Modulation of body fluids and angiotensin II receptors in a rat model of intra-uterine growth restriction

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We previously reported that sodium restriction during pregnancy reduces plasma volume expansion and promotes intra-uterine growth restriction (IUGR) in rats while it activates the renin–angiotensin–aldosterone system (RAAS). In the present study, we proceeded to determine whether expression of the two angiotensin II (ANGII) receptor subtypes (AT_1 and AT_2) change in relation to maternal water–electrolyte homeostasis and fetal growth. To this end, pregnant (gestation day 15) and non-pregnant Sprague-Dawley rats were randomly assigned to two groups fed either normal, or Na^+-restricted diets for 7 days. At the end of the treatment period, plasma aldosterone and renin activity as well as plasma and urine electrolytes were measured. Determinations for AT_1 and AT_2 mRNA and protein were made by RNase protection assay and photoaffinity labelling, respectively, using a number of tissues implicated in volume regulation and fetal growth. In non-pregnant rats, Na^+ restriction decreases Na^+ excretion without altering plasma volume, plasma Na^+ concentration or the expression of AT_1 and AT_2 mRNA or protein in the tissues examined. In normally fed pregnant rats when compared to non-pregnant controls, AT_1 mRNA increases in the hypothalamus as well as pituitary and declines in uterine arteries, while AT_1 protein decreases in the kidney and AT_2 mRNA declines in the adrenal cortex. In pregnant rats, Na^+ restriction induces a decrease in plasma Na^+, an increase in plasma urea, as well as a decline in renal urea and creatinine clearance rates. Protein levels for both AT_1 and AT_2 in the pituitary and AT_2 mRNA in the adrenal cortex are lower in the Na^+-restricted pregnant group when compared to normally fed pregnant animals. Na^+ restriction also induces a decrease in AT_1 protein in the placenta. In conclusion, these results suggest that pregnancy may increase sensitivity to Na^+ depletion by the tissue-specific modulation of ANGII receptors. Finally, these receptors may be implicated in the IUGR response to low Na^+.

Maternal haemodynamic adaptation and marked physiological changes occur throughout pregnancy in both humans and rats to ensure the normal evolution of mother and fetus. During normal pregnancy in the rat, remodelling of the uterine vasculature results in longer, larger calibre, and more distensible vessels (Osol & Cipolla, 1993) that can then accommodate the increased blood flow to the uterine artery (Ahokas *et al.* 1983) that helps support fetal growth. Furthermore, maternal extracellular or plasma volume expansion occurs during pregnancy concomitant with the activation of the renin–angiotensin–aldosterone system (RAAS) (Verkeste *et al.* 1998). Despite the marked increase in plasma volume, blood pressure decreases significantly during gestation in the rat (St-Louis & Massicotte, 1985) due to a major reduction of peripheral vascular resistance

(Dowell & Kauer, 1997). In addition, while the glomerular filtration rate and renal plasma flow are known to increase in the rat during pregnancy (Baylis, 1987), Na^+ retention is markedly elevated, particularly during the last week of gestation (Barron, 1987), the time for maximum fetal growth (Beaudoin, 1980). However, decreasing dietary Na^+ during the last week of gestation was reported by our laboratory to significantly reduce plasma volume expansion and promote intra-uterine growth restriction (IUGR) while further activating the RAAS (Roy-Clavel *et al.* 1999). Comparable alterations in plasma volume are observed in women with idiopathic IUGR (Salas *et al.* 1993).

Several factors contribute to the haemodynamic changes observed during pregnancy. One of the most important is certainly the RAAS (Barron, 1987) through the vascular

and adrenal effects of angiotensin II (ANGII) and the anti-natriuretic action of aldosterone. The RAAS normally plays an important role in maintaining vascular tone as well as fluid and electrolyte balance. The electrolyte-regulating steroid, aldosterone, released from adrenal cortical cells stimulated by ANGII, promotes Na^+ resorption at the expense of K^+ and Mg^{2+} excretion in kidney and colon as well as sweat and salivary glands (Weber, 2003). The biological activity of renin–angiotensin is mediated primarily by the octapeptide ANGII through its binding to and activation of specific transmembrane G protein-coupled ANGII receptors, type I (AT_1) and type II (AT_2).

The AT_1 receptor subtype, was cloned in 1991 (Sasaki *et al.* 1991; Murphy *et al.* 1991) and shown to mediate most of the classical effects of ANGII such as vasoconstriction as well as electrolyte and water homeostasis. Consistent with this, AT_1, although ubiquitous in the adult, is most abundantly expressed in blood vessels (where it induces vasoconstriction), adrenal cortex (where it promotes aldosterone release), kidney (where it controls water and Na^+ retention), liver (where it acts to control glycogen metabolism) and brain (where it promotes vasopressin release, thirst and salt appetite and controls blood pressure regulation and sympathetic output).

In contrast, while AT_2 is expressed ubiquitously in fetal tissues, it is maintained at high levels in adulthood in vascular endothelium, adrenal gland, uterus, ovary and certain brain areas (Timmermans *et al.* 1993). Expression patterns for this receptor *in vivo* as well as findings from a number of *in vitro* studies and transgenic/knockout animal models strongly suggest important roles for AT_2 in development and repair as well as to counterbalance or oppose the effects of AT_1 (Dzau *et al.* 1994). In this regard, AT_2 receptor activation has been shown to promote neuronal cell morphological differentiation (Gendron *et al.* 1999), induce apoptosis (Gallinat *et al.* 1999) and activate kinin and nitric oxide systems so to promote vasorelaxation (Tsutsumi *et al.* 1999). Finally, although expression of both ANGII receptor types are subject to modulation in a number of pathophysiological states, there is ample evidence, particularly for AT_2, that these receptors may undergo reversible physiological changes throughout life to facilitate adaptation and modulation of the RAAS to distinct physiological events. One such change has been demonstrated in the human myometrium during pregnancy (de Gasparo *et al.* 1994).

Despite marked activation of the RAAS during pregnancy, sensitivity to ANGII has been shown to be somewhat altered. A diminished vasoconstrictive response to ANGII has been reported in normotensive pregnant women when compared to non-pregnant women (Gant *et al.* 1994). This decreased sensitivity has also been demonstrated in the rat in isolated vessels such as the aorta (Hart, 1982) and mesenteric arteries (Massicotte *et al.* 1987). In agreement with these findings, ANGII receptor density has been documented to decrease in mesenteric arteries and renal glomeruli of pregnant rabbits (Brown & Venuto, 1986). In contrast, Bird *et al.* (1997) reported an increase in AT_1 expression in the uterine artery endothelium, which remained unaltered in arterial smooth muscle in pregnant sheep. On the other hand, the number and affinity of ANGII receptors have been shown not to change during gestation in the mesenteric vascular bed (Parent *et al.* 1991), isolated hepatocytes (Massicotte *et al.* 1990) and adrenal cortex of rats (Forcier *et al.* 1995) and rabbits (Brown & Venuto, 1986).

Alterations in Na^+ intake or plasma ANGII levels have been repeatedly shown to affect the regulation of ANGII receptors. Indeed, Na^+ restriction has been documented to promote increases in AT_1 receptor levels in the adrenal cortex in rats (LeHoux *et al.* 1997). Low dietary Na^+ has been reported to variably affect AT_1 receptor levels in the kidney (Jo *et al.* 1996; Schmid *et al.* 1997). In cultured rat endothelial cells, ANGII has been reported to down-regulate AT_2 mRNA expression via AT_1 binding (De Paolis *et al.* 1999). Co-treatment of cells transfected with the AT_2 gene promoter with an AT_1 antagonist and ANGII was shown to increase AT_2 mRNA expression (De Paolis *et al.* 1999). In nephrectomized rats on Na^+-restricted diets, the administration of DuP753, a specific AT_1 antagonist, was found to promote notable decreases in AT_1 mRNA and concomitant increases in AT_2 mRNA in the adrenal cortex (Gigante *et al.* 1997) suggesting once again the existence of an important functional 'cross-talk' between ANGII receptor types, one that may be particularly sensitive to Na^+.

