Interactions between subtotal nephrectomy and salt: effects on blood pressure and renal function in pregnant and nonpregnant ewes

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Submitted 4 December 2006; accepted in final form 23 January 2008

Recent studies by ourselves (1) and others (2, 3, 4) have shown that several well-established models for hypertension in the nonpregnant sheep also cause hypertension when induced acutely in the pregnant ewe. These models include one-clip, one-kidney hypertension (13) and infusion of ACTH (12).

Recent studies have shown that the observed increase in glomerular filtration rate (P < 0.03) and depression of fractional proximal sodium reabsorption (P = 0.003) that occurred in STNxP, but not in STNxNP, in response to high salt may have contributed to this protection. As well, the increased production of vasorelaxants in pregnancy may selectively protect against the occurrence of salt-sensitive hypertension in pregnancy.

IN THE PAST WE HAVE SHOWN that several well-established models for hypertension in the nonpregnant sheep also cause hypertension when induced acutely in the pregnant ewe. These models include one-clip, one-kidney hypertension (13) and infusion of ACTH (12).

It is well recognized in a number of species that high salt intake results in hypertension in nonpregnant animals with reduced kidney mass (3). Similarly, in oophorectomized ewes, it has been reported that hypertension in response to ingestion of ~15 mmol·kg⁻¹·day⁻¹ of salt is exacerbated by prior surgical removal of ~70% of renal mass (20).

The Dahl rat strain can be salt sensitive or salt resistant, depending on blood pressure response to a high-salt diet. Interestingly, while Dahl salt-sensitive (SS) virgin rats had higher blood pressures than Dahl resistant (R) virgin rats, there was no difference in the blood pressures of Dahl SS and Dahl R pregnant rats measured at midgestation (19). Thus pregnancy appears to protect this strain of rat from salt-sensitive hypertension. Therefore we decided to expose our pregnant intact (IntP) and STNxP (STNxNP) ewes to a high salt intake (~20 mmol·kg⁻¹·day⁻¹) to see whether their blood pressures increased and whether the effect was more severe in STNxP. We also studied a cohort of nonpregnant intact ewes (IntNP) and STNx ewes (STNxNP).

METHODS

These experiments were approved by the University of New South Wales Animal Care and Ethics Committee.

Surgery

All surgeries were carried out under general anesthesia induced by intravenous injection of 1–2 g of sodium thiopentone (Pentothal, Abbott Australasia) and maintained with 2–3% halothane (Fluothane, Zeneca) in oxygen. At the end of surgery, incisions were infiltrated with 0.5% bupivacaine HCl (Marcain, Astra Pharmaceuticals). Ewes were given 600 mg of penicillin (Ilium Propen, Troy Laboratories) and 288 mg of oxytetracycline (Alamycin, Norbrook Laboratories) intramuscularly at induction of anesthesia and at completion of surgery. After catheter surgery, ewes also received these antibiotics (im or iv) for at least 2 days postoperatively.

Unilateral nephrectomy and ligation of a branch of a renal artery.

Nonpregnant ewes underwent STNx as described previously (6). Briefly, one kidney was removed through a paravertebral incision. Through another paravertebral incision, at least one branch of a renal artery in the hilus of the other kidney was ligated so that there was a color change in 30–50% of the kidney surface. Animals were held until recovery from surgery and then returned to pasture a week later. STNx and age-matched intact ewes from the same flock were then timed in groups at various times over the next 2 yr. STNx ewes (STNxNP) became pregnant at 9.5–17 mo after nephrectomy. In a separate study, another group of nonpregnant STNxP ewes (STNxNP) were studied at 2–29 mo (mean 9 ± 12 mo) after nephrectomy. An age-matched group of intact ewes (IntNP) were also studied.

Catheter surgery in pregnant and nonpregnant ewes.

