant.

Zoccali & Mallamaci 2008, Ross *et al.* 2010, Tsoukas *et al.* 2011). However, the cyclic hexapeptide sequence in UII carboxyl-terminus, which is responsible for its biological activity, is highly conserved between species (Itoh *et al.* 1987, Conlon *et al.* 1990, Coulouarn *et al.* 1998, Douglas *et al.* 2004). Hence, it is unlikely that the physiological function of UII is dependent on its source (Douglas *et al.* 2000).

Data presented here indicate that intrarenal arterial administration of hUII elevates RVR and MAP in newborn pigs. However, a previous study showed that intrarenal arterial infusion of hUII did not alter MAP in adult rats (Zhang *et al.* 2003). These conflicting findings could be due to species differences and/or animal age. Urantide induced a small but significant decrease in MAP in newborn pigs, suggesting that endogenous UII regulates blood pressure in the piglets. However, urantide did not alter basal RBF, RVR, and regional renal perfusion in the piglets. These results raise the possibility that the hypotensive effect of urantide may be due to its systemic but not localized renal vasoregulatory actions. Neurons of the brain and spinal cord express UII (Coulouarn *et al.* 1998, Douglas *et al.* 2004, Ross *et al.* 2010). Studies have also shown that UII can modulate cardiovascular homeostasis via central neural mechanisms (Gibson *et al.* 1986,

Lu *et al.* 2002, Lin *et al.* 2003). However, the expression and function of UII system in renal plexus have not been characterized. Hence, future studies are needed to explore whether hUII alters neonatal blood pressure and renal hemodynamics via regulation of renal sympathetic nerve activity.

Renal perfusion exhibits considerable regional heterogeneity. Changes in renal CBF are not always replicated in the medullary or papillary regions because medullary hemodynamics can be regulated independently of renal CBF (Pallone *et al.* 1990, Navar *et al.* 1996, Mattson 2003). Vasoactive agents, including AngII, dopamine, arginine vasopressin, atrial natriuretic peptide, and bradykinin have been demonstrated to induce renal region-specific hemodynamic changes (Takezawa *et al.* 1987, Pallone *et al.* 1990, Nobes *et al.* 1991, Mattson & Cowley 1993, Heyman *et al.* 1995, Badzyska *et al.* 2002, Igbe *et al.* 2012). However, the effect of UII on regional RBF was unclear. In this study, we show that hUII-induced activation of UTR dose dependently reduced CBF but elevated MBF in newborn pigs, suggesting that hUII differentially regulates neonatal regional renal perfusion. Although hUII-induced elevation in MBF in the pigs occurred at the highest dose tested, it is likely that the medullary actions of UII result in localized vasodilation. Endogenous mediators of renal vasodilation, including nitric oxide (NO) and vasodilatory PGs, exert larger vasoregulatory

Figure 5

PLC activation mediates hUII-induced pressor effect and renal hemodynamic changes in newborn pigs. (A, B, C and D) Mean (±S.E.M.) data signifying that hUII (100 ng/kg)-induced changes in MAP, RBF, RVR, and CBF were inhibited by U-73122 (10 μg/kg) in newborn pigs. (E) Mean (±S.E.M.) data illustrating that U-73122 did not alter hUII-induced MBF elevation in newborn pigs. hUII and U-73122 were co-administered to the piglets ~10 min after intrarenal arterial bolus injection of U-73122. *n* = 5 each; #*P* < 0.05 when compared with basal values; **P* < 0.05 when compared with hUII-induced responses; NS, not significant.

