Maternal Protein Restriction Reduces Angiotensin II AT₁ and AT₂ Receptor Expression in the Fetal Rat Kidney

Saleh H. Alwasel^a Irram Kaleem^b Vandana Sahajpal^b Nick Ashton^b

^aDepartment of Zoology, College of Science, King Saud University, Riyadh, Saudi Arabia; ^bFaculty of Life Sciences, University of Manchester, Manchester, UK

Key Words

Angiotensin II receptor · Maternal low protein · Developmental programming

Abstract

Maternal dietary protein restriction during pregnancy results in an increase in offspring blood pressure in the rat. The kidneys of the low protein (LP) rat have fewer nephrons, increased hemodynamic sensitivity to angiotensin II and lower glomerular filtration rate, suggesting altered activity of the renin-angiotensin system. Angiotensin II plays a role in nephrogenesis through the AT₁ and AT₂ receptor subtypes. The aim of this study was to determine expression levels of both subtypes during nephrogenesis. Pregnant Wistar rats were fed either a control 18% protein diet or a low 9% protein (LP) diet. A 35% reduction in nephron number ($p < 0.05$) associated with a 50% reduction in total glomerular volume ($p < 0.001$) was seen in LP rats. Renal AT₁ ($p < 0.0001$) and AT₂ ($p < 0.0001$) receptor protein expression were significantly lower in LP rats from E18 to day 10. AT₁ expression in LP rat kidneys tended to increase over time while AT₂ expression declined until day 10, when it began to increase again. Angiotensin II-regulated cell proliferation may be perturbed in the LP rat kidney during nephrogenesis which could contribute to the reduction in nephron number and the elevation in blood pressure observed in this model of programmed hypertension.

Copyright © 2010 S. Karger AG, Basel

Introduction

Numerous epidemiological studies have shown an association between poor nutrition during pregnancy and an increased risk of developing cardiovascular disease in adulthood [1]. Similarly, in the laboratory rat poor nutrition in the form of modest protein restriction during pregnancy results in the development of hypertension in the offspring [2]. This phenomenon has become known as the developmental programming of cardiovascular disease.

The kidney is central to the long-term regulation of blood pressure and appears to be susceptible to the effects of programming in utero. In man, low birth weight is associated with a reduction in nephron number [3] and increased risk of end-stage renal disease associated with hypertension, diabetes and cardiovascular disease [4]. Ultrasound studies have shown that the kidneys of intra-uterine growth restricted fetuses are vulnerable to growth failure [5] and have an immature pattern of renin distribution within the renal cortex [6]. Rats exposed to a low protein (LP) diet in utero have proportionally smaller kidneys [7] in which the total number of nephrons is reduced by 30–40% [7–9].

Several studies have shown that the renin-angiotensin system is perturbed in the LP rat model. Renal renin levels are reduced at birth [8]; however, by weaning renin, angiotensin II (Ang II) and aldosterone concentrations

are restored to control levels [10]. Furthermore, renal Ang II AT₁ [9] and AT₂ [10] receptor expression is increased, leading to increased glomerular Ang II binding [10]. In vivo, the increased Ang II receptor expression results in greater haemodynamic sensitivity to exogenous Ang II [9]. Taken together, these data suggest that the renin-angiotensin system of the LP rat is suppressed at birth and then becomes overactive at weaning.

A number of workers have assessed the impact of exposure to an adverse intrauterine environment on renal angiotensin II receptor expression in the rat; however, these studies have focused on animals in which nephrogenesis was complete [9, 11–13]. In contrast, less is known about the programming of AT₁ and AT₂ expression during nephrogenesis in the rat [14]. Ang II plays an important role in regulating growth of the developing kidney through both the AT₁ and AT₂ receptor subtypes [15]. Both subtypes are expressed as early as E14 [16], but the AT₂ receptor is more abundant until birth at 22 days. It is found in the nephrogenic regions of the developing metanephros [16] where it regulates cell proliferation by stimulating apoptosis [17]. The AT₁ subtype is confined to the more mature regions of the metanephros [16] where it stimulates proliferation of a number of cell types, including glomerular endothelial cells [18] and mesangial cells [19]. Hence changes in the expression of either receptor subtype may potentially contribute to the reduction in nephron number or altered function of the LP rat kidney. Accordingly, the aim of this study was to quantify AT₁ and AT₂ receptor expression in fetal and neonatal LP rat kidneys. We also assessed kidney morphology and glomerular number and dimensions to determine whether changes in angiotensin receptor expression are associated with altered renal morphology.