At 108–114 days of gestation (term = 150 days), STNxP and IntP were anesthetized and polyvinyl catheters (2.7-mm OD, 1.5-mm ID) were inserted into a maternal femoral artery and vein as described previously (18). Similarly, one femoral artery and both femoral veins were catheterized in STNxNP and IntNP. All vascular catheters were flushed daily with 0.15 M heparinized saline (100 IU heparin/ml, Heparin Injection, Pharmacia & Upjohn). Ewes were housed in metabolic cages, and

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room temperature was maintained between 18 and 23°C. They were given free access to 8 liters of water, 1.2 kg of lucerne chaff, and 300 g of oats per day. No experiments were carried out for ≥6 days after surgery. After an initial experiment carried out while ewes were on a normal salt intake (i.e., no salt was added to their diet), ewes were given a high-salt diet (up to 8 l/day of 0.17 M NaCl in place of their normal drinking water) for 5 days and the experiment was then repeated. In pregnant ewes these experiments were carried out at 122 ± 0.1 days on a low-salt diet and at 127 ± 0.1 days on a high salt intake.

**Experimental Protocol**

Food and fluid intake and urine and feces output were measured every day. Samples of urine were collected and stored at −20°C for further analysis. Ewes were removed from their cages on the mornings of the experiments, and a 14-gauge Foley catheter was inserted into the bladder via the urethra. They were returned to their cages and given an intravenous dose of 150 μmol/kg lithium chloride and 4.8 mg/kg of para-aminophenylurate [PAH, Merck, Sharpe & Dohme (Australia) or Sigma-Aldrich (Australia)]. During the course of the experiment, intravenous infusions of 10 μmol·kg⁻¹·h⁻¹ lithium and 810 mg/h PAH were delivered through a 0.2-μm filter (Minisart) at a rate of 6.3 ml/h (pregnant ewes) or 10 ml/h (nonpregnant ewes). After at least 45 min, urine was collected at 30-min intervals for 1.5–2 h.

Arterial blood samples (5 ml) were taken at the midpoint of the second and third or fourth periods, and 20 IU/ml of heparin were added. Blood pressures were recorded throughout the course of the experiment with a pressure transducer (Easy Vent Deadender Cap, Ohmeda BOC) connected to a Grass polygraph (Quincy, MA) and an IBM-compatible PC.

After the high-salt experiment, ewes were weighed and killed by intravenous injection of 4–5 g of sodium pentobarbate (Lethabarb, Virbac). Kidneys and hearts were removed and weighed.

**Biochemical Analysis**

**Arterial blood samples.** Arterial sodium, potassium, and chloride concentrations were measured with a blood gas analyzer (ABL 715, Radiometer Pacific). Hematocrits were determined in duplicate with a microhematocrit centrifuge and reader (Hettich, Tutlingen, Germany). The remaining blood was centrifuged for 10 min at 1,100 g and 4°C. Plasma and urine samples were stored at −20°C until biochemical analysis.

**Plasma and urine.** Effective renal plasma flow (ERPF) was measured as the renal clearance of PAH by methods already described (1, 6). Urinary levels of sodium and potassium were measured with an FLM3 Flame Photometer (Radiometer). Plasma and urinary osmolalities were measured with a Fiske One-Ten Osmometer (Fiske Associates). The clearance of lithium was used to determine the amounts of sodium reabsorbed by the proximal and distal nephron (14). Lithium is freely reabsorbed by the proximal tubule but not by the distal tubule. Urinary and plasma lithium concentrations were determined with a Varian-Technicon AA5 Atomic Absorption Spectrophotometer (Melbourne, Australia).

Glomerular filtration rates (GFRs) were measured as the renal clearance of endogenous creatinine. Creatinine levels in urine and plasma were measured by Laverty Pathology (Sydney, Australia) or with methods described by Haeckel (7). In pregnant ewes, urinary protein concentrations were determined by the method of Lowry et al. (9). In pregnant ewes, plasma renin levels were measured as the rate of formation of angiotensin I in nanograms per milliliter per hour at 37°C and pH 7.5 in the presence of added nephrectomized sheep substrate (15). Plasma angiotensinogen levels were measured as the amount of angiotensin I formed in plasma in the presence of an excess of human renin (15). Angiotensin I was measured by radioimmunoassay (11).

**Data Analysis and Statistics**

The sodium content of normal drinking water was zero. Sodium intake in food was calculated based on a sodium content in chaff of 33 mmol/kg (4). Twenty-four-hour urine collections were used to determine salt and water balance over the day.