In view of the above, we propose that some of the important haemodynamic and vascular adaptive mechanisms that come into play during normal pregnancy to ensure maternal well-being and fetal growth implicate changes in the expression of ANGII receptor subtypes in certain target tissues. Furthermore, Na^+ restriction during the last week of gestation in the rat may reduce plasma volume expansion and promote IUGR despite increased activation of the RAAS by differentially altering levels of AT_1 and AT_2 in these specific target tissues. Similar alterations in these receptors may be found in women with idiopathic IUGR. Therefore, the aim of the present investigation was to study, in the face of an important RAAS activation, the effects of Na^+ restriction during pregnancy on ANGII receptor regulation in selected tissues thought to be involved in the maintenance of vascular tone, haemodynamic homeostasis and fetal growth. Changes in ANGII receptor expression will be measured and compared to the state of activation of the RAAS, important renal clearance indices, uterine artery measurements and fetal growth.

Methods

Animals, experimental design, diet, housing and physiological measurements

Experimental procedures were reviewed and approved by the local Animal Care Committee, accredited by the Canadian Council on Animal Care. A total of 96 female Sprague-Dawley rats (Charles River Canada, St-Constant, Québec, Canada) were used. Fifty-six pregnant near-term rats (15 days gestation, weighing 225–250 g) obtained as detailed previously (Roy-Clavel *et al.* 1999), and 40 age-matched non-pregnant females (chosen randomly throughout their oestrous cycle) were randomly assigned to two groups that were placed on normal or Na$^+$-restricted diets for 7 days. This 7-day experimental period corresponded to the last week of gestation for the pregnant animals.

The four groups of animals were housed under controlled illumination on a 12 h light–dark cycle under ambient temperature and fed and watered *ad libitum.* The normal diet consisted of 0.2% sodium (Basal Diet 5755; PMI Feed Inc., Ren's Feed and Supplies, Oakville, Ontario, Canada) and tap water. The diet restricted in Na$^+$ contained 0.03% sodium (Low-sodium Diet 5881; PMI Feed Inc.) and demineralized water.

Animals were housed in metabolic cages for the last three experimental days so to measure 24 h water intake and urine output. At the end of the treatment period (day 7), all animals were killed by decapitation (at 9.00–9.30 h), body weight was recorded, blood was collected for hormonal and electrolyte analyses, and tissues (hypothalamus, pituitary, adrenal cortex, kidney, uterine arteries and placenta) were removed as previously described (Beausejour *et al.* 2003) for receptor measurements. Weights of fetoplacental units were recorded after the animals were killed, but before further tissue dissection. The number of animals used for measuring the numerous parameters varied according to specific procedural and technical requirements.

Haematology, clinical biochemistry and urine analysis

The following haematological and clinical indices were measured as previously described (Beauséjour *et al.* 2003). For haematocrit measures, blood drawn into microhaematocrit capillary tubes (Fisherbrand, Fisher) was centrifuged at 800 *g* for 3 min (Triac Centrifuge, Becton-Dickinson, Franklin Lakes, NJ, USA) and the result was read manually. For all other analyses, blood was drawn, at the time the animal was killed, into heparinized vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ, USA) and centrifuged at 800 *g* for 20 min at 4°C for collection of plasma. Aliquots of plasma and 24-h urine samples collected during the last three experimental days from animals housed in metabolic cages were used for the following determinations: Na$^+$ and K$^+$ concentrations using specific electrodes, urea levels using conductibility electrodes, creatinine concentration by the standard enzymatic colorimetric test, and osmolality using a vapour osmometer.

Plasma renin activity, aldosterone and corticosterone analyses

Plasma renin activity (PRA) was determined indirectly as already described (Roy-Clavel *et al.* 1999). Briefly, angiotensin I, generated per 2-h incubation period, was quantified by radioimmunoassay using rabbit anti-human ANG I (RAS 7001) that cross-reacts with rat, human, fowl and bullfrog ANG I (Peninsula, Inc., Belmont, CA, USA). Aldosterone extracted from plasma by solid phase using C18 September – Pak cartridges (Millipore; Waters, Montréal, Québec, Canada) was measured by radioimmunoassay (Roy-Clavel *et al.* 1999). Cortisterone plasma levels, determined to assess possible stress inflicted by animal manipulation, were determined using a commercially available radioimmunoassay kit (Medicorp, Montréal, Québec, Canada). Fetal corticosterone plasma determinations were performed similarly on pooled blood samples from each litter. Number of Na$^+$-restricted pregnant rats was smaller not because of death or abortion but because samples were lost in storage.

RNA isolation

Total cellular RNA from excised tissues was extracted using TRIzol Reagent according to the procedure detailed by the manufacturer (Invitrogen Canada Inc., Burlington, Ontario, Canada). Final RNA pellets were dissolved in appropriate volumes of 100% deionized formamide, and stored at −20°C until further use. Concentration of RNA was determined from absorbance measurements at 260 nm, and sample quality verified by ethidium bromide fluorescence.

RNase protection assay

Preparation of probes. A 182-base *Dra*I fragment excised from pcDNAI plasmid was used for the preparation of a high-activity RNA antisense probe for the AT$_1$ receptor (Murphy *et al.* 1991). A 215-base *Hin*dIII-*Xba*I fragment excised from pcDNAI/Amp plasmid was cloned into the multiple cloning sites of the Bluescript vector, linearized with *Pvu*II and used for the preparation of a high-activity RNA antisense probe for the AT$_2$ receptor (Hunyady *et al.* 1994). A 334-base fragment of β-actin gene (Ambion RNA Company, Austin, TX, USA) served as an internal control. The antisense probes were prepared by transcription *in vitro* by the Ambion Maxiscript Technique (Ambion RNA Company) in the presence of T7 polymerase for AT$_2$, SP6 polymerase for AT$_1$ and β-actin, and [α-^{32}P]UTP (Mandel, Boston, MA, USA). The radioactive probes

were separated from free $[\alpha\text{-}^{32}P]$UTP by washing with 75% ethanol−25% sodium acetate.

RNase protection. Total RNA (25 μg) was incubated at 65°C for 10 min with the antisense probes (100 000 counts min^{-1} for AT$_1$ and AT$_2$, 15 000 counts min^{-1} for β-actin) in 5 X Pipes buffer (200 mM 1,4-piperazine diethanesulphonic acid, pH 6.4, 2 M NaCl, 5 mM EDTA) and 80% deionized formamide. After denaturation of RNA at 85°C for 5 min, hybridization was performed overnight at 47°C. Non-hybridized RNA was digested using RNase T1/A (RNase cocktail from Ambion RNA Company) in RNase digestion buffer containing 1 M Tris-HCl (pH 7.5), 0.5 M EDTA and 0.3 M NaCl for 1 h at 30°C. After treatment with 20% sodium dodecyl sulphate (SDS) and 10 mg ml^{-1} proteinase K for 15 min at 37°C, the hybridized RNA was purified by phenol–chloroform extraction, precipitated in 100% ethanol and resuspended in 10 μl of electrophoresis buffer containing 80% deionized formamide, 1 mM EDTA, 0.1% bromophenol blue, 0.1% xylene cyanol and 0.1% SDS. The RNA was denatured at 85°C for 5 min and then separated by electrophoresis on 5% acrylamide−7 M urea gels at 40 W. After separation, the gel was fixed for 15 min in 10% methanol−5% acetic acid and dried for 1 h. The dried gel was exposed to X-ray film (Kodak X-Omat AR5) using intensifying screens at −80°C for 1–7 days. Relative intensities of AT$_1$, AT$_2$ and β-actin bands were determined by gel analysis with Scion Image computer software (Scion Corporation, National Institutes of Health, Frederick, MD, USA). The steady-state mRNA levels for AT$_1$ and AT$_2$ were expressed in arbitrary units, and standardized by comparison with hybridization results obtained from the housekeeping β-actin gene.