Methods

All experiments were performed in accordance with the UK Animals (Scientific Procedures) Act 1986 and received local ethical approval.

Animals

Wistar rats (Harlan UK Ltd., Belton, UK) were paired in individual breeding cages and held in a room at 22–24°C with a 12 h light:12 h dark cycle. As soon as mating was confirmed by the presence of a plug, the female's diet was switched from standard chow (Rat & Mouse Standard Diet; Bantin & Kingman Ltd., Hull, UK) to one which contained either 9% protein (LP) or 18% protein (control). The semi-synthetic diets were formulated as described previously [9]. Those dams which proceeded to term were switched back to the standard rat chow immediately after birth. Animals were weighed on a daily basis throughout pregnancy.

As the primary experimental manipulation was of the dams rather than the pups, offspring were sampled from 4–7 litters in each of the methodologies described below. For Western blotting it was necessary to pool tissue from fetuses in the same litter; however, for postnatal animals, no more than 2 rats per litter were studied. Kidneys were harvested from male fetal and neonatal rats at embryonic days E18, E19 and E21, where E0 is taken as the day that the sperm plug was found, and postnatal days 5, 10 and 28. Pregnant dams or neonates were killed by cervical dislocation. Kidneys were then rapidly removed from each fetus or neonate and either snap frozen in liquid nitrogen and stored at –80°C or fixed in neutral-buffered formalin (4% formaldehyde, 35 mM sodium phosphate, monobasic and 45 mM sodium phosphate, dibasic) or minced for the calculation of glomerular number.

Renal AT₁ and AT₂ Receptor Expression

AT₁ and AT₂ receptor expression were determined by Western analysis. Fetal and neonatal kidneys were harvested from control (n = 4–7 litters) and LP (n = 4–7 litters) rats at E18, E19, E21, D5 and D10. Each sample is representative of a single litter; however, it was necessary to pool the kidneys from littermates to yield sufficient protein per sample for each litter (number of pooled kidneys per sample was E18: 8, E19: 6, E21: 4, D5: 2, D10: 2). Membranes were isolated by homogenization and centrifugation, as described previously [9]. The protein concentration of the resuspended membrane pellet was determined using the Bradford method (Bio-Rad Assay Reagent; Bio-Rad Laboratories, Hercules, Calif., USA). 50 µg of tissue homogenate were then denatured at 80°C for 5 min and fractionated using an 8% SDS-polyacrylamide gel. Equality of protein loading was confirmed by staining with Coomassie blue prior to incubation with the primary antibody. Blots were incubated with primary antibody (1:300, rabbit polyclonal anti-AT₁ (N-10) or goat polyclonal anti-AT₂ (C-18); Santa Cruz Biotechnology, Inc., Santa Cruz, Calif., USA), horseradish peroxidase conjugated anti-rabbit secondary antibody (1:5,000) and developed with the ECL plus detection kit (Amersham Pharmacia Biotech UK Ltd., Little Chalfont, UK) as described previously [9]. Negative controls were performed by omitting the primary antibody. Immunoreactivity was quantified by densitometry and normalized to the control bands.

Renal Morphology

Glomerular number was determined using the acid digestion method [20], as described previously [9]. Briefly, kidneys from day 28 control (n = 7 from n = 5 litters) and LP (n = 7 from n = 5 litters) rats were minced and digested in 1 M HCl for 4 h. The kidney homogenate was transferred to 50 ml deionized water, macerated mechanically and mixed for 8–10 h to produce a suspension containing isolated, intact glomeruli. The number of glomeruli present in 40 µl of the suspension was counted in 20 replicates per kidney. Inter- and intra-assay variabilities were 11.4 and 13.5%, respectively.

Semiquantitative estimates of glomerular dimensions (diameter and volume) were determined in formalin-fixed kidneys from control (n = 6 from n = 5 litters) and LP rats (n = 6 from n = 5 litters) at day 28, according to the method of Souster and Emery [21]. The kidneys were halved longitudinally and embedded in paraffin wax. Sections were cut at 4 µm and stained with hematoxylin and eosin. In order to determine glomerular diameter, 10 sections per kidney were examined: these sections were taken in

a systematic manner from the mid-portion of each kidney, sampling at every 5th section cut. For each section, 10 outer cortical glomeruli were chosen at random for measurement of the renal corpuscle diameter across its centre using a calibrated eyepiece. Images were collected on an Axioskop upright microscope using 10×/0.50 Fluor and 20×/0.75 Plan Apochromat objectives and captured using a Axiocam colour CCD camera through Axiovision software.

