The effect of an angiotensin-converting enzyme inhibitor on water and electrolyte balance in water-restricted sheep

R A Meintjes[a] and H Engelbrecht[a]

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 osmolarity 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.

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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[14]. 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[18]. 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[7]. 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[9].

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[13,19,20]. 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[13]. However, infusion of dehydrated sheep with the ACE inhibitor, D-3 -mercapto-2 methylpropanoyl-L-proline (Captopril, Bristol-Myers Squibb), did produce a rise in urinary sodium excretion over the 60–80-minute monitoring period following infusion[20].

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[17]. Many of the hormones that act on the nephron tubule also act on the colon with similar effects to those in the kidney[4].

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$^{0.75}$, 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

[a]Department of Veterinary Physiology, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort, 0110 South Africa.

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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)[11]. 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)[2]. Osmolarity of the urine and plasma samples was measured by the freezing-point depression method using a micro-osmometer (Roebling, type 12/12DR, Hermann Roebling 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 (C_{osm}), 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 follows[15]:

$C_{osm} = U_{vol}.U_{osm} / Pl_{osm}$ ℓ/d,

where U_{vol} = volume of urine (ℓ/d); U_{osm} and Pl_{osm} are the osmolarities (mOsm/ℓ) of urine and plasma respectively.

Free water clearance (C_{H_2O}) 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 tubule[15].

$C_{H_2O} = C_{osm} - U_{vol}$ ℓ/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.*

Urine 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 1: **Concentrations of sodium** ($Pl_{[Na]}$) **and potassium** ($Pl_{[K]}$) **in plasma and plasma osmolarity** (Pl_{osm}) **in sheep during phase 1** (control – *ad libitum* water)**, phase 2** (water restriction) **and phase 3** (water restriction + ACE inhibitor) **of the experiment.**

	Phase 1	Phase 2	Phase 3
$Pl_{[Na]}$ (mmol ℓ)	140[a] (0.8)	147[b] (2.4)	149[b] (1.9)
$Pl_{[K]}$ (mmol/ℓ)	4.3[a] (0.3)	4.4[a] (0.3)	4.4[a] (0.3)
Pl_{osm} (mOsmol/ℓ)	298[a] (1.9)	311[b] (6.9)	316[b] (5.4)

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

Table 2: **Daily urine volume** (U_{vol}) **, concentrations of sodium** ($U_{[Na]}$) **and potassium** ($U_{[K]}$) **in urine, total sodium** ($U_{vol}.U_{[Na]}$) **and mass specific sodium** ($U_{vol}.U_{[Na]}$/kg*) **excretion per day *via* the urine, total potassium** ($U_{vol}.U_{[K]}$) **and mass specific potassium** ($U_{vol}.U_{[K]}$/kg*) **excretion per day *via* the urine and urine osmolarity** (U_{osm})**, in sheep during phase 1** (control – *ad libitum* water)**, phase 2** (water restriction) **and phase 3** (water restriction + ACE inhibitor) **of the experiment.**

	Phase 1	Phase 2	Phase 3
U_{vol} (ℓ/d)	1.002[a] (0.109)	0.808[b] (0.081)	1.014[a] (0.048)
U_{vol}/kg* (ℓ/d)	0.078[a] (0.008)	0.059[b] (0.006)	0.071[a] (0.004)
$U_{[Na]}$ (mmol/ℓ)	14.2[a] (8.7)	24.8[a] (9.1)	37.9[b] (9.8)
$U_{[K]}$ (mmol/ℓ)	601[a] (45)	686[b] (57)	645[a] (32)
$U_{vol}.U_{[Na]}$ (mmol/d)	14[a] (9)	23[a] (8)	41[b] (11)
$U_{vol}.U_{[Na]}$ /kg*(mmol/d/kg)	1.10[a] (0.68)	1.67[a] (055)	2.87[b] (0.71)
$U_{vol}.U_{[K]}$ (mmol/d)	600[ab] (63)	551[a] (43)	652[b] (37)
$U_{vol}.U_{[K]}$ /kg*(mmol/d/kg)	46.4[a] (4.8)	40.5[b] (3.1)	45.7[a] (3.0)
U_{osm} (mOsm/ℓ)	1827[a] (82)	2442[b] (159)	2104[c] (105)

Mean values [(SD); $n = 6$] over 3 days are given.
[a,b]Values in rows with different superscripts differ significantly from each other at the $P < 0.05$ level.
*Per kg metabolic mass.