Data are expressed as means ± SE; n is the number of animals. SPSS (Statistical Package for the Social Sciences; SPSS Inc; Chicago, IL) was used to determine means and SE. Values for each variable measured in each experimental period over 1.5–2 h were averaged. Data from pregnant ewes and nonpregnant ewes were analyzed separately. For both pregnant ewes and nonpregnant ewes, simple factorial ANOVA was carried out with SPSS, with experimental treatment (high and low salt intake) and renal integrity (STNx and intact) as the two factors. We determined whether there were differences between intact and STNx ewes, whether salt intake had an effect, and whether the effects were different in STNx compared with intact ewes (i.e., whether there was a 2-way interaction between salt intake and renal integrity). Significance was set at 5%. Linear regression analysis was performed with SPSS.

**RESULTS**

**Morphology**

**Pregnant ewes.** STNxP were heavier than IntP [61.7 ± 2.0 (n = 9) vs. 53.1 ± 3.6 (n = 7) kg; P < 0.05]. They had bigger hearts (307 ± 12 vs. 266 ± 9 g; P < 0.05) and left ventricles (114 ± 4 vs. 90 ± 6 g; P < 0.01). However, there were no differences between the two groups in heart weight-to-body weight ratio or in left ventricular-to-body weight ratio. The left ventricular-to-heart weight ratio was higher in STNxP than in IntP (0.37 ± 0.01 vs. 0.34 ± 0.01; P < 0.05). Total kidney mass was less in STNxP than in IntP (127 ± 2 vs. 171 ± 10 g; P < 0.001), as was the kidney-to-body mass ratio (2.0 ± 0.1 vs. 3.3 ± 0.2 g/kg; P < 0.001).

**Nonpregnant ewes.** Body weight was similar in the two groups [STNxNP 59.6 ± 2.9 (n = 8) and IntNP 57.4 ± 1.9 (n = 7) kg], as were heart and left ventricular weights and left ventricular-to-heart weight ratio. Total kidney mass was lower in STNxNP than in IntNP (100 ± 7 vs. 161 ± 8 g; P < 0.001), as was kidney-to-body mass ratio (1.7 ± 0.1 vs. 2.8 ± 0.1 g/kg; P < 0.001). There was no relationship between time since nephrectomy and kidney-to-body mass ratio in STNxNP.

**Arterial Pressure and Heart Rate**

**Pregnant ewes.** STNxP had higher systolic, mean, and diastolic pressures than IntP (P = 0.02, P < 0.02, P = 0.005; Fig. 1 and Table 1). However, there was no effect of a high salt intake on arterial pressures or heart rates.

**Nonpregnant ewes.** STNxNP had higher mean and diastolic pressures than IntNP (P = 0.03, P = 0.02). They also tended to have higher systolic pressures (P = 0.07). High-salt diet tended to increase diastolic pressure in STNxNP (P = 0.08), and when data from both low- and high-salt diets were combined there was a positive relationship between diastolic pressure and salt balance in STNxNP (r = 0.497, n = 16, P = 0.05; Fig. 2). This relationship was not present in IntNP (r = 0.094, n = 14, P = 0.75). IntP (r = 0.009, n = 11, P = 0.98), or STNxP (r = 0.038, n = 15, P = 0.894).

One STNxNP consumed only 10.7 mmol·kg⁻¹·day⁻¹ of salt when on the high-salt diet, whereas the average of the other STNxNP was 21.0 ± 1.9 mmol·kg⁻¹·day⁻¹ (n = 7). If this...
animal is excluded because its salt intake was too low, the rise in blood pressure with high-salt diet in STNxNP was significant. Systolic pressure rose from $133 \pm 5$ mmHg on low salt to $146 \pm 4$ mmHg on high salt ($n = 7$, $P = 0.03$ by paired t-test); diastolic pressure rose from $86 \pm 3$ to $97 \pm 3$ mmHg ($P < 0.02$); mean arterial pressure rose from $104 \pm 3$ mmHg on low salt to $116 \pm 3$ mmHg ($P < 0.02$). There was no relationship between time since nephrectomy and arterial pressure on either low- or high-salt diet, whether or not this sheep was included.