From glomerular diameter (μm) measurements, the individual glomerular volume and total glomerular volume were estimated:

Individual glomerular volume ($10^6 \mu\text{m}^3$) = $((r^3 \times \pi) \times 4/3)$.
Total glomerular volume (mm^3) = $((r^3 \times \pi) \times 4/3) \times$ estimated glomerular number.

Statistical Analysis
All data are presented as the mean ± SEM. Statistical analysis was by t test, repeated measures or one-way ANOVA followed by Duncan's test, as appropriate. The association between AT₁/AT₂ receptor expression and age in the LP rat was tested using a 2-tailed Pearson correlation coefficient. Significance was ascribed at the 5% level (SPSS for Windows, version 16.0; SPSS UK Ltd., Surrey, UK).

Results

Maternal and Pup Growth
Maternal body weights at day 1 of gestation were comparable between control and low protein-fed dams (table 1). The pattern of maternal weight gain was similar between the two groups of dams throughout gestation (fig. 1); hence, there was no difference in maternal body weight at day 22 (table 1). Consistent with these data, maternal food intake did not differ between dams. Length of gestation (table 1) and litter sizes (table 2) did not differ between control and low protein-fed dams. LP pups were significantly heavier ($p < 0.01$) than their control counterparts at E18–E21, but by day 1 their growth had slowed such that they were lighter than control pups ($p < 0.05$). The LP pups subsequently underwent catch-up growth and were of a similar weight to that of controls by day 10 (table 2).

Renal AT₁ and AT₂ Receptor Expression
The polyclonal anti-AT₁ receptor antibody detected a protein band at 45 kDa. AT₁ receptor expression (fig. 2) was significantly lower in LP rats across the ages studied ($F_{9,42} = 4.96$, $p < 0.001$). LP AT₁ receptor expression tended to increase over time, although this pattern did not achieve statistical significance in a correlation analysis (Pearson correlation, $r = 0.48$, $p = 0.1$). The lowest relative expression was observed at E19 ($29.4 \pm 8.2\%$ of the aver-

Fig. 1. Maternal body weight gain during pregnancy in control (C) and low protein (LP) rats (n = 12 per group). Data are shown as the mean ± SEM. Statistical comparisons were by repeated-measures ANOVA.

Table 1. Maternal weight and food intake by dams fed a control (n = 12) or low protein (n = 12) diet during pregnancy

	Control diet	LP diet
Maternal weight day 1, g	249.1 ± 8.4	253.8 ± 4.4
Maternal weight day 22, g	334.9 ± 13.0	330.45 ± 13.4
Maternal weight gain, g/24 h	6.4 ± 0.2	6.0 ± 0.2
Maternal food intake, g/24 h	17.7 ± 0.7	17.3 ± 0.6
Length of gestation, days	22.5 ± 0.2	22.3 ± 0.3

Data are shown as the mean ± SEM. Statistical comparisons were by independent samples t test.

Table 2. Litter sizes and weights of control (C, n = 5–12) and low protein (LP, n = 5–12) rats

Age	Litter size		Mean pup weight, g	
	C	LP	C	LP
E18	13.5 ± 0.3	12.1 ± 0.5	1.15 ± 0.01	1.94 ± 0.06**
E19	15.2 ± 0.9	13.3 ± 0.8	1.76 ± 0.02	2.84 ± 0.04**
E21	12.5 ± 1.2	14.6 ± 1.8	2.92 ± 0.06	3.37 ± 0.1**
Day 1	13.1 ± 0.5	12.6 ± 0.5	5.6 ± 0.1	5.1 ± 0.2*
Day 5	–	–	15.3 ± 0.6	11.4 ± 0.5*
Day 10	–	–	17.0 ± 0.7	13.2 ± 0.9
Day 28	–	–	99.4 ± 1.6	103.5 ± 1.6

Data are shown as the mean ± SEM. Statistical comparisons were by independent samples t test. * $p < 0.05$, ** $p < 0.01$ control vs. LP at the same gestational age.

