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The urinary excretion of purine derivatives in sheep is not influenced by long term treatment with vasopressin*

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ABSTRACT

This study examines the influence of vasopressin (1-desamino-8-D-arginine vasopressin (dDAVP)) on the urinary excretion of purine derivatives in sheep. Subcutaneous injections of 12.5 μg of dDAVP given twice daily started 10 days before the measurements of renal functions. The urinary excretion of total purine derivatives was significantly lower in the dDAVP group (1.43 ± 0.08 vs 2.18 ± 0.18 $\mu mol \cdot min^{-1}$, $P<0.01$). Similar results were obtained for excretion of allantoin (0.90 ± 0.07 vs 1.44 ± 0.10 $\mu mol \cdot min^{-1}$, $P<0.001$), uric acid (0.30 ± 0.02 vs 0.45 ± 0.05 $\mu mol \cdot min^{-1}$, $P<0.05$) and xanthine (0.015 ± 0.002 vs 0.008 ± 0.002 $\mu mol \cdot min^{-1}$, $P<0.05$). The urinary excretion of hypoxanthine was unaffected by dDAVP administrations (0.22 ± 0.01 vs 0.28 ± 0.03 $\mu mol \cdot min^{-1}$, NS). The clearance protocol showed a decreased urine flow rate (1.40 ± 0.12 vs 3.58 ± 0.44 $ml \cdot min^{-1}$, $P<0.001$) without changes in glomerular filtration rate due to dDAVP. The urine urea concentration was significantly higher in the dDAVP group (18.71 ± 1.49 vs 39.81 ± 4.90 $mmol \cdot l^{-1}$, $P<0.01$). We conclude that vasopressin did not affect excretion of purine derivatives by the kidneys of sheep. The lower amount of total purine derivative excretion after treatment with vasopressin is a reflection of the urinary flow rate and water reabsorption.

KEY WORDS: purine derivatives, allantoin, vasopressin, kidney, water reabsorption, sheep

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INTRODUCTION

In ruminants dietary nucleic acids are extensively degraded in the rumen, and duodenal purines appear to originate predominantly from rumen microorganisms (McAllan and Smith, 1973). Nucleic acid bases, nucleosides and nucleotides present in duodenal digesta are degraded during passage through the intestinal mucosa and generally absorbed as nucleosides (McAllan, 1982). The metabolism of purine bases into allantoin consists of a series of reactions involving hypoxanthine, xanthine, uric acid and allantoin, all of which are excreted in urine, with allantoin constituting the greatest proportion. A number studies (Topps and Elliot, 1965; Antoniewicz et al., 1980; Verbič et al., 1990) have demonstrated that measurements of urinary excretion of allantoin, hypoxanthine, uric acid and xanthine could potentially be used as the basis for a non-invasive index of microbial protein supply in sheep and dairy cows. However, not all purine metabolites excreted in ruminant urine originate from metabolism of absorbed purines. During tissue nucleic acid turnover a proportion of purine bases are not salvaged and reutilized but enter catabolic pathways, constituting an endogenous loss. Allantoin, uric acid, xanthine and hypoxanthine can all be recovered from the urine of ruminants in varying proportions depending on the nutrition of the animal. More recent work has confirmed a close correlation between the intake of digestible dry matter or organic matter and urinary allantoin excretion in cattle (Vercoe, 1976), sheep (Antoniewicz et al., 1981) and goats (Lindberg, 1985). Lindberg (1989) showed that the endogenous urinary excretion of purine derivatives in a young growing ruminant was only marginally affected by large variations in protein supply, and also by the level of intake of milk. We demonstrated in our previous paper (Faix et al., 1988) that the kidneys of sheep regulate urea excretion (the main protein metabolite in ruminants) dependent upon protein and energy intake. During a low protein diet, renal excretion of urea is reduced by a decreased filtered load of urea and an enhanced capacity for urea reabsorption from tubules and the renal pelvis (Cirio and Boivin, 1990). The effect of highly-concentrated urine induced by vasopressin administration in sheep which are fed a high protein diet does not result in a rise of glomerular filtration rate, but this is a possible response to recycling of urea nitrogen through the digestive tract (Boldižárová et al., 1999).