Preparation of tissue membranes

To prepare each tissue-specific membrane the following amount of tissue was needed: adrenal cortices from four rats, pituitaries from two rats, 120 mg of the kidney cortex and placenta pooled from each experimental pregnant group. Tissues were minced with scissors and then homogenized in 0.25 M sucrose by 2×10 s pulses with a Polytron at setting 8 (Brinkman, Rexdale, Ontario, Canada). The homogenates were centrifuged at 1500 g for 10 min at 4°C and supernatants thus collected centrifuged at 50 000 g for 30 min at 4°C. The resulting particulate fractions were resuspended in buffer (25 mM Tris-HCl, 120 mM NaCl, 5 mM MgCl$_2$, 0.0002% NaN$_3$, 5 mM EDTA, 1 μM leupeptide, 1 μM aprotinin, 1 μM pepstatin, and 100 μM phenylmethylsulfonyl fluoride, pH 7.4) and membrane protein concentrations measured by the Bradford colorimetric method. Membranes were then diluted to a final protein concentration of 1 μg μl^{-1} and stored at 80°C until further use.

Photoaffinity labelling procedures

Iodination of peptides. Purified ^{1}Sar-^{5}Val-^{8}Bpa-ANGII was radiolabelled using Na^{125}I by the iodogen method as described by Fraker & Speck (1978). Radio-labelled peptides were purified to homogeneity by high performance liquid chromatography (reverse phase C-18), and specific radioactivity measured using a gamma counter (Packard Cobra II Model 5003, Canberra Packard Canada, Mississauga, Ontario, Canada). Specific activity for ^{125}I-ANGII-Bpa was calculated at 2000 Ci mmol^{-1}.

Photoaffinity labelling. Briefly, tissue membranes (100 μg of protein) were incubated with 2 μCi (or 5 nM) of ^{125}I-labelled ^{1}Sar-^{5}Val-^{8}Bpa-ANGII in 500 μl of binding buffer (25 mM Tris-HCl 100 mM NaCl, 5 mM MgCl$_2$, 1 mM EDTA, 0.1% bovine serum albumin (BSA), 2 mg ml^{-1} bacitracin and 50 μg ml^{-1} soybean trypsin inhibitor, pH 7.4) for 45 min in the dark at room temperature. To selectively label AT$_1$ or AT$_2$ receptors, 1 μM PD123319 (AT$_2$ antagonist) or L-158 809 (AT$_1$ antagonist) was added to the incubation mixture, respectively. Non-specific binding was determined by incubating in the presence of 10^{-5} M ANGII. Following incubation, the membranes were washed by centrifugation (13 000 g for 10 min) with binding buffer prepared without BSA and then irradiated under UV light (365 nm) (Long Wave B-100SP, UVP Inc., Upland, CA, USA) for 45 min at 4°C. Non-covalently bound ligand was removed by successive washes with citrate buffer (pH 5.5). The washed pellets were then incubated for 60 min at 37°C in denaturing buffer (60 mM Tris-HCl, 10% (v/v) glycerol, 2% (w/v) SDS, 0.3% bromophenol blue and 125 mM dithiothreitol, pH 6.8) and denatured photolabelled protein complexes (40 μl) separated electrophoretically on polyacrylamide gels (9% running and 5% stacking) at 25 mA (stacking) and 50 mA (running) for approximately 5 h. For comparative analyses, samples from each experimental animal group were routinely separated on the same gel. Gels were dried and then exposed to Kodak X-Omat AR film for 1–3 days at −80°C. Relative intensities of the AT$_1$ and AT$_2$ bands for all samples from all four groups of animals were determined by gel analysis using Scion Image Computer Software (Scion Corporation, National Institutes of Health) and expressed in arbitrary units relative to values from non-pregnant rats fed a normal diet which were set constant at 1 arbitrary unit.

Mechanical uterine vessel measurement

Vessel mechanical measurements were performed as previously described (St-Louis *et al.* 1997, 2001). An arcuate artery, at the midpoint of the uterine arcade, was cleaned of adhering tissues. A section (1.5 mm) was isolated and two tungsten wires (20 μm) were

inserted into the lumen to secure the vessel to supports of the myograph, one attached to a force transducer and the other to a micrometer device. Force was recorded through data acquisition package (Workbench, Kent Scientific, Litchfield, CT, USA). The artery was bathed in 7 ml oxygenated (95% O_2−5% CO_2) Krebs bicarbonate solution (KBS). The arterial segment was stretched in steps of ∼30 μm in length (internal circumference) until ∼1.5 mN mm^{-1} was reached (1 mN = 102 mg) and held at each length for 2–3 min while wall tension at peak response to stretch was recorded. The passive length–tension relationship generated was fitted to an exponential equation that intercepted the line of Laplace (pressure, 50 mmHg) at the diameter used as passive tension.

Statistical analysis

Data were compared by two-way analysis of variance. Unpaired Student's *t* test was performed group by group if interaction between gestation and diet was significant. Otherwise, *post hoc* Newman–Keuls test was used. Minimum level of significance of $P < 0.05$ was used. The results were expressed as means ± s.e.m. with numbers in parentheses corresponding to numbers of animals studied. Where the data were not normally distributed (PRA, aldosterone and corticosterone), they were analysed using the Kruskal–Wallis test. In this case, data are presented as their median values and interquartile ranges.

Drugs and chemicals

All salts used in the experiments were of analytical grade and obtained from Fisher Scientific (Montreal, Quebec, Canada) or Sigma Chemical (St Louis, MO, USA).

Results

### Physiological changes induced by Na$^+$ restriction and pregnancy

Sodium restriction promotes significant decreases in mean body weights in both non-pregnant (277 ± 4 g (14) on normal *versus* 254 ± 5 g (13) on low-Na$^+$ diet, $P < 0.001$) and pregnant rats (370 ± 7 g (13) on normal *versus* 325 ± 6 g (12), on low-Na$^+$ diet, $P < 0.001$). In addition, the mean fetoplacental unit weight is significantly ($P < 0.01$) lower in pregnant rats on Na$^+$-restricted diet (85 ± 2 g (12)) when compared to those from rats on normal diet (101 ± 4 g (13)). Furthermore, Na$^+$ restriction appears to induce premature delivery as seven of the 28 rats on the low-Na$^+$ diet delivered the morning of gestational day 22. In contrast, none of the 28 pregnant rats on the normal diet delivered before they were killed. Nevertheless, Na$^+$ restriction does not affect litter size (14.5 ± 0.6 pups (13) for normally fed

animals *versus* 15.0 ± 0.6 pups (12), for rats on low-Na$^+$ diet).