Fig. 2. a Typical immunoblots for the AT₁ receptor in the kidney of control (C) and low protein (LP) rats. Receptor expression is shown at E18, E19, E21, day 5 and day 10. **b** Renal AT₁ receptor expression in control (open bars, n = 4–7 pooled kidney samples) and LP rats (solid bars, n = 4–7 pooled kidney samples) at E18, E19, E21, day 5 and day 10. Data normalized to the control bands and presented as mean $\pm$ SEM. Statistical comparisons were by one-way ANOVA and Duncan's test. * $p < 0.05$ control vs. LP rats.

Fig. 3. a Typical immunoblots for the AT₂ receptor in the kidney of control (C) and low protein (LP) rats. Receptor expression is shown at E18, E19, E21, day 5 and day 10. **b** Renal AT₂ receptor expression in control (open bars, n = 4–7 pooled kidney samples) and LP rats (solid bars, n = 4–7 pooled kidney samples) at E18, E19, E21, day 5 and day 10. Data normalized to the control bands and presented as mean $\pm$ SEM. Statistical comparisons were by one-way ANOVA and Duncan's test. ** $p < 0.01$, *** $p < 0.001$ control vs. LP rats.

age control expression, $p = 0.03$) and the highest at postnatal day 5 ($72.2 \pm 1.5\%$ of the average control expression, $p = 0.04$).

The polyclonal anti-AT₂ receptor antibody detected a protein band at 45 kDa. AT₂ expression (fig. 3) was also significantly lower in LP rats across the ages studied

($F_{9,61} = 6.98$, $p < 0.001$). LP AT₂ receptor expression declined over time (Pearson correlation, $r = -0.502$, $p = 0.01$), with the highest relative expression observed at E18 ($76.9 \pm 2.7\%$ of the average control expression, $p < 0.001$) and the lowest at postnatal day 5 ($31.8 \pm 15.7\%$ of the average control expression, $p = 0.02$). This was followed by

Fig. 4. Hematoxylin and eosin-stained kidney sections from control and LP rats at day 28. There are no obvious morphological differences between the cortices of control (a) and LP (b) rat kidneys. Original magnification $\times 100$. Scale bar = 100 μm . Higher power ($\times 200$, scale bar = 50 μm) images show that the glomeruli of control (c) and LP (d) rats are similar in structure. Similarly, there are no differences in the gross structure of the medullae of control (e) and LP (f) kidneys. $\times 200$. Scale bar = 50 μm . G = Glomerulus; PCT = proximal convoluted tubule; DCT = distal convoluted tubule; tL = thin limbs of Henle's loop; CD = collecting duct.

Fig. 5. Estimated glomerular dimensions of control (open bars, $n = 7$) and LP (solid bars, $n = 7$) rat kidneys at 4 weeks of age. Number of glomeruli per gram kidney weight (**a**), diameter of glomeruli (**b**), individual glomerular volume (**c**), and total glomerular volume (**d**). Data shown as the mean $\pm$ SEM. Statistical comparisons were by independent samples t test. ** $p < 0.01$, *** $p < 0.001$ control vs. LP.

a significant increase in AT_2 expression at day 10 (day 5, $n = 4$, 31.8 ± 15.7 vs. day 10, $n = 7$, $71.4 \pm 8.1\%$, $p = 0.03$).

Renal Morphology

There was no difference in kidney weight (C 1.10 ± 0.02 , $n = 6$ vs. LP 1.15 ± 0.02 g, $n = 6$, $p = 0.1$) or kidney to body weight ratio (C 1.11 ± 0.02 vs. LP $1.11 \pm 0.01\%$, $p = 1.0$) between control and LP rats on day 28. There were no obvious differences in the gross morphology of kidney sections taken from control and LP rats at E19, E21 (data not shown) and day 28 (fig. 4). However, when the glomeruli were subject to quantification, several differences became apparent. Consistent with our earlier report [9], glomerular number per gram kidney weight was significantly lower in LP rats (fig. 5, $p < 0.01$). This was also the case for absolute glomerular number (C $68,168 \pm 5,151$, $n = 7$ vs. LP $47,262 \pm 1,291$ g, $n = 7$, $p < 0.001$). Estimated glomerular diameter ($p < 0.001$) and individual glomerular volume ($p < 0.001$) were also significantly smaller in LP rats; hence total glomerular volume of the LP rat kidney was 50% lower than that of control animals ($p < 0.001$).