**Fluid Balance**

*Pregnant ewes.* STNxP drank more fluid ($P < 0.001$) than IntP [STNxP low salt $4.9 \pm 0.4 (n = 9)$ and high salt $7.8 \pm 0.2 (n = 7)$ l/day; IntP low salt $3.7 \pm 0.4 (n = 7)$ and high salt $5.8 \pm 0.6 (n = 7)$ l/day]. The addition of salt to the drinking water stimulated drinking ($P < 0.001$) by the same extent in each group $[1.8 \pm 0.2$-fold increase ($n = 7$) in STNxP vs. $1.6 \pm 0.1$-fold increase ($n = 7$) in IntP]. The difference between daily fluid intake and the amount of fluid excreted as urine (fluid balance) was remarkably similar in STNxP and IntP and was not affected by high salt intake [STNxP low salt $2.8 \pm 0.3 (n = 9)$ and high salt $2.6 \pm 0.3 (n = 7)$ l/day; IntP low salt $2.2 \pm 0.2 (n = 7)$ and $2.7 \pm 0.3 (n = 7)$ l/day].

*Nonpregnant ewes.* STNxP drank more fluid ($P < 0.01$) than IntP [STNxNP low salt $4.2 \pm 0.4$ and high salt $6.7 \pm 0.7$ l/day ($n = 8$); IntP low salt $2.5 \pm 0.2$ and high salt $4.9 \pm 0.8$ l/day ($n = 7$)]. The addition of salt stimulated drinking ($P < 0.001$) by a similar extent in both groups $[1.6 \pm 0.1$-fold

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**Table 1. Mean arterial pressure, heart rate, and composition of maternal blood in intact pregnant and subtotally nephrectomized pregnant ewes on low and high salt intake**

<table>
<thead>
<tr>
<th></th>
<th>IntP Low Salt $n = 7$</th>
<th>IntP High Salt $n = 6$</th>
<th>STNxP Low Salt $n = 9$</th>
<th>STNxP High Salt $n = 6$</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP, mmHg*</td>
<td>$90 \pm 7$ (6)</td>
<td>$84 \pm 5$ (5)</td>
<td>$103 \pm 6$</td>
<td>$104 \pm 4$</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>$126 \pm 9$ (6)</td>
<td>$121 \pm 6$ (5)</td>
<td>$112 \pm 4$</td>
<td>$110 \pm 8$</td>
</tr>
<tr>
<td>Chloride, mmol/l†‡</td>
<td>$112 \pm 2$ (4)</td>
<td>$115 \pm 2$ (4)</td>
<td>$110 \pm 7$</td>
<td>$120 \pm 2$ (4)</td>
</tr>
<tr>
<td>Potassium, mmol/l</td>
<td>$3.8 \pm 0.2$</td>
<td>$3.7 \pm 0.2$</td>
<td>$3.8 \pm 0.1$</td>
<td>$3.9 \pm 0.1$</td>
</tr>
<tr>
<td>Plasma Na-to-K ratio</td>
<td>$39.0 \pm 1.9$</td>
<td>$41.9 \pm 3.3$</td>
<td>$39.1 \pm 1.4$</td>
<td>$39.7 \pm 1.0$</td>
</tr>
<tr>
<td>Bicarbonate, mmol/l</td>
<td>$25.9 \pm 0.8$</td>
<td>$24.0 \pm 1.0$</td>
<td>$28.5 \pm 0.3$</td>
<td>$25.4 \pm 0.6$</td>
</tr>
<tr>
<td>Hemoglobin, g/l†</td>
<td>$83 \pm 3$</td>
<td>$75 \pm 4$</td>
<td>$85 \pm 4$</td>
<td>$75 \pm 6$</td>
</tr>
<tr>
<td>Hematocrit, %†</td>
<td>$24.3 \pm 1.0$</td>
<td>$21.7 \pm 1.0$</td>
<td>$24.6 \pm 1.1$</td>
<td>$21.5 \pm 1.5$</td>
</tr>
<tr>
<td>Creatinine, mmol/l</td>
<td>$0.05 \pm 0.003$</td>
<td>$0.04 \pm 0.003$</td>
<td>$0.07 \pm 0.003$</td>
<td>$0.07 \pm 0.005$</td>
</tr>
<tr>
<td>Plasma renin levels, ng ANG I·mL⁻¹·h⁻¹†‡</td>
<td>$1.7 \pm 0.4$</td>
<td>$0.6 \pm 0.2$</td>
<td>$1.3 \pm 0.6$ (8)</td>
<td>$0.4 \pm 0.1 (6)$</td>
</tr>
<tr>
<td>Angiotensinogen, µg/ml</td>
<td>$1.8 \pm 0.2$</td>
<td>$1.5 \pm 0.1$</td>
<td>$1.8 \pm 0.1$ (8)</td>
<td>$1.8 \pm 0.2$ (5)</td>
</tr>
</tbody>
</table>