The aim of this study was to investigate the effect of the formation of highly-concentrated urine induced by a vasopressin administration on the urinary excretion of purine derivatives in sheep.

MATERIAL AND METHODS

Animals and diets

The experiments were carried out on twelve female sheep of Polish Lowland breed weighing from 40 to 45 kg. The sheep were housed individually and had free access to water and minerals. The animals were fed a diet for at least 3 weeks

before the measurement of kidney functions. The total daily ration for each sheep consisted of 40 g of rapeseed oilmeal, 400 g of barley and 800 g of hay, containing 1103 g of dry matter and 148.3 g of crude protein.

Experimental design

The animals were divided into two groups, six control sheep and six sheep treated with vasopressin analogue. The experimental group was given subcutaneous injections of 12.5 μg 1-desamino-8-D-arginine vasopressin (dDAVP, Adiuretin-SD, Ferring-Léčiva a.s., Prague), a synthetic analogue of vasopressin with prolonged effects, twice daily in 125 μl of glycerol. The treatment began ten days before the measurements of kidney functions. The control group received injections of glycerol for the same period only. The renal functions were measured by a standard clearance technique on conscious sheep fixed in cages. The right jugular vein was cannulated with a polyethylene capillary and used for the infusion of pyrogen-free inulin (Sigma) dissolved in sterile 0.15 mol NaCl. The priming dose of inulin solution (1g in 50 ml) was injected through a jugular cannula and then a continuous infusion of inulin (6.6 $mg \cdot ml^{-1} \cdot min^{-1}$) was initiated.

Sample collection

Samples of urine were quantitatively collected into calibrated glass cylinders through a Foley catheter placed in the urinary bladder. Three collection periods were set up for every animal. The first urine collection period began 90 min after the start of inulin infusion to allow its equilibrium in the extracellular space. Each collection period lasted 30 min. Blood was sampled from the left jugular vein into heparinized tubes at the mid-point of every urine collection period.

Analytical methods

Inulin was measured in urine and blood plasma samples using the Heyrovsky method (1957) and urea using the Conway method (1962). Separation and quantification of purine derivatives (allantoin, uric acid, xanthine and hypoxanthine) in urine were achieved using the modified HPLC method using two Nova-Pak C_{18} columns (Czauderna and Kowalczyk, 2000). The osmolality of plasma and urine were determined cryoscopically on a Knauer osmometer.

Statistical analysis

The statistical significance of the differences between control values and values obtained from the dDAVP treated group was assessed using Student's t-test. The results are given as the arithmetic means and standard error of means (SEM).

RESULTS AND DISCUSSION

The study of the urinary excretion of purine derivatives by sheep (Table 1) shows that urine concentrations of allantoin, uric acid and hypoxanthine were significantly higher after vasopressin administration. The urine concentration of xanthine was also higher, but without significant differences compared with control sheep. Higher concentrations of purine derivatives are the result of more concentrated urine, which is documented by higher urine osmolality (241.30 ± 36.85 vs 411.40 ± 26.41 mosm · kg^{-1} H_2O; $P<0.01$).

TABLE 1

The effect of vasopressin analogue (dDAVP) administration on the urine concentration and urine excretion of purine metabolites in sheep