Discussion

This study extends previous reports of angiotensin receptor expression at single time points during and after nephron development to show temporal changes in AT_1

and AT_2 expression throughout nephrogenesis in the LP rat. The main outcome is that both AT_1 and AT_2 expression in the LP rat kidney are reduced throughout the period of nephron development. Later, at 4 weeks of age AT_1 and AT_2 receptor expression are significantly increased in the LP rat kidney [9, 10], suggesting that there is an increase in angiotensin II receptor expression as the LP rat matures. In addition to changes in angiotensin receptor expression, we also observed that LP rats had a marked reduction in their total glomerular volume, in part due to a reduction in nephron number but also because the glomeruli were smaller. These two changes in the LP rat kidney may have arisen separately as a consequence of a shared environmental insult: nephrogenesis is regulated by a plethora of growth factors. Nonetheless, angiotensin II contributes to nephron formation via its two receptor subtypes, so altered AT_1 and AT_2 expression may play a role in the development of the LP rat kidney.

The AT_2 receptor is the predominant subtype in the fetal rat kidney. Both AT_1 and AT_2 receptors have been identified using RT-PCR as early as E14 [16, 22], although expression levels of AT_1 remain low until E19 [16, 22]. Relative expression studies have shown that the ratio of AT_2/AT_1 decreases in a log-linear fashion during maturation, from 6.7 on E14 to 0.2 on postnatal day 14 [22]. AT_1 mRNA increases 5-fold from E17 to postnatal day 5 [23], reaching a plateau at around postnatal day 10–20 [15]. In general, the AT_2 receptor is associated with areas of the

kidney undergoing growth and differentiation, whereas the AT₁ receptor is primarily found in more mature areas. Thus, at E14, the AT₂ receptor is found in undifferentiated mesenchyme, spreading to differentiating epithelial structures at E17 and the medulla, juxtamedullary glomeruli and the differentiating nephrogenic regions of the cortex at E20. The few AT₁ receptors present at E14 are also located in the undifferentiated mesenchyme, but by E17 they have migrated to the mature glomeruli in the deep cortex and at E20 they are found almost exclusively in the mature juxtamedullary and cortical glomeruli, with little or no expression in the nephrogenic regions [16].

As we used the relative expression levels of control rat AT₁ or AT₂ receptor within each individual gel to normalize the LP data, rather than a housekeeping marker such as β -actin, we are not able to compare relative AT₁/AT₂ expression or absolute changes over time which is a limitation of our study. However, we can comment on the overall pattern of change in AT₁ and AT₂ expression in LP kidneys relative to the expected temporal changes in control expression. Thus for the AT₂ receptor, which would have been declining over the period E18 to day 10 in the control rats, there was a proportionately greater reduction in expression in LP rats up to day 5. However, at day 10, AT₂ expression relative to control rats began to increase and by week 4 it was higher than that of control rats [9]. In contrast, the relative expression of the AT₁ receptor in the LP kidney showed a steady increase over time. AT₁ expression levels still remained lower than those of the control rats throughout, but later at week 4 AT₁ expression was significantly higher than that of controls [9, 10].

A somewhat different pattern of responses has been reported in the various ovine models of developmental programming, dependent in part upon the timing of the insult. Maternal dexamethasone treatment early in gestation, which leads to hypertension in the offspring, resulted in increased renal AT₁ and AT₂ mRNA in fetal lambs near term [24]. In contrast, maternal nutrient restriction during mid-gestation resulted in lower AT₁ expression whereas AT₂ was unaffected. Late-gestation nutrient restriction had no effect on either AT₁ or AT₂. Furthermore, nephron number was only reduced in males following late-gestation nutrient restriction [25]. A further study showed that intra-uterine growth restriction in late gestation has no effect on fetal kidney AT₁ and AT₂ mRNA expression, yet nephron number was reduced [26]. A number of factors may account for the differences in renal angiotensin receptor expression reported in the

LP rat herein and those in earlier studies in the sheep, including duration of the insult, the relative immaturity of the rat kidney at term compared with the sheep and other potential species differences.

The AT₂ receptor is thought to play a role in regulating cell proliferation and early differentiation of the nephron [15], as it has been shown to enhance apoptosis and has an antiproliferative action [17]. The current study reaffirms previous reports that the LP rat kidney has 30–40% fewer nephrons than that of control rats [7–9], which could reflect the observed reduction in AT₂ expression during the early stages of metanephros development. AT₂ null mice (*Agtr2*^{-/-}), for example, display a variety of kidney and urinary tract malformations which are preceded by delayed apoptosis of undifferentiated mesenchymal cells [27]. Welham et al. [28] have reported that maternal protein restriction is associated with increased apoptosis in mesenchymal cells at E13, before the AT₂ receptors normally appear, but there was no difference at E15. Later at 8 weeks of age, Vehaskari et al. [29] observed increased apoptosis without increased cell proliferation. It has been suggested that deregulation of apoptosis at key stages of nephrogenesis contributes to the reduction in nephron formation [28]. Hence the reduction in AT₂ receptor expression in LP rats could result in an altered pattern of apoptosis during development of the metanephros contributing to a reduction in nephrogenesis.

