ABSTRACT
The importance of angiotensin II in the regulation of water and electrolyte balance in sheep is questionable. In this trial the effects of an angiotensin-converting enzyme (ACE) inhibitor were quantified in sheep on restricted water intake. Comparing the phase of water restriction only with that of water restriction plus ACE inhibition, significant increases were observed during the latter phase in urine volume, sodium and potassium excretion via the urine, sodium concentration in the plasma and osmolar clearance. Urine osmolality decreased with inhibition of angiotensin II formation while variables such as water, sodium and potassium loss via the faeces were unaffected. Most of the renal effects of ACE inhibition, except the increase in urinary potassium excretion, were explicable in terms of the established functions of angiotensin II. Furthermore, results of this trial indicate that angiotensin II has no significant effect on the intestine in regulating water and electrolyte excretion via the faeces.

Key words: angiotensin-converting enzyme inhibition, angiotensin II, colon, potassium, renal function, sheep, sodium, water.


INTRODUCTION
The importance of the renin–angiotensin–aldosterone cascade in the humoral control of electrolyte and water homeostasis has for many years been recognised in both humans and animals. The subject has received particular attention in humans because of the ultimate effect of the cascade on mean arterial pressure, especially in the long term. Inhibition of the effects of angiotensin II, either by preventing its formation or by antagonising its binding at receptor level, is a frequently used mechanism to combat hypertension in humans. Not only does angiotensin II directly and indirectly (via its effect in stimulating aldosterone release) promote sodium and water retention, but it is also one of the most powerful vasoconstrictors so far discovered in the physiological sciences.

Several experiments in the past have addressed the effects of angiotensin-converting enzyme (ACE) inhibition on renal function in sheep under various conditions such as in the dehydrated animal, in sodium-restricted sheep and in sheep treated with adrenocorticotropic hormone. Failure of angiotensin II inhibition to result in an increase in urinary sodium excretion in sheep on a low sodium diet led to speculation that sheep may be less dependent on angiotensin II in regulating sodium excretion than are other species. However, infusion of dehydrated sheep with the ACE inhibitor, D-3 -mercapto-2 methylpropio- noyl-L-proline (Captopril, Bristol-Myers Squibb), did produce a rise in urinary sodium excretion over the 60–80-minute monitoring period following infusion.

The mammalian colon and the distal part of the nephron tubule are similar in that the final modification of water and sodium, in the digesta and renal filtrate respectively, occurs in these regions, according to the hydration and sodium status of the animal. Many of the hormones that act on the nephron tubule also act on the colon with similar effects to those in the kidney.

In the current trial we investigated the effects of inhibiting angiotensin II on several plasma variables and on both the urinary and faecal excretion of water and electrolytes over several days in sheep that were restricted in their water intake.

MATERIALS AND METHODS
South African Mutton Merino wethers (n = 6) were used in this trial. The animals were approximately 12 months of age and weighed between 29.6 and 36.5 kg.

The sheep were individually housed in metabolic crates in a room where the ambient temperature was maintained at 22 °C. They were fed a diet of lucerne hay ad libitum throughout the trial. Each animal was fitted with a faecal collection bag and urine was collected in a refrigerated container under the crate.

The sheep were allowed 1 week to become accustomed to conditions in the crates. Over this period they were allowed free access to drinking water. The duration of the trial itself was 3 weeks, each successive phase lasting 5 days with a 2-day interval between phases.

During the 1st week (Phase 1), drinking water was available ad libitum. The daily water intake of each animal was recorded, and at the end of the week the average water intake per sheep per day was calculated. During the 2nd week (Phase 2), the daily water intake of the animals was restricted to half. The same water restriction was imposed during the 3rd week (Phase 3), but during this week each sheep was also treated twice daily with 25 mg Captopril given intravenously via the jugular vein. Between phases 2 and 3 the sheep were allowed free access to drinking water.