As shown in Fig. 1, pregnancy alone is associated with an increase in both water intake (Fig. 1*A*) and urine output (Fig. 1*B*). These increases are significant and independent of diet. During the last experimental day, water intake significantly decreased in both pregnant ($P < 0.01$; Fig. 1*A*) and non-pregnant ($P < 0.05$; Fig. 1*A*) animals on the low-Na$^+$ diet while urinary output only decreased significantly ($P < 0.01$; Fig. 1*B*) in the pregnant rats on the low-Na$^+$ diet.

Effects of Na$^+$ restriction and pregnancy on plasma hormone levels

As shown in Table 1, Na$^+$ restriction promotes increases in PRA and plasma aldosterone in non-pregnant and pregnant rats. Pregnancy increases both PRA and
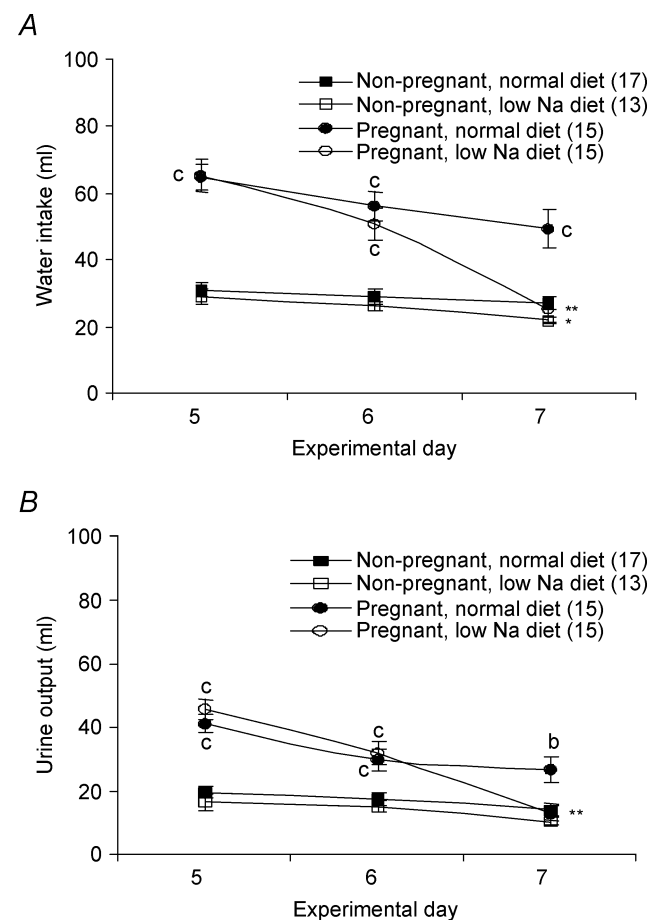
Figure 1. Effects of low-Na$^+$ diet and pregnancy on water homeostasis: water intake (*A*) and urine output (*B*)
Experimental days 5 to 7 correspond to days 19 to 21 of gestation for the pregnant animals. Results are expressed as means ± s.e.m. with numbers in parentheses representing the number of rats. Values significantly different from those obtained for animals (same group) fed a normal diet indicated by *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. Values significantly different from those obtained from non-pregnant animals (same diet) indicated by ^{a}$P < 0.05$, ^{b}$P < 0.01$, ^{c}$P < 0.001$.

Table 1. Effects of low-Na$^+$ diet and pregnancy on plasma hormone levels

	Non-pregnant rats		Pregnant rats	
	Normal	Low-Na$^+$	Normal	Low-Na$^+$
Plasma renin activity	2.1	2.8	5.0*	9.8
((pmol angiotensin I) ml^{-1} h^{-1})	[1.5–2.7]	[1.1–5.3]	[4.2–8.3]	[5.0–26.5]
	(12)	(12)	(12)	(8)
Aldosterone (pmol ml^{-1})	2.2	4.8	6.8[a]	24.0*[b]
	[1.7–2.4]	[3.6–6.5]	[4.7–8.5]	[18.1–28.2]
	(14)	(13)	(12)	(10)
Corticosterone (nmol ml^{-1})	1.3	1.5	1.2	1.1
	[0.8–1.8]	[1.0–1.6]	[0.9–1.4]	[0.9–1.3]
	(13)	(12)	(13)	(11)

Results are expressed as medians and interquartile ranges. Numbers in parentheses represent the number of rats. Significantly different from animals (same group) fed a normal diet indicated by *$P < 0.05$. Significantly different from non-pregnant animals (same diet) indicated by [a]$P < 0.05$, [b]$P < 0.01$.

Table 2. Impact of low-Na$^+$ diet and pregnancy on plasma osmolality, solute concentrations and haematocrit

	Non-pregnant rats		Pregnant rats	
	Normal	Low-Na$^+$	Normal	Low-Na$^+$
Osmolality (mosmol (kg H$_2$O)$^{-1}$)	295 ± 2 (13)	292 ± 3 (13)	279 ± 3[c] (14)	259 ± 5***[c] (12)
Sodium (mmol l^{-1})	139 ± 1 (13)	140 ± 1 (12)	132 ± 1[c] (11)	118 ± 2***[c] (12)
Potassium (mmol l^{-1})	6.4 ± 0.2 (10)	6.2 ± 0.2 (11)	5.7 ± 0.7 (8)	6.6 ± 0.8 (9)
Urea (mmol l^{-1})	5.1 ± 0.2 (13)	4.9 ± 0.2 (12)	4.0 ± 0.5[a] (14)	9.2 ± 0.6***[c] (11)
Creatinine (μmol l^{-1})	35 ± 1 (14)	37 ± 2 (12)	46 ± 3[a] (13)	55 ± 2* [a] (11)
Haematocrit (%)	42 ± 1 (14)	46 ± 1* (11)	33 ± 1[a] (13)	39 ± 1*[a] (12)

Results are expressed as means ± S.E.M. Numbers in parentheses represent the number of rats. Significantly different from animals (same group) fed a normal diet indicated by *$P < 0.05$, ***$P < 0.001$. Significantly different from non-pregnant animals (same diet) indicated by [a]$P < 0.05$, [c]$P < 0.001$.

aldosterone levels in rats fed normal or low-Na$^+$ diets (see Table 1). In contrast, as shown in Table 1, plasma corticosterone levels are not affected by diet or gestation. However, fetal corticosterone levels are significantly higher ($P < 0.05$) in fetuses from rats on the low-Na$^+$ diet (1.07 ± 0.07 nmol ml^{-1}, $n = 3$ litters) when compared to fetuses from rats on the normal diet (0.82 ± 0.07 nmol ml^{-1}, $n = 3$ litters).

Effects of Na$^+$ restriction and pregnancy on haematocrit, electrolyte and solute measurememts

As shown in Table 2, Na$^+$ restriction promotes a significant ($P < 0.05$) increase in haematocrit in the non-pregnant rat group without affecting plasma osmolality (P_{osm}) or solute concentrations. Pregnancy alone is found to significantly decrease plasma Na$^+$, urea and osmolality as well as haematocrit levels while significantly increasing plasma creatinine values in rats fed a normal diet (Table 2). Sodium restriction during pregnancy significantly decreases plasma Na$^+$ concentrations and osmolality further while promoting significant increases in both creatinine and haematocrit values (see Table 2). These

findings clearly show that although Na$^+$ restriction elicits a contraction of extracellular fluid volume in both non-pregnant and pregnant rats, volume contraction is greatest in the pregnant rat group.