Values are means ± SE for $n$ animals (for a value given in parentheses if different from that stated at top of column). MAP, mean arterial pressure. *$P < 0.05$ for differences between intact pregnant ewes (IntP) and subtotally nephrectomized pregnant ewes (STNxP); †$P < 0.05$ for effects of altered salt intake; ‡$P < 0.05$ for interaction between STNx and salt. Actual $P$ values are cited in the text.

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(n = 8) in STNxNP compared with 2.0 ± 0.3-fold (n = 7) in IntNP. Fluid balance was greater (P < 0.001) in STNxNP than IntNP but was not altered by salt intake [STNxNP low salt 2.7 ± 0.3 and high salt 2.7 ± 0.4 l/day (n = 8); IntNP low salt 1.1 ± 0.1 and high salt 1.7 ± 0.1 l/day (n = 7)].

Salt Balance

Pregnant ewes. On a low-salt diet, the intake of sodium was similar in STNxP and IntP [0.59 ± 0.04 (n = 9) and 0.62 ± 0.07 (n = 7) mmol·kg⁻¹·day⁻¹, respectively]. With addition of salt to the water, it increased (P < 0.001) by a similar extent in both groups, so that on the high-salt diet it was 23 ± 1 mmol·kg⁻¹·day⁻¹ (n = 7) in STNxP and 20 ± 3 mmol·kg⁻¹·day⁻¹ (n = 7) in IntP. Although on a low-salt diet sodium balance (measured as the difference over a 24-h period between sodium ingested and sodium excreted in the urine) was close to zero in STNxP (−0.01 ± 0.19 mmol·kg⁻¹·day⁻¹, n = 9) but positive in IntP (0.41 ± 0.14 mmol·kg⁻¹·day⁻¹, n = 7), this difference was not significant. On the high-salt diet, salt balance increased (P < 0.001) and became strongly positive in both groups [STNxP 2.88 ± 0.88 mmol·kg⁻¹·day⁻¹ (n = 7); IntP 3.24 ± 65 mmol·kg⁻¹·day⁻¹ (n = 7)]. It should be noted that these “balance” measurements do not account for any fecal sodium losses, so the degree of positive balance is likely to be overestimated.

Nonpregnant ewes. Salt intake was not affected by renal integrity, but salt intake rose (P < 0.001) when salt was added to the drinking water [STNxNP low salt 0.61 ± 0.04 and high salt 19.7 ± 2.1 mmol·kg⁻¹·day⁻¹ (n = 8); IntNP low salt 0.32 ± 0.03 and high salt 15.2 ± 2.6 mmol·kg⁻¹·day⁻¹ (n = 7)]. Similarly, salt balance (measured as described above) rose (P < 0.001) on high salt [STNxNP low salt 0.13 ± 0.14 and high salt 4.35 ± 0.64 mmol·kg⁻¹·day⁻¹ (n = 8); IntNP low salt 0.21 ± 0.08 and high salt 3.14 ± 0.18 mmol·kg⁻¹·day⁻¹ (n = 7)].