Table 3: **Osmolar clearance** (C_{osm}) **and solute free water reabsorbed** (C_{H_2O})**, in sheep during phase 1** (control – *ad libitum* water)**, phase 2** (water restriction) **and phase 3** (water restriction + ACE inhibitor) **of the experiment.**

	Phase 1	Phase 2	Phase 3
Cosm (ℓ/d)	6.118[a] (0.467)	6.255[a] (0.383)	7.030[b] (0.358)
C_{H_2O} (ℓ/d^1)	–5.115[a] (0.373)	–5.452[ab] (0.322)	–5.998[b] (0.334)

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

Table 4: **Faecal mass per day** (Faec. mass)**, faecal moisture content** (Faec H_2O)**, water loss *via* faeces** (Faec H_2O loss)**, faecal Na and K concentration** ($Faec_{[Na]}$ and $Faec_{[K]}$, respectively) **and daily loss of Na and K *via* the faeces** (Naex.faec and Kex.faec respectively) **in sheep during phase 1** (control – *ad libitum* water)**, phase 2** (water restriction) **and phase 3** (water restriction + ACE inhibitor) **of the experiment.**

	Phase 1	Phase 2	Phase 3
Faec. mass (g/sheep/d)	1789[a] (411)	1476[b] (138)	1421[b] (181)
Faec H_2O (%),			
Faec H_2O loss (mℓ/sheep/d)	71[a] (4)	66[b] (4)	65[b] (4)
$Faec_{[Na]}$ (% m/m)*	0.2[a] (0.095)	0.16[a] (0.073)	0.17[a] (0.044)
$Faec_{[K]}$ (% m/m)*	0.4[a] (0.231)	0.21[a] (0.051)	0.27[a] (0128)
Na ex.faec (mmol/sheep/d)	46[a] (24)	36[a] (15)	35[a] (9)
K ex.faec (mmol/sheep/d)	53[a] (32)	27[a] (7)	33[a] (16)

Mean values [(SD); n = 6 sheep] over 3 days are given.
[a,b]Values in rows with different superscripts differ significantly from each other at the $P < 0.05$ level.
*On a dry matter basis.

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[12], plasma concentrations of angiotensin II are more likely to be elevated in water-restricted animals. It follows that the effect of inhibiting angiotensin II formation would be more marked in such animals than in animals with free access to water.

A 'cross-over' type design was specifically avoided because environmental conditions regarding temperature and humidity were kept constant throughout all phases. More importantly however, in our experience, housing animals together in 1 room, under conditions where some animals have free access to drinking water while others are water-restricted, imposes severe stress on the latter group. Plasma cortisol levels are predictably raised in animals under stress, a factor that in itself could affect water and electrolyte balance[3].

In previous experiments where the effect of an ACE inhibitor on renal function was investigated in sheep, samples were taken either during the continuous i.v. infusion of inhibitor[13], or very shortly (less than 200 minutes) after the bolus dosage of inhibitor[19,20]. However, Fitzpatrick *et al.*[6] compared the effects of intermittent (twice daily) and continuous Captopril administration on some aspects of renal function. They concluded that, although serum ACE concentrations fluctuated markedly in the intervals between bolus dosages of the enzyme inhibitor, there were no significant differences between the renal effects of intermittent and continuous ACE inhibition[6].

The effects of an ACE inhibitor on sodium excretion *via* the urine appears to vary according to the conditions of the experiment. When Captopril was administered to sheep on a low sodium diet, no change in urinary sodium excretion was observed[13]. Similarly, an inability to demonstrate an increase in urinary sodium excretion after Captopril administration to normal sheep and to sheep pretreated with adreno-corticotrophic hormone (ACTH), led to the suggestion that sheep may be less dependent than other species on the role of angiotensin II in sodium homeostasis[19].