Indices	Control group	dDAVP group	P<
Allantoin, μmol · l^{-1}	420 ± 60	690 ± 80	0.05
Amount of allantoin excreted, μmol · min^{-1}	1.44 ± 0.10	0.90 ± 0.07	0.001
Uric acid, μmol · l^{-1}	140 ± 30	230 ± 30	0.05
Amount of uric acid excreted, μmol · min^{-1}	0.45 ± 0.05	0.30 ± 0.02	0.05
Xanthine, μmol · l^{-1}	4.3 ± 1.3	5.8 ± 1.1	NS
Amount of xanthine excreted, μmol · min^{-1}	0.015 ± 0.002	0.008 ± 0.002	0.05
Hypoxanthine, μmol · l^{-1}	80 ±10	170 ± 20	0.01
Amount of hypoxanthine excreted, μmol · min^{-1}	0.28 ± 0.03	0.22 ± 0.01	NS
Total purine derivates, μmol · l^{-1}	640 ± 100	1100 ± 160	0.05
Amount of total purine derivatives excreted, μmol · min^{-1}	2.18 ± 0.18	1.43 ± 0.08	0.01

values are means ± SEM, n=6 for each group

According to our results it is evident that not only intake and nutrient status have effects on the concentrations of purine derivatives in the urine, but a significant role is also played by urine volume and urinary flow rate. It would be interesting to find out the effect of a diet with high and low nitrogen and digestible energy contents on the excretion of purine derivatives, as our previous study (Faix et al., 1988) showed that different dietary nitrogen and digestible energy intakes have no effects on urine flow rate, but that glomerular filtration rate decreased when sheep were fed a low protein diet. Parasitkusol et al. (2002) demonstrated that glomerular filtration rate was an important factor affecting the amount of urinary allantoin recovered, and that incomplete recovery of plasma ^{14}C allantoin in the urine indicated losses of plasma ^{14}C allantoin *via* non-renal routes, which is supported by the presence of ^{14}C in saliva. Osuji et al. (1993) demonstrated the potential of purine derivative concentration in total urine as a predictor of intake and nutrient status in sheep. The experiments of Parasitkusol et al. (2002) with intravenously administered ^{14}C allantoin proved that the recovery of ^{14}C allantoin showed no relation to the level of feed intake. Likewise, the sampling period had no influence on the concentration of purine derivatives in urine, when urine was collected over three periods in the

morning, at noon and in the evening (Nsahlai et al., 2000). Urinary and plasma purine derivatives in fed and fasted llamas were examined by Bakker et al. (1996). They showed that daily urinary excretion of purine derivatives decreased with feed intake and leveled on the last 3 days of fasting. Chen et al. (1992a) suggest that the changes in endogenous allantoin excretion may reflect remodeling of the metabolic state of the animal during periods when protein supply fluctuates. Chen et al. (1992b) also show that dry matter intake and body weight have an effect on purine derivatives excretion and microbial protein supply in sheep. Surra et al. (1997) concluded that the urinary excretion of purine derivatives is independent both of the supply of nucleic acids to the caecum and of the extent of hind gut fermentation, although it may be affected by variations in the flow of undigested fibre along the small intestine. The increase in microbial N flow at the duodenum may be provoked by higher levels of rumen NH_3 concentration (Balcells et al., 1993).

Data in Table 2 shows that ten-day administration of vasopressin highly significantly reduced urinary flow rate ($P<0.001$) and fractional excretion of water ($P<0.01$), which is conditioned by the antidiuretic role of the arginine vasopressin (Wood et al., 1986). Plasma urea concentration (P_{urea}) and renal urea reabsorption ($Reab_{urea}$) showed higher values in the dDAVP group in comparison with the control group, but without significant differences compared with our previous paper, where these differences were significantly higher (Boldižárová et al., 1999). The explanation lies in the number of sheep which were used for statistical analysis, when in our previous experiments we used 11 sheep for each group and now 6 sheep for each group. Similar results were observed in the amount of urea excreted and fractional urea excretion, when we measured lower values in the dDAVP group compared with the control group, but without significant differences (NS). Significantly higher urine osmolality ($P<0.01$) is proof of the formation of highly-concentrated urine induced by exogenous administration of vasopressin. Vasopressin regulates urine osmolality by increasing cortical and medullary collecting duct luminal membrane permeability to water by activation of V2 receptors. Vasopressin contributes to