The AT₁ receptor has a dual role, early in nephron differentiation and later in the development of renal function [15]. It has been shown to stimulate proliferation of glomerular endothelial cells [18], mesangial cells [19], medullary thick ascending limb epithelia [30] and vascular smooth muscle [31]. Administration of the AT₁ receptor antagonist losartan to the pregnant rat dam between E15 and E20 induces renal abnormalities in the developing fetus [32]. Similarly, combined AT_{1A} and AT_{1B} null mice (*Agtr1*^{-/-}) display papillary atrophy and delayed glomerular growth, although those lacking AT_{1B} alone (*Agtr1b*^{-/-}) are phenotypically normal [33]. Postnatal AT₁ inhibition with losartan also reduces renal cell proliferation, leading to the development of fewer, smaller glomeruli which have shorter, thicker afferent arterioles [34]. The kidneys of LP rats did not show marked histological abnormalities typical of embryonic AT₁ inhibition, suggesting that the reduction in AT₁ expression observed during late gestation probably had little impact on nephrogenesis.

In addition to a reduction in the total number of glomeruli in LP rat kidneys, glomerular diameter and volume were also reduced. As a result, the total glomerular

volume of LP rat kidneys was 50% less than that of control animals. These observations contrast with those made in growth restricted rats [35] and in low birth weight babies [3] in which glomerular number was reduced but volume was increased. These differences may reflect a lack of compensatory enlargement in LP rats at 4 weeks of age: further study is required to determine whether glomerular volume increases in LP rats as they mature and blood pressure increases.

One other notable effect of maternal protein restriction was a change in the growth pattern of the fetal and neonatal LP rat. In common with an earlier report [36], fetal LP rats were heavier than their control counterparts from E18–E21. Increased maternofetal substrate transfer via a larger placenta has been proposed as an adaptive response to the reduction in amino acid availability during protein restriction in mid- to late gestation [36]. However, this placental adaptation does not appear to be able to provide sufficient nutrients during the rapid phase of growth immediately prior to term, as growth in LP rats slows, resulting in birth weights comparable with those in controls. Interestingly growth continued at a slower rate in the LP rats for the first 5–10 days after birth before catching up with control animals from day 10 onwards. The reason(s) for the delayed growth followed by a catch-up phase are unclear but it is interesting to note that catch-up growth is associated with accelerated senescence in both the kidneys and hearts of LP rats which has been linked to cardiovascular and renal disease [37].

In summary, this study has shown that both AT₁ and AT₂ receptor expression are reduced in the kidneys of LP rats during late gestation and in early postnatal life before nephrogenesis is complete. Both receptor subtypes show the expected temporal patterns of expression up to postnatal day 5; AT₁ receptors tend to increase whereas AT₂ receptors decline. However, at day 10 there was an upturn in AT₂ expression. By week 4, both AT₁ and AT₂ expression are higher in the LP rat [9, 10], suggesting that the period of fetal/early postnatal suppression is followed by an increase in expression. This is paralleled by suppression of other components of the renin-angiotensin system at birth [8] followed by an increase in renin activity at 4 weeks [9]. The reduction in AT₂ expression prior to day 10 in particular may contribute to the development of fewer nephrons by the LP rat; however, further work is required to establish a casual link between these two observations.

Acknowledgements

This work was supported by funds from the Fetal Programming of Disease Research Chair, King Saud University (SHW). V.S. was supported by an MRC studentship. The Bioimaging Facility microscopes used in this study were purchased with grants from BBSRC, Wellcome and the University of Manchester Strategic Fund. Special thanks goes to Robert Fernandez for help with the microscopy.