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[20]. Also, in ovine models where heart failure was induced by rapid ventricular pacing, low urinary sodium output was significantly increased by ACE inhibition[6].

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[10]. 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[1].

Urine 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[16]. As aldosterone release is stimulated by angiotensin II[5], 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[6,19,20]. 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[8]. 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.

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REFERENCES

1. Bie P 1980 Osmoreceptors, vasopressin and control of renal water excretion. *Physiological Reviews* 60: 981–1048
2. Bock R 1979 Oxidising procedures. In *A Handbook of decomposition methods in analytical chemistry* (1st edn). International Textbook Company, Glasgow: 142–144
3.. Boissy A, Le Neidre P 1997 Behaviour, cardiac and cortisol response to brief peer separation and reunion in cattle. *Physiology and Behaviour* 61: 693–699
4. Dawson C D 1991 Ion channels and colonic salt transport. *Annual Review of Physiology* 53: 321–329.
5. Elliot M E, Siegel F L, Hadjokas N E, Goodfriend T L 1985 Angiotensin effects on calcium and steroidogenesis in adrenal glomerulosa cells. *Endocrinology* 116: 1051–1059
6. Fitzpatrick M A, Rademaker M T, Frampton C M, Espiner E A, Yandle T G, A'Court G, Ikram H 1990 Renal effects of ACE inhibition in ovine heart failure: a comparison of intermittent and continuous ACE inhibition. *Journal of Cardiovascular Pharmacology* 16: 629–635
7. Gans R O B, Hoorntje S J, Donker A J M 1988 Renal effects of angiotensin-I converting enzyme inhibitors – a review. *Netherlands Journal of Medicine* 32: 247–264
8. Guyton A 1991 *Textbook of medical physiology* (8th edn.) W B Saunders, Philadelphia
9. Handa R K, Johns E J 1985 Interaction of the renin angiotensin system and the renal nerves in the regulation of rat kidney function. *Journal of Physiology* 369: 3111–3121
10. Harris P J, Navar G 1985 Tubular transport responses to angiotensin. *American Journal of Physiology* 248: F621–F630
11. Jones C E 1984 Animal feeds. In *Official methods of analysis of the association of official analytical chemists* (14th edn). American Organisation of Analytical Chemists, Washington DC: 152–159
12. Kirscheim H, Ehmke H, Persson P 1990 Role of blood pressure in the control of renin release. *Acta Physiolgica Scandinavica* 139: Supplement no. 591: 40–45
13. Nelson M A, Stewart K W, Coghlan J P, Denton D A, Fei D T W, Scoggins B A 1982 A comparison of the effects of two angiotensin converting enzyme inhibitors, SQ 14 225 and MK 422 in Na-restricted sheep. *Clinical and Experimental Pharmacology and Physiology* Supplement no. 7: 87–91
14. Reid I A, Morris B J, Ganong W F 1978 The renin–angiotensin system. *Annual Review of Physiology* 40: 377–410
15. Rose B D 1986 New approach to disturbances in the plasma sodium concentration. *American Journal of Medicine* 81: 1033–1040
16. Sansom S C, O'Neil R G 1985 Mineralocorticoid regulation of apical cell membrane sodium and potassium transport of the cortical collecting duct. *American Journal of Physiology* 248: F858–F868
17. Schultz S G 1984 A cellular model for active sodium absorption by the mammalian colon. *Annual Review of Physiology* 46: 435–451
18. Vander A, Sherman J, Luciano D 1998 *Human physiology* (7th edn). McGraw-Hill, Boston
19. Whitworth J A, Hammond T G, Stewart K W, Mason R T, Schneider E G, Denton D A, Coghlan J P, Scoggins B A 1982 Effects of converting enzyme inhibition with Captopril on renal function in normal and ACTH treated sheep. *Clinical and Experimental Pharmacology and Physiology* 9: 505–509
20. Yesberg N E, Henderson M, Dallemagne C, Law S, Hamilton D, Cross R B 1984 Converting-enzyme inhibition and l-sarcosine-8-isoleucine-angiotensin II: effects on renal function in the dehydrated sheep. *Quarterly Journal of Experimental Physiology* 69: 133–143