The effects of both diet and gestation on urinary parameters measured on the last experimental day corresponding to gestational day 22 for the pregnant rat groups are presented in Table 3. Restricting dietary Na$^+$ significantly ($P < 0.001$; Table 3) reduces Na$^+$ excretion in non-pregnant rats. Pregnancy significantly ($P < 0.01$; Table 3) increases urinary volume while significantly decreasing Na$^+$ ($P < 0.001$; Table 3), potassium ($P < 0.001$; Table 3) and urea ($P < 0.01$; Table 3) excretion in the normally fed animals. However, Na$^+$ restriction significantly depresses urinary volume ($P < 0.01$; Table 3), Na$^+$ ($P < 0.05$; Table 3) and creatinine excretion ($P < 0.001$; Table 3) and significantly increases urinary excretion in pregnant rats ($P < 0.01$; Table 3).

Creatinine clearance is not affected by restricting Na$^+$ intake in non-pregnant rats (Fig. 2A). In contrast, while pregnancy significantly decreases creatinine clearance from rats fed either diet, decreases were most marked (see

Table 3. Effects of low-Na$^+$ diet and pregnancy on urinary parameters measured on the last experimental day

	Non-pregnant rats		Pregnant rats	
	Normal	Low-Na$^+$	Normal	Low-Na$^+$
Urine volume (ml day^{-1})	14.1 ± 1.7 (17)	10.4 ± 1.3 (14)	26.7 ± 4.1^b (16)	$13.0 \pm 2.2^{**}$ (15)
Osmolality (mosmol (kg H$_2$O)$^{-1}$)	1393 ± 121 (16)	1347 ± 104 (14)	476 ± 60^c (15)	$806 \pm 121^{*b}$ (16)
UV_{osmol} (μmol day^{-1})	17.3 ± 1.6 (16)	$12.5 \pm 1.0^*$ (14)	10.0 ± 0.9^a (15)	8.1 ± 0.6^a (16)
UV_{Sodium} (μmol day^{-1})	0.89 ± 0.12 (13)	$0.09 \pm 0.01^{***}$ (12)	0.29 ± 0.06^c (13)	$0.12 \pm 0.02^*$ (13)
$UV_{Potassium}$ (μmol day^{-1})	1.24 ± 0.15 (13)	1.59 ± 0.17 (12)	0.82 ± 0.11^c (14)	0.81 ± 0.07^c (13)
UV_{Urea} (μmol day^{-1})	8.88 ± 1.32 (12)	6.78 ± 0.61 (12)	5.24 ± 1.18^b (14)	3.81 ± 0.29^b (13)
$UV_{Creatinine}$ (μmol day^{-1})	0.075 ± 0.007 (13)	0.065 ± 0.003 (12)	0.079 ± 0.003 (14)	$0.051 \pm 0.003^{***b}$ (13)

Results are expressed as means $\pm$ S.E.M. with numbers in parentheses representing the number of rats. UV, urinary excretion. Significantly different from animals (same group) fed a normal diet indicated by $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. Significantly different from non-pregnant animals (same diet) indicated by $^aP < 0.05$, $^bP < 0.01$, $^cP < 0.001$.

Fig. 2A) in rats on the low Na$^+$ diet. Urea clearance is not affected by diet in non-pregnant rats nor is it altered by pregnancy alone. However, Na$^+$ restriction significantly ($P < 0.001$; Fig. 2B) decreases urea clearance from pregnant animals.

Restricting Na$^+$ differentially affects uterine artery measurements

Diameter of the uterine arcuate arteries was measured to get an index of blood flow to the fetoplacental unit. In non-pregnant rats, Na$^+$ restriction is found to produce a non-significant increase in mean uterine artery diameter from $121 \pm 7 \mu$m ($n = 11$) in normally fed animals to $140 \pm 10 \mu$m ($n = 11$). Pregnancy significantly ($P < 0.001$) increases mean artery diameter in normally fed animals to $269 \pm 11 \mu$m ($n = 10$). However, Na$^+$ restriction significantly ($P < 0.01$) reduces mean artery diameter measured in the pregnant animals to $198 \pm 15 \mu$m ($n = 10$).

Pregnancy and Na$^+$ restriction affect AT$_1$ and AT$_2$ protein and mRNA expression

The effects of pregnancy and restricting dietary Na$^+$ on AT$_1$ and AT$_2$ mRNA and protein levels in several tissues that are implicated in volume regulation (hypothalamus, pituitary, adrenal cortex and kidney) and fetal growth (uterine artery and placenta) are depicted in Fig. 3. Sensitivity of the RNase protection assay limited detection of AT$_2$ mRNA to the adrenal cortex. In addition, receptor proteins could not be measured in hypothalamus and uterine arteries due to limited availability of these tissues.

Hypothalamus. In non-pregnant rats, Na$^+$ restriction does not alter the AT$_1$ receptor steady-state mRNA levels in the hypothalamus (Fig. 3A). However, pregnancy does promote a significant ($P < 0.01$; Fig. 3A) increase in AT$_1$ mRNA levels in the hypothalamus that is not affected further by the low Na$^+$ diet.

Pituitary. Both ANGII receptor subtypes are present in the pituitary (Fig. 3A, B and D). In non-pregnant rats, restricting dietary Na$^+$ does not alter expression of AT$_1$ (mRNA and protein) or AT$_2$ (protein). Furthermore, pregnancy does significantly ($P < 0.05$; Fig. 3A) increase AT$_1$ mRNA levels in the pituitary without affecting receptor protein (Fig. 3B). Finally, AT$_1$ and AT$_2$ protein levels in pituitaries of pregnant rats on Na$^+$-restricted diets are both significantly ($P < 0.05$; Fig. 3B and D) lower than those from normally fed pregnant animals.

Adrenal cortex. As shown in Fig. 3, AT$_1$ receptor steady-state mRNA and protein levels in the adrenal cortex are not affected by diet, gestation or the combination of Na$^+$ restriction and pregnancy. Although Na$^+$ restriction does not alter AT$_2$ mRNA or protein in the adrenal cortex of non-pregnant rats, pregnancy significantly ($P < 0.05$; Fig. 3C) decreases AT$_2$ steady-state mRNA levels in normally fed animals. Furthermore, Na$^+$ restriction significantly ($P < 0.05$; Fig. 3D) decreases AT$_2$ mRNA levels in the adrenal cortex of pregnant rats further.

Kidney. As shown in Fig. 3, neither Na$^+$ restriction nor pregnancy affect AT$_1$ mRNA and AT$_2$ protein levels in the rat kidney. In contrast, pregnancy promotes a significant ($P < 0.05$; Fig. 3B) decrease in AT$_1$ protein in the kidney that is not affected further by restricting dietary Na$^+$.

Uterine arteries. While pregnancy significantly ($P < 0.05$; Fig. 3A) decreases AT$_1$ mRNA levels in uterine arteries, restricting dietary Na$^+$ is without effect. For the reasons detailed earlier, neither mRNA for AT$_1$ and AT$_2$ nor protein values for AT$_2$ in uterine arteries could be obtained.

Placenta. Finally, while restricting dietary Na$^+$ does not affect AT$_1$ mRNA or AT$_2$ protein levels in the placenta, it significantly ($P < 0.05$; Fig. 3B) decreases AT$_1$ protein values.

Discussion

In the present study we show that maternal adaptive haemodynamic changes that take place during normal pregnancy in the rat, are noted concurrently with alterations in the expression of ANGII receptors in some tissues involved in volume regulation and fetal development. However, restricting dietary Na^+ during the last week of gestation disrupts some of these adaptive vascular changes, impedes fetal growth, and induces modifications in the expression of the ANGII receptors that are in some instances greater, and in others, distinct, from those noted during normal pregnancy. Finally, although extracellular volume contraction is also seen in non-pregnant rats on a low-Na^+ diet, plasma electrolyte levels, kidney clearance indices and ANGII receptor expression in all the tissues examined in these animals are unaltered.