Blood Composition: Plasma Electrolytes and Osmolality

Pregnant ewes. Plasma sodium was higher in STNxP than IntP (Fig. 3, P = 0.001). High salt intake was associated with increased plasma sodium and chloride levels (P < 0.001), and there was a significant interaction between renal integrity and dietary salt intake on sodium and chloride concentrations (P = 0.002, P < 0.03) such that the highest levels were present in STNxP on high salt (Fig. 3, Table 1). There was an interaction between renal integrity and salt intake on plasma osmolality (P = 0.04, Fig. 3) so that plasma osmolality was higher in STNxP than IntP (P = 0.009). Plasma potassium levels and plasma Na-to-K ratio were similar in STNxP and IntP and were not affected by high salt. STNxP had higher plasma bicarbonate levels than IntP (P = 0.008, Table 1). Bicarbonate levels fell with high salt intake (P = 0.001). Nonpregnant ewes. STNxP ewes had higher plasma sodium (P = 0.001) and osmolality (P < 0.001) but lower plasma chloride (P = 0.05) than IntNP (Fig. 3, Table 2). The levels of plasma potassium and bicarbonate and plasma Na-to-K ratio were similar in the two groups. High-salt diet was associated with an increase in sodium (P = 0.003), potassium (P = 0.001), chloride (P < 0.001), and osmolality levels (P = 0.013) but a fall in plasma bicarbonate (P < 0.001) and Na-to-K ratio (P = 0.004). There was an interaction between renal integrity and salt intake on plasma chloride levels (P = 0.05), so that on high salt intake levels were similar in STNxNP and IntNP (Table 2).

Blood Composition: Hemoglobin, Hematocrit, Creatinine, Renin, and Angiotensinogen

Pregnant ewes. Similar declines in hemoglobin levels (P < 0.05) and hematocrit (P = 0.02) occurred in both STNxP and IntP on high salt (Table 1). Plasma creatinine levels were greater in STNxP than IntP (P < 0.001) and did not change with salt. Plasma renin levels were suppressed in each group by high salt (P < 0.03), but angiotensinogen levels did not change (Table 1).

Nonpregnant ewes. Both hemoglobin levels and hematocrit were lower in STNxP than IntNP (P = 0.006 and P = 0.03), but there was no significant effect of high salt intake (Table 2). Plasma creatinine levels were greater in STNxP than IntNP (P < 0.001) and did not change with salt.

Renal Function

Pregnant ewes. STNxP had higher urine flows and sodium and osmolar excretions than IntP (P < 0.004, P = 0.003, P = 0.002; Table 3). High salt intake increased these values in both groups (P < 0.001) but to a greater extent in STNxP (P <
Table 2. Mean arterial pressure, heart rate, and composition of blood in intact nonpregnant and subtotally nephrectomized nonpregnant ewes on low and high salt intake

<table>
<thead>
<tr>
<th></th>
<th>IntNP</th>
<th>STNxNP</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Low Salt (n = 7)</td>
<td>High Salt (n = 7)</td>
</tr>
<tr>
<td>MAP, mmHg*</td>
<td>101 ± 5</td>
<td>106 ± 5</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>80 ± 3</td>
<td>89 ± 0.6</td>
</tr>
<tr>
<td>Chloride, mmol/L†‡</td>
<td>114 ± 1</td>
<td>109 ± 1</td>
</tr>
<tr>
<td>Potassium, mmol/L</td>
<td>3.7 ± 0.1</td>
<td>3.7 ± 0.1</td>
</tr>
<tr>
<td>Plasma Na-to-K ratio†</td>
<td>39.5 ± 1.4</td>
<td>39.5 ± 0.5</td>
</tr>
<tr>
<td>Bicarbonate, mmol/L</td>
<td>28.0 ± 0.2</td>
<td>28.4 ± 0.5</td>
</tr>
<tr>
<td>Hematocrit, %*</td>
<td>29.2 ± 1.0</td>
<td>24.9 ± 1.1</td>
</tr>
<tr>
<td>Creatinine, mmol/L</td>
<td>0.04 ± 0.003</td>
<td>0.09 ± 0.01</td>
</tr>
</tbody>
</table>

Values are means ± SE for n animals. * P < 0.05 for differences between intact nonpregnant ewes (IntNP) and subtotally nephrectomized nonpregnant ewes (STNxNP); † P < 0.05 for effects of altered salt intake; ‡ P < 0.05 for interaction between STN and salt. Actual P values are cited in the text.
ever, unlike in the pregnant ewes, there was no significant effect of high-salt diet on fractional reabsorption of sodium by the proximal tubule.