References

- 1 Barker DJ, Bagby SP: Developmental antecedents of cardiovascular disease: a historical perspective. *J Am Soc Nephrol* 2005;16: 2537–2544.
- 2 Langley SC, Jackson AA: Increased systolic pressure in adult rats induced by fetal exposure to maternal low protein diet. *Clin Sci* 1994;86:217–222.
- 3 Manalich R, Reyes L, Herrera M, Melendi C, Fundora I: Relationship between weight at birth and the number and size of renal glomeruli in humans: a histomorphometric study. *Kidney Int* 2000;58:770–773.
- 4 Hoy WE, Mathews JD, McCredie DA, Pugsley DJ, Hayhurst BG, Rees M, Kile E, Walker KA, Wang Z: The multidimensional nature of renal disease: rates and associations of albuminuria in an Australian Aboriginal community. *Kidney Int* 1998;54:1296–1304.
- 5 Konje JC, Bell SC, Morton JJ, de Chazal R, Taylor DJ: Human fetal kidney morphometry during gestation and the relationship between weight, kidney morphometry and plasma active renin concentration at birth. *Clin Sci* 1996;91:169–175.
- 6 Kingdom JC, Hayes M, McQueen J, Howatson AG, Lindop GB: Intrauterine growth restriction is associated with persistent juxta-medullary expression of renin in the fetal kidney. *Kidney Int* 1999;55:424–429.
- 7 Langley-Evans SC, Welham SJ, Jackson AA: Fetal exposure to a maternal low protein diet impairs nephrogenesis and promotes hypertension in the rat. *Life Sci* 1999;64:965–974.
- 8 Woods LL, Ingelfinger JR, Nyengaard JR, Rasch R: Maternal protein restriction suppresses the newborn renin-angiotensin system and programs adult hypertension in rats. *Pediatr Res* 2001;49:460–467.
- 9 Sahajpal V, Ashton N: Renal function and angiotensin AT₁ receptor expression in young rats following intrauterine exposure to a maternal low-protein diet. *Clin Sci (Lond)* 2003;104:607–614.
- 10 Sahajpal V, Ashton N: Increased glomerular angiotensin II binding in rats exposed to a maternal low protein diet in utero. *J Physiol* 2005;563:193–201.
- 11 Mao C, Wu J, Xiao D, Lv J, Ding Y, Xu Z, Zhang L: The effect of fetal and neonatal nicotine exposure on renal development of AT₁ and AT₂ receptors. *Reprod Toxicol* 2009;27: 149–154.
- 12 Samuelsson AM, Alexanderson C, Molne J, Haraldsson B, Hansell P, Holmang A: Prenatal exposure to interleukin-6 results in hypertension and alterations in the renin-angiotensin system of the rat. *J Physiol* 2006; 575:855–867.