The sheep were weighed weekly. Where excretion of a substance was expressed on a mass-specific basis (e.g. Na excreted via urine per kg per day), the metabolic mass, i.e. mass, was used to minimise the effect of rumen fill on body mass.

Samples were collected daily (early morning) over 3 successive days in each phase of the trial. The 1st samples were taken only 48 hours after the sheep had been on a particular treatment to allow time for the treatment to take effect and to ensure that the samples were in fact representative of that treatment.

Feed intake, water intake (Phase 1), and
faecal output were recorded daily. Samples of faeces were retained daily for estimation of faecal moisture content and sodium and potassium concentrations. Urine output was recorded daily and samples were retained for later analyses of sodium and potassium concentrations and osmolarity.

Blood was collected daily (08:00–09:00) by venepuncture of the jugular vein into chilled 10 ml evacuated tubes containing Li-heparin (Vacutainer, Becton Dickenson Vacutainer Systems), and plasma was obtained by centrifugation in a refrigerated centrifuge.

Faecal moisture content was measured according to the method of Jones (1984)15. Dry faecal samples from each sheep were pooled for a phase, and sodium and potassium concentrations were determined on these pooled samples using flame spectrophotometry (Flame Analyser FH-500, Gallenkamp). Osmolarity of the urine and plasma samples was measured by the freezing-point depression method using a micro-osmometer (Roebeling, type 12/12DR, Hermann Roebeling Messtechnik). Sodium and potassium concentrations in plasma and urine were determined by a selective ion electrode method (Instrumentation Laboratory System 501, Instrumentation Laboratory).

Calculations

Osmolar clearance (Cosm), a value equal to the amount of urine that would have been produced had it been of the same osmolarity as plasma, was calculated as follows16:

\[
C_{\text{osm}} = \frac{U_{\text{osm}} \times U_{\text{vol}}}{P_{\text{osm}}}
\]

where \(U_{\text{osm}}\) = volume of urine (l/d); \(U_{\text{vol}}\) and \(P_{\text{osm}}\) are the osmolalities (mOsm/l) of urine and plasma respectively.

Free water clearance (\(C_{\text{fvol}}\)) is equal to the difference between osmolar clearance and urine volume and the value represents the volume of solute-free water resorbed, i.e. water not resorbed by osmotic drag (if a negative value) or that fails to be resorbed (if a positive value) by the nephron tubule17.

\[
C_{\text{fvol}} = C_{\text{osm}} - U_{\text{vol}} \times V/d.
\]

Statistical analysis

Results are given as mean values ±SD for all the sheep over a particular phase. The results were analysed using a 1-way repeated measures analysis of variance as an indication of significant differences between treatments. Differences were accepted as being significant at values of \(P < 0.05\). To isolate treatments that differ significantly from each other, a multiple comparison procedure was used. The Microsoft Jandel Sigma Stat programme was used in the statistical analyses.

Ethical considerations

Permission to proceed with this trial was obtained from the Ethics Committee of the Faculty of Veterinary Science, University of Pretoria.

RESULTS

Water intake during phase 1 (unrestricted drinking water), averaged 4.373 (± 0.639) l per sheep per day. During the phases of water restriction each sheep received only 2.5 l of drinking water per day, all of which was always consumed, amounting to intakes of 0.4–0.6 of their ad libitum intakes.

Feed intake dropped by approximately 100 g per day per sheep as a result of water restriction, little difference being apparent as a result of ACE inhibition imposed on water restriction. The values for plasma variables appear in Table 1.

Inhibition of the angiotensin-converting enzyme had no significant effect on plasma sodium concentration or plasma osmolarity in the water-restricted sheep. However, when the water restriction phases (phases 2 and 3) were compared with the control phase (phase 1), water restriction was shown to cause a significant rise in both variables. Plasma potassium concentrations remained constant irrespective of treatment.

Urine volumes and osmolarities, as well as electrolyte excretion via the urine, appear in Table 2. Inhibition of angiotensin II formation during the period of water restriction restored urine volumes back to similar values obtained when water was available ad libitum.