Figure 2. Effects of low-Na^+ diet and pregnancy on clearance of creatinine (*A*) and urea (*B*)
Results are expressed as means ± S.E.M. with numbers in parentheses representing the number of rats. Values significantly different from those obtained for animals (same group) fed a normal diet indicated by *$P < 0.05$, ***$P < 0.001$. Values significantly different from those obtained for non-pregnant animals (same diet) indicated by [a]$P < 0.05$, [c]$P < 0.001$.

Changes in ANGII receptor expression in the hypothalamus

In specific areas of the hypothalamus (thirst centre), ANGII has been shown to stimulate water intake via AT_1 (Beresford & Fitzsimons, 1992) and AT_2 receptor activation (Hogarty *et al.* 1992; Li *et al.* 2003). In our present study, we found that AT_1 expression in the rat hypothalamus increased significantly during pregnancy. This higher expression may account for the increase in water intake we note in our animals during the last few days of pregnancy. Similar increases in water intake and decreases in urine output have been reported during the last week of gestation in the rat as well (Atherton *et al.* 1982). However, we find that although water intake declines significantly during these last days of gestation in the pregnant rats on the low-Na^+ diet, high levels of AT_1 are maintained in the hypothalamus and the RAAS is significantly activated in these animals. In this instance, Na^+ restriction may in itself stimulate these changes in hypothalamic receptor levels. In support of this, Jo *et al.* (1996) have shown that mRNA for both AT_{1a} and AT_{1b}, the two isoforms of the rat AT_1 receptor, increased in the non-pregnant rat hypothalamus following Na^+ restriction. In our study, Na^+ restriction alone does not promote increases in AT_1 receptor levels in the non-pregnant rat hypothalamus. A likely explanation may be that if Na^+ restriction had been prolonged, as in the study by Jo *et al.* (1996), to 20 instead of 7 days, statistical significance could have been reached. Alternatively, gestation and Na^+ restriction may differentially modulate AT_2 receptor levels in the hypothalamus leading to increases and decreases in water intake, respectively. These changes may be more important to fluid intake than those noted in AT_1. In this regard, the importance of the AT_2 receptor in the thirst response in rodents is clearly illustrated by Hein *et al.* (1995) who showed that water intake was significantly reduced in AT_2 receptor gene knock-out mice. Nevertheless, an assay with greater sensitivity than the RNase protection assay that was used in our study is needed to test the importance of this receptor subtype in the rat hypothalamus.

ANGII receptor expression in the pituitary

In response to ANGII, the posterior pituitary releases vasopressin (AVP) which in turn enhances water and salt reabsorption in the renal tubules (Bonjour & Malvin, 1970). However, the role of each ANGII receptor subtype in the control of AVP release from the rat pituitary is somewhat controversial. In this regard, Qadri *et al.* (1993) reported that ANGII-induced AVP release in the rat is attenuated by the central administration of losartan (an AT_1 antagonist) and unaffected by centrally administered PD123177 (an AT_2 antagonist), while blockade of either AT_1 or AT_2 was shown to suppress ANGII-induced increases in plasma AVP (Hogarty *et al.* 1992).
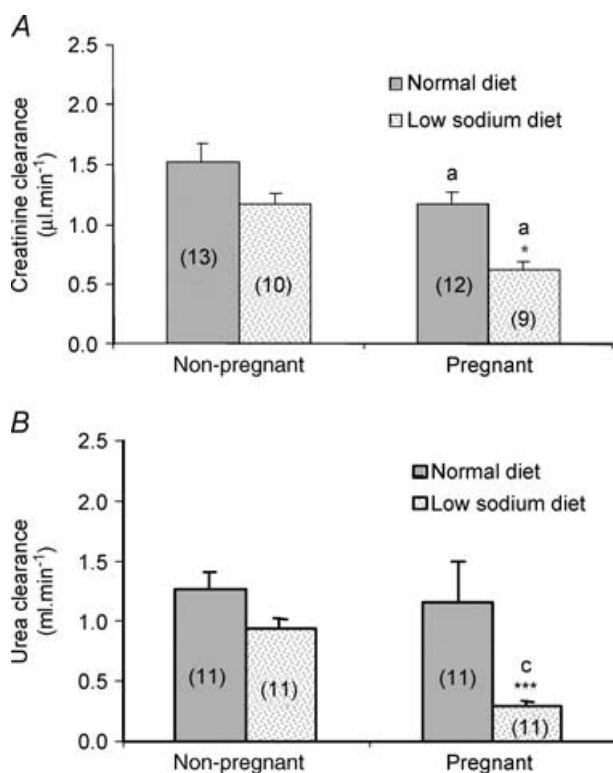
During normal pregnancy in the rat, circulating AVP is found in sufficiently high enough levels to promote water retention (Lindheimer *et al.* 1987) and thus contribute to maternal extracellular fluid expansion. In the present study, we found that expression of AT_1 mRNA increased