- 13 McMullen S, Langley-Evans SC: Sex-specific effects of prenatal low-protein and carbenoxolone exposure on renal angiotensin receptor expression in rats. *Hypertension* 2005;46:1374–1380.
- 14 Singh RR, Cullen-McEwen LA, Kett MM, Boon WM, Dowling J, Bertram JF, Moritz KM: Prenatal corticosterone exposure results in altered AT₁/AT₂, nephron deficit and hypertension in the rat offspring. *J Physiol* 2007;579:503–513.
- 15 Aguilera G, Kapur S, Feuillan P, Sunar-Akbasak B, Bathia AJ: Developmental changes in angiotensin II receptor subtypes and AT₁ receptor mRNA in rat kidney. *Kidney Int* 1994;46:973–979.
- 16 Norwood VF, Craig MR, Harris JM, Gomez RA: Differential expression of angiotensin II receptors during early renal morphogenesis. *Am J Physiol* 1997;272:R662–R668.
- 17 Yamada T, Horiuchi M, Dzau VJ: Angiotensin II type 2 receptor mediates programmed cell death. *Proc Natl Acad Sci USA* 1996;93:156–160.
- 18 Wolf G, Ziyadeh FN, Zahner G, Stahl RA: Angiotensin II is mitogenic for cultured rat glomerular endothelial cells. *Hypertension* 1996;27:897–905.
- 19 Ray PE, Aguilera G, Kopp JB, Horikoshi S, Klotman PE: Angiotensin II receptor-mediated proliferation of cultured human fetal mesangial cells. *Kidney Int* 1991;40:764–771.
- 20 Hellmann H, Davis JM, Thurau K: Blood pressure and indices of glomerular filtration area in hypertensive and normotensive Prague rats. *Kidney Blood Press Res* 2000;23:385–392.
- 21 Souster LP, Emery JL: The sizes of renal glomeruli in fetuses and infants. *J Anat* 1980;130:595–602.
- 22 Garcia-Villalba P, Denkers ND, Wittwer CT, Hoff C, Nelson RD, Mauch TJ: Real-time PCR quantification of AT₁ and AT₂ angiotensin receptor mRNA expression in the developing rat kidney. *Nephron Exp Nephrol* 2003;94:e154–e159.
- 23 Tufro-McReddie A, Harrison JK, Everett AD, Gomez RA: Ontogeny of type 1 angiotensin II receptor gene expression in the rat. *J Clin Invest* 1993;91:530–537.
- 24 Moritz KM, Johnson K, Douglas-Denton R, Wintour EM, Dodic M: Maternal glucocorticoid treatment programs alterations in the renin-angiotensin system of the ovine fetal kidney. *Endocrinology* 2002;143:4455–4463.
- 25 Gilbert JS, Ford SP, Lang AL, Pahl LR, Drumhiller MC, Babcock SA, Nathanielsz PW, Nijland MJ: Nutrient restriction impairs nephrogenesis in a gender-specific manner in the ovine fetus. *Pediatr Res* 2007;61:42–47.
- 26 Zohdi V, Moritz KM, Bubb KJ, Cock ML, Wreford N, Harding R, Black MJ: Nephrogenesis and the renal renin-angiotensin system in fetal sheep: effects of intrauterine growth restriction during late gestation. *Am J Physiol Regul Integr Comp Physiol* 2007;293:R1267–1273.
- 27 Nishimura H, Yerkes E, Hohenfellner K, Miyazaki Y, Ma J, Hunley TE, Yoshida H, Ichiki T, Threadgill D, Phillips JA 3rd, Hogan BM, Fogo A, Brock JW 3rd, Inagami T, Ichikawa I: Role of the angiotensin type 2 receptor gene in congenital anomalies of the kidney and urinary tract, CAKUT, of mice and men. *Mol Cell* 1999;3:1–10.
- 28 Welham SJ, Wade A, Woolf AS: Protein restriction in pregnancy is associated with increased apoptosis of mesenchymal cells at the start of rat metanephrogenesis. *Kidney Int* 2002;61:1231–1242.
- 29 Vehaskari VM, Aviles DH, Manning J: Prenatal programming of adult hypertension in the rat. *Kidney Int* 2001;59:238–245.
- 30 Wolf G, Ziyadeh FN, Helmchen U, Zahner G, Schroeder R, Stahl RA: Ang II is a mitogen for a murine cell line isolated from medullary thick ascending limb of Henle's loop. *Am J Physiol* 1995;268:F940–F947.
- 31 Gibbons GH, Pratt RE, Dzau VJ: Vascular smooth muscle cell hypertrophy vs. hyperplasia: autocrine transforming growth factor- β 1 expression determines growth response to angiotensin II. *J Clin Invest* 1992;90:456–461.
- 32 Spence SG, Allen HL, Cukierski MA, Manson JM, Robertson RT, Eydeloth RS: Defining the susceptible period of developmental toxicity for the AT₁-selective angiotensin II receptor antagonist losartan in rats. *Teratology* 1995;51:367–382.
- 33 Oliverio MI, Kim HS, Ito M, Le T, Audoly L, Best CF, Hiller S, Kluckman K, Maeda N, Smithies O, Coffman TM: Reduced growth, abnormal kidney structure, and type 2 (AT₂) angiotensin receptor-mediated blood pressure regulation in mice lacking both AT_{1a} and AT_{1b} receptors for angiotensin II. *Proc Natl Acad Sci USA* 1998;95:15496–15501.
- 34 Tufro-McReddie A, Romano LM, Harris JM, Ferder L, Gomez RA: Angiotensin II regulates nephrogenesis and renal vascular development. *Am J Physiol* 1995;269:F110–F115.
- 35 Merlet-Benichou C, Gilbert T, Muffat-Joly M, Lelievre-Pegorier M, Leroy B: Intrauterine growth retardation leads to a permanent nephron deficit in the rat. *Pediatr Nephrol* 1994;8:175–180.
- 36 Langley-Evans SC, Gardner DS, Jackson AA: Association of disproportionate growth of fetal rats in late gestation with raised systolic blood pressure in later life. *J Reprod Fertil* 1996;106:307–312.
- 37 Luyckx VA, Compston CA, Simmen T, Mueller TF: Accelerated senescence in kidneys of low birth weight rats after catch-up growth. *Am J Physiol Renal Physiol* 2009.