

THE RENAL RESPONSE OF SHEEP FED A HIGH PROTEIN DIET TO TREATMENT WITH VASOPRESSIN ANALOGUE

K. BOLDIŽÁROVÁ, Š. FAIX, L. LENG

Institute of Animal Physiology, Slovak Academy of Sciences, Košice, Slovak Republic

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Abstract

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This experiment was designed to test whether the long-term formation of highly concentrated urine rises the glomerular filtration rate in sheep like in simple stomach animals. Experiments were carried out on young sheep fed a high protein diet for 3 weeks. In order to produce animals with highly concentrating kidneys, the experimental group was treated with 1-desamino-8-D-arginine vasopressin (dDAVP, Adiuretin-SD, Ferring, Prague). Subcutaneous injections of 12.5 µg dDAVP given twice daily started one week before the measurements of renal function. The clearance protocol showed a decreased urine flow rate (from 3.19 ± 0.50 to 0.33 ± 0.03 ml·min⁻¹, $P < 0.001$) without changes in glomerular filtration rate (80.18 ± 6.36 vs. 77.86 ± 6.26 ml·min⁻¹, NS) due to dDAVP. The plasma level of urea was significantly increased (from 5.76 ± 0.39 to 7.28 ± 0.25 mmol·l⁻¹, $P < 0.01$) concomitantly with the larger renal urea reabsorption (from 239.72 ± 26.16 to 390.53 ± 33.25 µmol·min⁻¹, $P < 0.01$) in dDAVP treated group. Both fractional urea excretion (48.44 ± 3.12 vs. 30.33 ± 1.94 %, $P < 0.001$) and the amount of urea excreted were significantly reduced (206.22 ± 9.04 vs. 170.41 ± 10.76 µmol·min⁻¹, $P < 0.05$). One week-lasting treatment with a vasopressin analogue resulted in the decreased urea clearance (from 36.95 ± 2.07 to 23.76 ± 1.97 ml·min⁻¹, $P < 0.001$) but it did not influence the osmotic clearance (1.77 ± 0.10 vs. 1.62 ± 0.08 ml·min⁻¹, NS). In conclusion, the kidneys of sheep do not respond to a long-term formation of highly concentrated urine *per se* with a rise in the glomerular filtration rate. A possible explanation is that urea recycling through digestive tract of ruminants is of an order of magnitude larger than that in simple stomach animals.

Sheep, kidney, GFR, urinary concentrating ability

It is well known that kidneys of ruminants play a considerable role in the nitrogen sparing during low protein intake. The adaptation ability of sheep kidneys to a reduced delivery of protein by food includes the morphological (Kočíšová et al. 1997), biochemical (Faixová et al. 1998) and functional response (Leng et al. 1985) in order to increase the renal urea reabsorption for its subsequent recycling through the digestive tract. The main change in renal function responsible for a high urea retention in sheep appears to be a significant reduction of glomerular filtration rate (GFR, Leng et al. 1985; Cirio and Boivin 1990). That is why the regulation of GFR in ruminants became an interesting research subject.

A decreased GFR during intake of low protein diet is associated in sheep with smaller amounts of microbial protein passing from forestomachs into abomasum and duodenum, and consequently also with a lower reabsorption of amino acids from digestive tract (Ergene and Pickering 1978). On the other side, the supply of full milk diet with an additional protein to calves during neonatal period (Skrzypczak et al. 1996) or the administration of casein hydrolysate into abomasum of sheep on a low protein diet (Szanyiová et al. 1996) increases GFR like in simple stomach animals (Lee and Summerill 1982). Many possible mediators of the amino acid-induced rise in renal haemodynamics, like glucagon (Hirschberg et al. 1988), liver-borne factor (Lang et al. 1991), cAMP (Bankir et al. 1997) and others have been proposed. In ruminants, the

Address for correspondence:

Mgr. Klaudia Boldižárová
Institute of Animal Physiology
Slovak Academy of Sciences
Soltésvej 4, 040 01 Košice, Slovak Republic

Phone: +421 95 6332048
Fax: +421 95 762162; 764535
E-mail: lleng@sake.sk
<http://www.vfu.cz/acta-vet/actavet.htm>

intravenous infusion of most amino acids increases the blood concentration of glucagon (Kuhara et al. 1991). Contrary to the simple stomach animals, either systemic or intraportal infusion of glucagon to sheep has been shown to significantly reduce both GFR and urine flow rate (Faix and Leng 1997).