Urinary sodium concentrations, as well as daily sodium excretion via the urine, were significantly increased by the administration of Captopril to the water-restricted sheep.

Although urine potassium concentrations were significantly lower with the imposition of ACE inhibition on water restriction, values for daily potassium excretion via the urine were significantly higher.

Urine osmolarity fell significantly with angiotensin II inhibition in the water-restricted sheep, although this variable was still significantly higher than in the control animals on ad libitum water intake. Calculated values of variables which more specifically relate to renal function are recorded in Table 3.

Osmolar clearance was significantly raised in response to ACE inhibition in the water-restricted sheep, while free water clearance underwent no significant change.

Table 4 contains values for variables

<table>
<thead>
<tr>
<th>Phase 1</th>
<th>Phase 2</th>
<th>Phase 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>PhNa (mmol/l)</td>
<td>140±(0.8)</td>
<td>147±(2.4)</td>
</tr>
<tr>
<td>PhK (mmol/l)</td>
<td>4.3* (0.3)</td>
<td>4.4* (0.3)</td>
</tr>
<tr>
<td>Plosm (mOsm/l)</td>
<td>298±(1.9)</td>
<td>311±(6.9)</td>
</tr>
</tbody>
</table>

Mean values [(SD); n = 6] over 3 days are given. *Values in rows with different superscripts differ significantly from each other at the \(P < 0.05\) level.

Table 2: Daily urine volume (Uvol), concentrations of sodium (UNa), and potassium (UK) in urine, total sodium (UNa) and mass specific sodium (UNa/mass) and potassium (UK/mass) excretion per day via the urine, total potassium (UPotassium) and mass specific potassium (UPotassium/mass) excretion per day via the urine and urine osmolarity (Uosm), in sheep during phase 1 (control – ad libitum water), phase 2 (water restriction) and phase 3 (water restriction + ACE inhibitor) of the experiment.

<table>
<thead>
<tr>
<th>Phase 1</th>
<th>Phase 2</th>
<th>Phase 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>UNa (l/d)</td>
<td>1.002* (0.109)</td>
<td>0.808* (0.081)</td>
</tr>
<tr>
<td>UKg* (g/d)</td>
<td>0.078* (0.008)</td>
<td>0.059* (0.006)</td>
</tr>
<tr>
<td>UNa (mmol/l)</td>
<td>14.2* (8.7)</td>
<td>24.8* (9.1)</td>
</tr>
<tr>
<td>UNa (mmol/d)</td>
<td>601* (45)</td>
<td>686* (57)</td>
</tr>
<tr>
<td>UK (mmol/d)</td>
<td>14* (9)</td>
<td>23* (8)</td>
</tr>
<tr>
<td>UNa/kg (mmol/kg)</td>
<td>1.10* (0.68)</td>
<td>1.67* (0.55)</td>
</tr>
<tr>
<td>UNa/kg (mmol/d/kg)</td>
<td>600* (63)</td>
<td>551* (45)</td>
</tr>
<tr>
<td>UK/kg (mmol/d/kg)</td>
<td>46.4* (4.8)</td>
<td>40.3* (3.1)</td>
</tr>
<tr>
<td>Uosm (mOsm/l)</td>
<td>1827* (82)</td>
<td>2442* (159)</td>
</tr>
</tbody>
</table>

Mean values [(SD); n = 6] over 3 days are given. *Values in rows with different superscripts differ significantly from each other at the \(P < 0.05\) level.
pertaining to faecal loss of water and electrolytes. No significant differences were observed in any of these variables between the water-restriction phases, irrespective of whether or not Captopril was administered.