**DISCUSSION**

On a high salt intake (~20 mmol·kg\(^{-1}\)·day\(^{-1}\)) neither IntP nor STNxP had any change in blood pressure, despite the fact that STNxP had higher arterial pressures than IntP on a normal salt intake (Fig. 1). There was strong evidence that a high salt intake was associated with expansion of the extracellular volume in both STNxP and IntP, because both hemoglobin and hematocrit fell, as did plasma renin levels ($P < 0.03$), and the difference between salt intake and salt excreted in the urine increased markedly ($P < 0.001$). The lack of any potentiating effect of high salt intake on the blood pressures of STNxP was especially striking because their plasma sodium levels and osmolalities were particularly increased on a high salt intake (Fig. 3) compared with levels measured in IntP. Furthermore, in STNxNP there was an increase in blood pressure when salt intake was high and there was a positive relationship between diastolic pressure and salt balance (Fig. 2).

The combination of a remnant kidney and a high salt intake is a well-established model of hypertension in species like the rat (3) and is cited in standard physiological texts as the experimental paradigm that underpins the long-standing theory that hypertension is caused by sustained extracellular volume expansion (17). Sheep have been used to demonstrate that the hypertension that occurs with remnant kidney and high salt intake is due to volume expansion rather than to any vasoactive properties of sodium itself (17). In our nonpregnant sheep we had clear evidence that a high sodium intake increased blood pressure in the presence of reduced renal function (Table 2, Fig. 2). This finding is similar to that of Whitworth et al. (20), who also found that in oophorectomized ewes the combination of high-salt diet and reduced renal mass had an additive effect on blood pressure. Although the reduction in renal mass alone did not cause hypertension in that study (20), GFR was not measured, so it is not clear whether the amount of renal impairment was as severe as in the STNxNP in the present study.

Therefore our observation that STNxP on a high salt intake did not show any further increase in arterial pressure was completely unexpected. The amount of sodium ingested by our pregnant ewes (~20 mmol·kg\(^{-1}\)·day\(^{-1}\)) was similar to that ingested by our nonpregnant ewes and greater than that which caused a rise in blood pressure on either low- or high-salt diet. Although the STNxP had been nephrectomized for a longer time period than many of the ewes in the STNxNP group (2–3 mo), this is unlikely to account for the finding that their arterial pressures were not salt sensitive, because two ewes in the STNxNP group had been nephrectomized for even longer (>2 yr). Furthermore, there was no relationship between time since nephrectomy and arterial pressure on either low- or high-salt diet.

There are several reasons why expansion of the extracellular volume of pregnant sheep may not cause a rise in blood pressure and why severe hypertension did not develop even in STNxP on high salt. First, the capacitance of the cardiovascular system is enormously increased in pregnancy (16), with the growth and development of the uteroplacental circulation (12),

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**Table 4. Renal function in intact nonpregnant and subtotally nephrectomized nonpregnant ewes on low and high salt intake**

<table>
<thead>
<tr>
<th></th>
<th>IntNP</th>
<th>STNxP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low Salt ($n = 7$)</td>
<td>High Salt ($n = 7$)</td>
</tr>
<tr>
<td>Urine flow, ml/min</td>
<td>2.6±0.6</td>
<td>2.8±0.8</td>
</tr>
<tr>
<td>Na(^+) excretion, μmol/min(†)</td>
<td>29±11</td>
<td>606±151</td>
</tr>
<tr>
<td>K(^+) excretion, μmol/min</td>
<td>440±50</td>
<td>371±71</td>
</tr>
<tr>
<td>Osmolar excretion, μosmol/min(†)</td>
<td>1,531±205</td>
<td>2,443±364</td>
</tr>
<tr>
<td>Free water clearance, ml/min(†)</td>
<td>-2.3±0.5</td>
<td>-5.4±0.6</td>
</tr>
<tr>
<td>Urinary Na-to-K ratio</td>
<td>0.06±0.02</td>
<td>2.9±1.3</td>
</tr>
<tr>
<td>ERPF, ml·min(^{-1})·kg body wt (^{-1})</td>
<td>13.9±1.9</td>
<td>15.4±1.4</td>
</tr>
<tr>
<td>GFR, ml·min(^{-1})·kg body wt (^{-1})</td>
<td>3.4±0.3</td>
<td>3.7±0.3</td>
</tr>
<tr>
<td>Fraction of filtered Na load reabsorbed, %(†)</td>
<td>99.89±0.04</td>
<td>97.68±0.60</td>
</tr>
<tr>
<td>Fraction of Na reabsorbed proximally, %(†)</td>
<td>86.2±1.4</td>
<td>79.9±1.8</td>
</tr>
<tr>
<td>Fraction of Na reabsorbed distally, %(†)</td>
<td>13.7±1.4</td>
<td>18.0±1.0</td>
</tr>
</tbody>
</table>