in the rat pituitary during pregnancy while AT_1 and AT_2 protein levels were unaltered. This increase in receptor expression in the rat during pregnancy is supported by the findings of Seltzer *et al.* (1992) who showed that oestrogen levels influenced ANGII receptor expression in the anterior
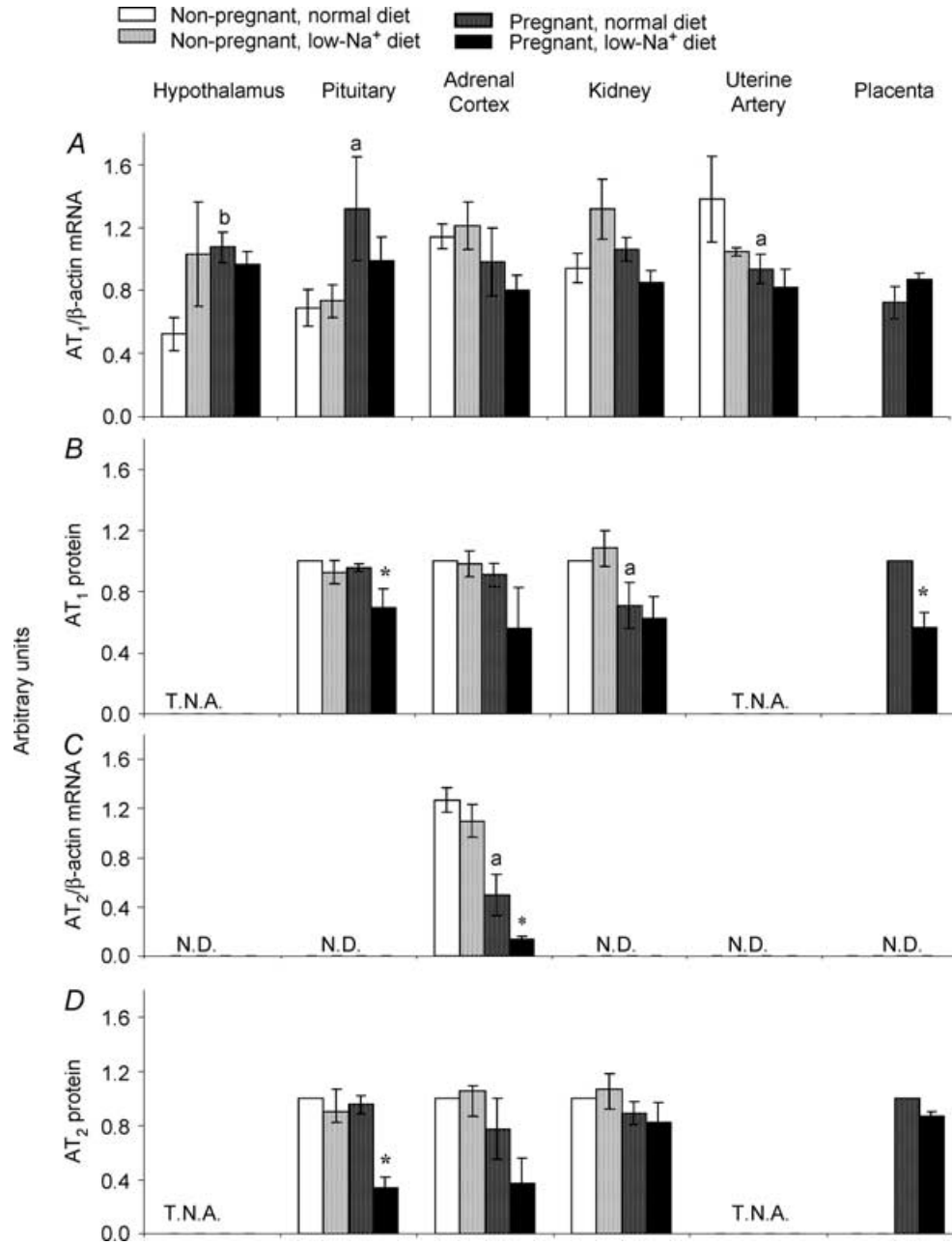
Figure 3. Effects of low-Na$^+$ diet on AT$_1$ and AT$_2$ receptor mRNA and protein expression in tissues from non-pregnant and pregnant rats
Data for mRNA are expressed as AT$_1$ or AT$_2$/β-actin ratio (arbitrary units) and results shown (*A* and *C*) represent means ± s.e.m. from five separate experiments. Membrane proteins were prepared and photoaffinity labelling performed as described in Methods. Data for receptor protein were compared to those from normal non-pregnant controls and results shown (*B* and *D*) represent means ± s.e.m. from three separate experiments. Significantly different from values obtained for non-pregnant rats fed a normal diet indicated by $^aP < 0.05$, $^bP < 0.01$. Significantly different from values obtained for pregnant animals on a normal diet indicated by $^*P < 0.05$. T.N.A., tissues not available. N.D., not detected.

pituitary of female rats. However, in pregnant rats on the low-Na$^+$ diet, AT$_1$ and AT$_2$ protein levels decreased by 25% and 64%, respectively. Should both ANGII receptor subtypes be implicated in promoting AVP release (see Hogarty *et al.* 1992), this decrease in receptor expression may underlie decreased AVP release and thus, decreased water retention (which we noted in the pregnant animals on the low-Na$^+$ diet).

Changes in ANGII receptor expression in the adrenal cortex

Aldosterone release from the adrenal cortex is stimulated by ANGII via AT$_1$ activation to promote Na$^+$ resorption and subsequently, water retention (Chung *et al.* 1999). Here we found that Na$^+$ restriction does not affect transcription or translation of either of the ANGII receptor subtypes in the adrenal cortex of the non-pregnant rats. Furthermore, in agreement with the earlier study of Forcier *et al.* (1995) we found that pregnancy did not affect AT$_1$ expression in the adrenal cortex of the rat. However, both AT$_2$ mRNA and protein levels declined in the adrenal cortex of our pregnant animals, most probably in response to the high plasma levels of ANGII. In this regard, it has been shown that ANGII can, via AT$_1$ binding, decrease AT$_2$ mRNA stability (Ouali *et al.* 1997). Finally, it is possible that the ratio of AT$_1$ to AT$_2$ is most important to the final adrenal response to ANGII. Therefore, when levels of AT$_2$ decrease during pregnancy, the AT$_1$: AT$_2$ ratio increases and stimulation of aldosterone release is favoured. This would explain the hyperaldosteronism we observed in the pregnant rats, irrespective of diet.

Renal function, osmoregulation and ANGII receptor expression

During pregnancy, the kidney has been shown to play an important role in resetting plasma tonicity in the rat to a new narrow range (Lindheimer *et al.* 1987). The decrease we observed in P_{osm} in the normally fed pregnant rats is consistent with such a re-adjustment. Furthermore, plasma volume expansion found during pregnancy is associated with a rise in the glomerular filtration rate in the rat (Baylis, 1987), which in turn increases Na$^+$ filtration and reabsorption rates. Increases in Na$^+$ reabsorption are observed during gestation in both proximal tubules and distal nephrons of the rat kidney (Atherton *et al.* 1988). In view of the above, the decrease we found in plasma urea concentration in the pregnant rats on the normal diet may be due to both extracellular fluid expansion and the decrease in P_{osm}. Furthermore, the decrease in Na$^+$ excretion we, and others (Atherton *et al.* 1982) observed in the pregnant rat, as well as the dilution of urine (indicated by a decrease in urinary osmolality) we noted in our pregnant animals may serve to maintain homeostasis during pregnancy.

In the kidney, water and salt reabsorption are stimulated in part by ANGII primarily via AT$_1$ activation (Chung *et al.* 1999). The effect of Na$^+$ restriction on renal ANGII receptors is indeed controversial. For example, low Na$^+$ intake has been shown to decrease expression of AT$_{1a}$ and AT$_{1b}$ mRNA in the renal afferent artery (Ruan *et al.* 1997). On the other hand, total renal expression of AT$_1$ is documented to increase in rats on a Na$^+$-restricted diet (Jo *et al.* 1996). In our present study, we found that Na$^+$ restriction did not affect AT$_1$ mRNA or AT$_1$ and AT$_2$ protein levels in the non-pregnant rats. However, and quite surprisingly, we found that AT$_1$ protein decreased rather than increased in the rat kidney during pregnancy, suggesting that the renal involvement in fluid retention during normal pregnancy may implicate physiological mediators other than ANGII such as AVP and aldosterone.

In pregnant rats on the low-Na$^+$ diet we found that osmoregulation was somewhat perturbed while urine volume and Na$^+$ excretion were reduced. Furthermore, although we expected Na$^+$ reabsorption to be greater in pregnant than non-pregnant rats on the low-Na$^+$ diet, in order to conserve the small amount of Na$^+$ available, Na$^+$ excretion was similar in both groups of animals. Therefore, the plasma constriction we observed in the pregnant rats on the low-Na$^+$ diet is apparently secondary to deficient renal Na$^+$ reabsorption. The kidney in this instance has probably reached its maximum capacity for Na$^+$ reabsorption.

Uraemia and decreased urea clearance were observed in the pregnant rats on the low-Na$^+$ diet. Plasma urea may increase in these rats as a result of decreased urinary flow that serves to counterbalance plasma volume constriction. We also found that expression (mRNA and protein) of the two ANGII receptor subtypes were not different in the kidneys from these rats compared to their pregnant controls. The high levels of plasma creatinine and low creatinine clearance rates in the pregnant rats on the low-Na$^+$ diet strongly suggest that the glomerular filtration rate declined in these animals as a result of plasma volume constriction. Taken together, the physiological changes we observed in the pregnant rats on the Na$^+$-restricted diet are consistent with a diagnosis of functional renal insufficiency. Functional renal insufficiency in humans is characterized clinically by urea/creatinine plasma ratio values > 100, $U_{osm} > 500$ mosmol (kg water)$^{-1}$ and urinary sodium < 20 mmol l^{-1} (Faber *et al.* 1993). Similar urinary and plasma values were seen in these rats.