**DISCUSSION**

The aim of the trial was to investigate the effects of inhibiting angiotensin II formation on water and electrolyte excretion in water-restricted sheep. It was reasoned that in water-restricted animals, the RAA axis would be activated in order to prevent or minimise a state of hypovolaemia and hypotension. As hypovolaemia is a stimulus for renin secretion and the subsequent formation of angiotensin II 

<table>
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<tr>
<th>Phase 1</th>
<th>Phase 2</th>
<th>Phase 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Faec. mass (g/sheep/d)</td>
<td>1789±(411)</td>
<td>1476±(138)</td>
</tr>
<tr>
<td>Faec H₂O (%)</td>
<td>71±(4)</td>
<td>66±(4)</td>
</tr>
<tr>
<td>Faec H₂O loss (m/sheep/d)</td>
<td>0.2±(0.095)</td>
<td>0.16±(0.073)</td>
</tr>
<tr>
<td>Faec NaCl (%) m/m%</td>
<td>0.4±(0.231)</td>
<td>0.21±(0.051)</td>
</tr>
<tr>
<td>Faec Na (mmol/sheep/d)</td>
<td>46±(24)</td>
<td>36±(15)</td>
</tr>
<tr>
<td>Faec K (mmol/sheep/d)</td>
<td>53±(32)</td>
<td>27±(7)</td>
</tr>
</tbody>
</table>

Mean values ([SD]; n = 6 sheep) over 3 days are given.

*Values in rows with different superscripts differ significantly from each other at the P < 0.05 level.

On the other hand, in sheep that were deprived of water for 3 days, urinary sodium excretion increased significantly over a 40-minute observation period during which Captopril was administered intravenously in bolus form.[6,19] Also, in ovine models where heart failure was induced by rapid ventricular pacing, low urinary sodium output was significantly increased by ACE inhibition.[5]

In the current experiment the administration of an ACE inhibitor to water-restricted sheep resulted in significantly increased natriuresis. One of the functions of angiotensin II is to increase sodium resorption from the proximal convoluted tubule (PCT). Most of the water resorption in the PCT occurs by osmotic drag caused by the diffusion of sodium ions into the renal tubular epithelium of this section of the nephron tubule.[30] Thus, with ACE inhibition, decreased sodium resorption not only accounts for the increased urinary sodium excretion, but also explains the significantly elevated values for urine volume and osmolar clearance observed in this trial. There was, however, no significant difference in solute-free water resorption between phases 2 and 3 of the trial. The values of this variable are positively correlated with plasma concentrations of anti-diuretic hormone (ADH), but these were not assessed in this trial.

Urinary potassium concentrations were significantly higher during phase 2 compared to phases 1 and 3. In spite of this, significantly more potassium was lost via the urine during phases 1 and 3 due to higher urine flow rates during these phases. Aldosterone stimulates counter-transport of sodium and potassium in the collecting duct, the sodium being reabsorbed and the potassium being excreted.[31] As aldosterone release is stimulated by angiotensin II, inhibition of angiotensin II formation would predictably result in lower concentrations of plasma aldosterone and therefore decreased loss of potassium. The lower urinary potassium concentrations obtained with angiotensin II inhibition (Phase 3), are in agreement with this function of aldosterone, but the higher loss of urinary potassium differs from results obtained in previous experiments.[6,19] In spite of this increase in kaliuresis during the phase of ACE inhibition, while dietary intake of potassium remained unchanged, plasma potassium concentrations did not change. This is possibly due to supplementation of plasma potassium from intracellular sources during the period of higher potassium loss.

From the results obtained for the faecal samples, namely similar values for sodium, potassium and water excretion via
the faeces in the water-restriction phases, irrespective of whether or not Captopril was dosed, it appears that angiotensin II has no nett effect on the reabsorption of these substances in the intestine.

Most of the results obtained in this experiment indicate that the effect of angiotensin II on kidney function in water-restricted sheep is in conformity with the known functions of angiotensin II in humans. The possible exception was the significantly higher kaliuresis obtained during angiotensin II inhibition, but this can be partially explained by the higher urine flow rates during this phase.

ACKNOWLEDGEMENTS

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REFERENCES