Values are means ± SE for $n$ animals. $*P < 0.05$ for differences between IntNP and STNxNP; $†P < 0.05$ for effects of altered salt intake; $‡P < 0.05$ for interaction between STNx and salt. Actual $P$ values are cited in the text.
the increase in renal blood flow (10), the increased vascularity associated with mammary gland development, and the relaxation of the venous circulation through the action of hormones like progesterone (16).

Second, both IntP and STNxP reduced their plasma renin levels in response to high salt. The consequent fall in angiotensin II levels would have led to a reduction in aldosterone secretion and increased sodium excretion and potassium retention, as evidenced by the rise in the urinary Na-to-K ratio (Table 3). These changes, plus the increase in GFR (Fig. 4) and the fall in fractional reabsorption of sodium by the proximal tubule (Table 3), thus limited the degree to which the extra ingested salt was retained. Together with reduced levels of circulating vasoactive factors (such as angiotensin II), this would have limited the degree of extracellular volume expansion and the amount of vasoconstriction within the circulation.

Third, the production of vascular endothelial factors like nitric oxide are increased in pregnancy, ensuring relaxation of the peripheral vasculature (8). Pregnancy has marked effects on the cardiovascular system of salt-sensitive rat strains (Dahl SS). Virgin Dahl SS rats have higher arterial pressures than virgin Dahl resistant strains (Dahl R), but in midgestation pregnant Dahl SS rats have blood pressures similar to pregnant Dahl R rats. In addition, pregnancy restores the renal vasodilator response to glycine in the Dahl SS rat. Dahl SS virgin rats only had a 12% fall in renal vascular resistance in response to glycine; Dahl R rats had a 30% fall, as did pregnant Dahl SS rats (19). It is claimed that in part this renal vasodilation in response to a high-salt diet protects the Dahl R rat from hypertension (19). Both IntP and STNxP had a rise in GFR on the high-salt diet (Fig. 4). By contrast, there was no increase in GFR in STNxNP (Fig. 4).

Interestingly, we can cause hypertension in pregnant sheep by inflation of an occluder around the renal artery in a remaining kidney and by infusion of ACTH, both well-known animal models of hypertension in the nonpregnant animal (12, 13). Therefore the protection conferred by pregnancy against salt-sensitive hypertension in rats (19) and STNx sheep may be specific for salt-sensitive hypertension.

As stated above, there was strong evidence that both groups of pregnant ewes retained more sodium when on high salt, because the difference between salt intake and urinary sodium loss increased and hematocrit, hemoglobin, and plasma renin levels fell. The greater increase in maternal plasma sodium and osmolality and the interaction between subtotal nephrectomy and high salt (Fig. 3) in STNxP suggested that they were less able (compared to IntP) to maintain osmolar balance (Fig. 3B), possibly because they had insufficient nephrons. Since STNx ewes had one kidney removed and at least 30% of the remaining kidney infarcted, the nephron number must have been <50%.

In conclusion, a high salt intake did not have any adverse effects on the blood pressures and renal function of IntP or STNxP. Thus it appears that pregnancy protects the mother from the hypertensinogenic effects of a high salt intake in the sheep, as is the case in other animals with salt-sensitive hypertension.

**Perspectives and Significance**

These findings may have implications for the pharmacological management of hypertension in human pregnancy. We suggest that because volume loading does not cause high blood pressure in pregnant ewes, even when they have renal impairment, antihypertensive therapies directed at vasodilatation might be more effective than antihypertensive therapies directed at volume depletion (e.g., diuretics) during pregnancy.

**ACKNOWLEDGMENTS**

We acknowledge the technical assistance of Pamela Bode, June Wu, and Vasumathy Kumarasamy.

**GRANTS**

This work was supported by a grant to Scientia Professor E. R. Lumbers from the National Health and Medical Research Council of Australia.

**REFERENCES**