Figure 6

IP₃R activation contributes to hUII-induced pressor effect and renal hemodynamic changes in newborn pigs. (A, B, C and D) Mean ($\pm$ S.E.M.) data showing that hUII (100 ng/kg)-induced changes in MAP ($n=6$), RBF ($n=4$), RVR ($n=4$), and CBF ($n=6$) were attenuated by 2-APB (2 mg/kg) in newborn pigs. (E) Mean ($\pm$ S.E.M.) data demonstrating that hUII-induced MBF ($n=5$) was unaltered by 2-APB in newborn pigs. hUII and 2-APB were co-administered to the piglets ~10 min after intrarenal arterial bolus injection of 2-APB. [#] $P<0.05$ when compared with basal values; ^{*} $P<0.05$ when compared with hUII-induced responses; NS, not significant.

effects in renal medulla than the cortex (Navar *et al.* 1996). UII stimulates NO, prostacyclin, and PGE₂ release in rat coronary arteries (Ishihata *et al.* 2005). UII also elevated NO production in rat renal artery endothelium (Zhang *et al.* 2003). Moreover, the physiological activity of NO and vasodilatory PGs are elevated in fetal and newborn kidneys compared with adults (Hook & Bailie 1979, Toth-Heyn *et al.* 2000). Conceivably, hUII elevates MBF in newborn pigs via localized generation of NO and/or vasodilatory PGs in the medulla. However, these possibilities require further investigations.

Studies have suggested that a functional cross talk exists between UII and AngII systems. UII-induced elevation in myocardial distensibility and negative inotropic and lusitropic effects in rabbits were inhibited by losartan (Fontes-Sousa *et al.* 2009). Furthermore, AngII-induced vasoconstriction was elevated in hUII-pretreated rat aortic segments (Wang *et al.* 2007). Data here show that at the dose that essentially abolished AngII-induced hypertension and renal hemodynamic changes, losartan did not alter pressor and renal hemodynamic responses of newborn pigs to hUII, indicating that renal actions of UII in newborn pigs are independent of AT₁ receptors.

In vascular smooth muscle cells, activation of PLC-coupled GPCRs by a wide variety of vasoconstrictors stimulates phosphoinositide hydrolysis, resulting in generation of IP₃ (Berridge 1993). IP₃ binds to

sarcoplasmic reticulum (SR)-localized IP₃Rs leading to Ca²⁺ release from SR Ca²⁺ store, an elevation in intracellular Ca²⁺, and vasoconstriction (Berridge 1993, Sanders 2001). In cerebral and mesenteric arteries, IP₃-mediated vasoconstriction can also occur independently of SR Ca²⁺ release and due to a molecular and functional coupling between smooth muscle cell type 1 IP₃R (IP₃R1) and canonical transient receptor potential 3 (TRPC3) channels (Xi *et al.* 2008, Adebiyi *et al.* 2010, 2012). However, it is unclear whether a coupling exists between IP₃R1 and TRPC3 channels in renal vascular smooth muscle cells. hUII constricts rabbit aortic smooth muscle by stimulating PLC-dependent elevation in inositol phosphates, suggesting that PLC/IP₃-mediated intracellular Ca²⁺ elevation contributes to hUII-induced vasoconstriction (Saetrum *et al.* 2000). Here, U-73122, a PLC inhibitor, and 2-APB, an IP₃R antagonist, attenuated hUII-induced MAP and RVR elevations, RBF and CBF reductions, but not MBF increase. These data suggest that PLC/IP₃-mediated renal vasoconstriction is involved in hUII-induced neonatal renal hemodynamic changes. Of note, 2-APB can also block store-operated Ca²⁺ (SOC) channels (Dobrydneva & Blackmore 2001, Bootman *et al.* 2002). Given that UII also induces vasoconstriction by activating store-operated Ca²⁺ entry (Dominguez-Rodriguez *et al.* 2012), SOC channel blockade may contribute to the inhibitory effect of 2-APB on hUII-induced neonatal renal hemodynamic changes.

In summary, data from our study indicate that UII regulates renal hemodynamics in neonatal pigs. Intrarenal arterial bolus administration of hUII elevates RVR and MAP but decreases total RBF in newborn pigs. In addition, hUII reduces CBF but increases MBF in the piglets. We also propose that hUII-induced activation of renal vascular UTR stimulates PLC/IP₃-mediated vasoconstriction, leading to an increase in RVR and MAP and a decrease in RBF and CBF in newborn pigs.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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