TABLE 2

The effect of vasopressin analogue (dDAVP) administration on the renal functions in sheep

Indices	Control group	dDAVP group	P<
Urinary flow rate, ml · min^{-1}	3.58 ± 0.44	1.40 ± 0.12	0.001
Glomerular filtration rate, ml · min^{-1}	55.30 ± 5.99	62.80 ± 8.39	NS
Fractional excretion of water, %	7.16 ± 1.20	2.43 ± 0.27	0.01
Plasma urea concentration, mmol · l^{-1}	5.88 ± 0.70	6.65 ± 0.56	NS
Urine urea concentration, mmol · l^{-1}	18.71 ± 1.49	39.81 ± 4.90	0.01
Amount of urea excreted, μmol · min^{-1}	62.69 ± 4.56	53.98 ± 3.87	NS
Plasma osmolality, mosm · kg^{-1} H_2O	302.10 ± 0.99	302.90 ± 1.76	NS
Urine osmolality, mosm · kg^{-1} H_2O	241.30 ± 36.85	411.40 ± 26.41	0.01
Osmotic clearance, ml · min^{-1}	2.58 ± 0.20	1.89 ± 0.15	0.05

values are means ± SEM, n = 6 for each group

further concentration of urine by increasing the medullary concentration gradient by activating a distinct urea transporter (Nielsen and Knepper, 1993). Vasopressin also induces a selective decrease in inner medullary blood flow without altering the cortical blood flow, which also contributes to the maximum concentrating ability of the kidney (Franchini and Cowley, 1996).

In conclusion, the results demonstrate that vasopressin has no direct effect on the urinary excretion of purine derivatives in sheep. The lower excretion of purine derivatives is a reflection of urinary flow rate and water reabsorption.

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STRESZCZENIE

Wydalanie pochodnych purynowych w moczu owiec nie zależy od długotrwałego podawania wazopresyny

Badano wpływ wazopresyny (1-desamino-8-D-arginina wazopresyna [dDAVP] na wydalanie pochodnych purynowych w moczu owiec. Na 10 dni przed pomiarem funkcji nerek dwa razy dziennie podawano podskórnie 12,5 mg dDAVP.

Całkowite wydalanie pochodnych purynowych w moczu w grupie owiec otrzymujących dDAVP było ($P<0,01$) mniejsze niż u owiec kontrolnych (1,43 ± 0,08 vs 2,18 ± 0,18 μmol · min^{-1}, odpowiednio). Podobnie wydalanie w moczu alantoiny (0,90 ± 0,07 vs 1,44 ± 0,10 μmol · min^{-1}, $P<0,1$), kwa-

su moczowego (0,30 ± 0,02 vs 0,45 ± 0,05 μmol · min^{-1}) i ksantyny (0,008 ± 0,002 vs 0,015 ± 0,002 μmol · min^{-1}; P<0,05) było w grupie otrzymującej wazopresynę mniejsze niż u owiec kontrolnych. Iniekcje dDAVP nie miały wpływu na wydalanie hipoksantyny w moczu (0,22 ± 0,01 vs 0,28 ± 0,03 μmol · min^{-1}).

Tempo przepływu moczu w grupie doświadczalnej było mniejsze w grupie doświadczalnej (1,40 ± 0,12 vs 358 ± 0,44 μmol · min^{-1}; P<0,001) niż w kontrolnej, bez zmian w filtracji kłębkowej, zależnych od dDAVP. Koncentracja mocznika w moczu była istotnie większa w grupie otrzymującej iniekcje dDAVP (39,81 ± 4,90 vs 18,71 ± 1,49 mmol · l^{-1}; P<0,01) niż kontrolnej.

Stwierdzono, że wazopresyna nie ma wpływu na wydalanie pochodnych purynowych w moczu owiec. Mniejsze całkowite wydalanie pochodnych purynowych po iniekcji wazopresyny jest odbiciem tempa przepływu moczu i reabsorbcji wody.