The IUGR response: changes in uterine diameter and ANGII receptor expression in the placenta

The mean uterine arcuate artery diameter increased significantly in the normally fed pregnant rats. This is

consistent with one aspect of remodelling of the uterine vasculature that normally takes place during pregnancy (Osol & Cipolla, 1993). This increase in artery diameter would allow for the higher uteroplacental blood flow (Ahokas *et al.* 1983) that is needed for adequate fetal growth. In support of this, the decrease we noted in mean artery diameter in the pregnant rats on the Na^+-restricted diet may contribute to the diminished mean fetoplacental unit weight.

To the best of our knowledge, there are no published data on ANGII receptor expression in the rat uterine artery, most probaby due to the low availability of tissue in this animal. In our present study we showed that AT_1 mRNA levels do decline during gestation in the rat uterine artery. This decrease is similar in pregnant animals on the Na^+-restricted diet. The significance of these changes in AT_1 receptor expression to those in vessel diameter is unclear. Furthermore, although functional studies have shown the presence of the AT_2 receptor subtype in rat uterine arteries (St-Louis *et al.* 2001) and this receptor subtype may play a role in cell proliferation (Rizkalla *et al.* 2003) and in turn influence vessel diameter, AT_2 could not, unfortunately, be detected in the rat uterine artery with our current techniques.

In the placenta, the predominant ANGII receptor subtype is AT_1 (Li *et al.* 1998). Although ANGII is not a potent vasoconstrictor in the human placenta (McCarthy *et al.* 1994) it may, via AT_1 receptor activation, play an important role in controlling fetoplacental circulation and thus, fetal growth. In this regard, it has been recently demonstrated that ANGII can, via AT_1 receptor activation, increase expression of vascular epidermal growth factor (VEGF) (Rizkalla *et al.* 2003; Zhang *et al.* 2004), a growth factor that plays an important role in the remodelling of the uteroplacental and fetoplacental vasculature (Ni *et al.* 1997). Of particular relevance to our study, increases in VEGF mRNA in the uterine tissues and placenta are most evident in late pregnancy (Ni *et al.* 1997), corresponding to the last gestational week in the rat and the time for maximum fetal growth. In addition, ANGII has been shown, via AT_1 activation, to stimulate human placental lactogen release (Kalenga *et al.* 1994) which can act directly, or indirectly, via insulin growth factor-1, to promote fetal growth. Therefore, in view of the above, Na^+ restriction during this crucial period of gestation in the rat may jeopardize fetal growth (as reflected by the decrease in mean fetoplacental weight) by decreasing levels of AT_1 in the placenta. Indeed levels of this ANGII receptor are significantly decreased in the rats on the low-Na^+ diet. This decrease in placental AT_1 protein noted in these animals on the low-Na^+ diet may translate into diminished synthesis of VEGF and placental lactogen that could, in turn, impede vascular growth, fetoplacental circulation and fetal growth. In support of this, as well as the relevance of this animal model for idiopathic IUGR in humans, Li *et al.* (1998) found that AT_1 mRNA and protein decreased in syncytiotrophoblasts and cytotrophoblasts of the placenta in human IUGR.

In summary, we report here that dietary Na^+ restriction has a greater impact on water/electrolyte homeostasis in the rat during pregnancy. Furthermore, Na^+ restriction during the last week of gestation significantly jeopardizes fetal growth and modifies the expression of ANGII receptors in a number of tissues involved in maintaining haemodynamics and sustaining fetal growth. We propose that some of these changes in ANGII receptor expression
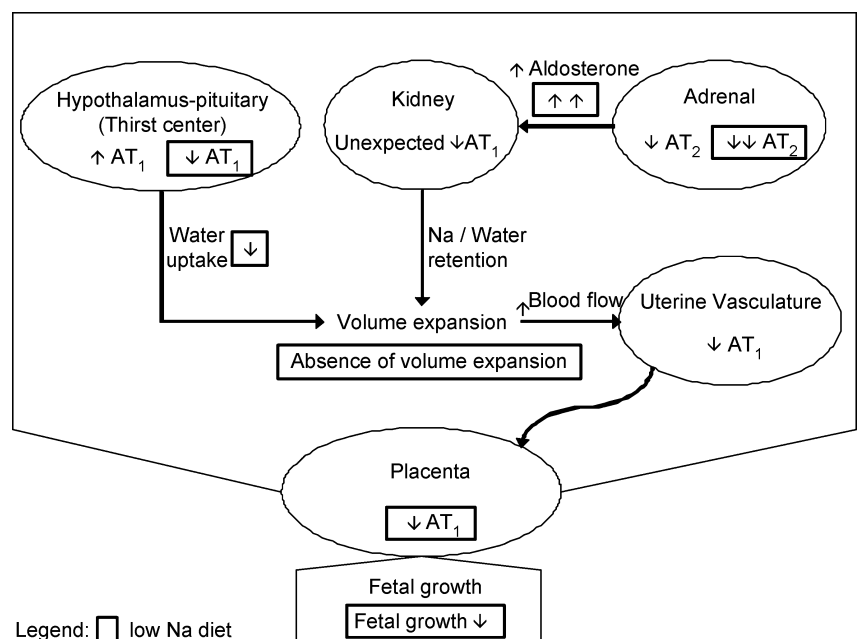
Figure 4. Schematic illustration of interactions between angiotensin receptors, maternal homeostasis and fetal growth
The modifications occurring in pregnant rats are compared to non-pregnant animals. The changes during low-Na^+ diet are illustrated by dotted boxes only if they differ from the pregnant animals on normal diet. See text for detailed discussion.

correlate well with the adaptive vascular mechanisms that come into play during normal gestation while others (noted in our pregnant animals where Na^+ intake is reduced) underlie a maladaptive process that leads to IUGR. In Fig. 4 we summarize some of our findings that show that the increase in AT_1 expression in the hypothalamic–pituitary axis in the normally fed pregnant rats may serve to increase water consumption. At the level of the kidney, the unexpected decrease in AT_1 we see during pregnancy may simply reflect a down-regulatory response. Other renal mechanisms implicating AVP and aldosterone may account for the increase in water retention at the level of the kidney during normal gestation in the rat. In the adrenal cortex, the decrease in AT_2 levels would favour ANGII binding to the AT_1 receptor subtype ultimately leading to an increase in aldosterone secretion which would act on the kidney to increase Na^+ and water retention. These three aforementioned phenomena favour the volume expansion that occurs during normal pregnancy. The increase in mean uterine artery diameter during normal gestation helps maintain normal fetal growth. On the other hand, when dietary Na^+ is restricted during pregnancy, the decrease in AT_1 levels in the hypothalamic–pituitary axis may serve to suppress thirst. At the level of the adrenal cortex, Na^+ restriction results in a greater decrease in AT_2 expression that correlates well with the increase in plasma aldosterone in these animals. However, this increase does not serve to compensate for the decrease in water intake and thus, volume expansion is dramatically reduced. The decreased mean artery diameter we measured in the pregnant rats on the low-Na^+ diet, combined with the diminished levels of AT_1 in the placenta, serve to inhibit fetal growth by restricting uteroplacental circulation and decreasing production of fetal growth factors, respectively. How these findings correlate with those taking place in any clinical conditions of human pregnancy is speculative, considering the present state of characterization of our animal models. However, the model allows the investigation of pregnancy-associated mechanisms, which cannot be studied in human.

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