The reduction of protein intake has been reported to be a factor significantly decreasing urinary concentrating ability in sheep and also in other ruminants (Schmidt-Nielsen 1979). On the other hand, the high protein intake which is associated with larger urea delivery to the kidney, increases the maximum urinary concentrating ability in simple stomach animals (Bouby et al. 1988). Recently Bankir et al (1996) reported that a chronic stimulation of the urinary concentrating activity with protein intake or the chronic administration of vasopressin led to the similar specific changes in renal morphology and functions, mainly to the increased GFR in animals with simple stomachs. In other words, they stated that the protein intake and vasopressin have the same or additive effects on GFR which are mediated or induced by changes in the urinary concentrating ability of kidneys.

The aim of this experiment was to investigate the effects of long-term formation of highly concentrated urine induced by a synthetic analogue of vasopressin on renal functions, mainly on GFR, in sheep fed a high protein diet.

Materials and Methods

The experiments were carried out on young female sheep (*Ovis aries*) of Merino breed weighing from 20 to 25 kg. The sheep were housed individually and they had free access to water and minerals. The animals were fed a high protein diet for at least 3 weeks before the measurements of kidney functions. The total daily ration for each sheep consisted of 500 g of hay, 300 g of barley and 250 g of wheat bran, containing 129.25 g of crude protein and 12.03 MJ of digestible energy. The animals were divided into two groups, eleven control sheep and eleven sheep treated with vasopressin analogue. The experimental group was given subcutaneous injection 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 one week 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 cannulated with a polyethylene capillary (o.d., 1.1 mm; i.d., 0.7 mm) was 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·ml⁻¹·min⁻¹) was initiated. Blood was sampled from a contralateral jugular vein. The samples of urine were quantitatively collected into calibrated glass cylinders through a Foley catheter (French size 14) placed in the urinary bladder.

The first collection period of urine began 90 min after the start of inulin infusion to allow its equilibration in the extracellular space. Each collection period lasted 30 minutes. Blood samples were collected into heparinized tubes at the mid-point of every urinary collection period. Two collection periods were carried out in every animal.

Concentrations of inulin (Vurek and Pegram 1966) and urea (Leng et al. 1986) were determined in plasma and urine samples by fluorometric methods. Plasma and urine levels of sodium, potassium, calcium and magnesium were measured using a Perkin Elmer atomic absorption spectrophotometer. The osmolality of plasma and urine was determined cryoscopically on a Knauer osmometer.

The urine flow rate, GFR, the clearance of osmotically active substances (C_{osm}), the amounts of urea and electrolytes excreted were determined for each 30 min period. The results from periods in the same animal were averaged because there were no significant changes in the values of all the parameters measured. The statistical analysis of the differences between control values and values obtained from dDAVP treated group was performed by Student's *t*-test. Results are quoted as the means \pm S.E.M.

Results

One week-lasting administration of vasopressin to sheep fed a high protein diet resulted in an increase of the plasma urea level (P_{urea} , 5.76 ± 0.39 vs. 7.28 ± 0.25 mmol·l⁻¹, $P < 0.01$). Obviously, the urine flow rate (V , 3.19 ± 0.50 vs. 0.33 ± 0.03 ml·min⁻¹, $P < 0.001$) was highly significantly reduced due to dDAVP treatment. The glomerular filtration rate (GFR) was not found to be influenced by formation of highly concentrated urine in dDAVP group (80.18 ± 6.36 vs. 77.86 ± 6.26 ml·min⁻¹, NS). Both the fractional urea excretion (FE_{urea} , 48.44 ± 3.12 vs. 30.33 ± 1.94 %, $P < 0.001$) and the amount of urea excreted ($U_{urea} V$, 206.22 ± 9.04 vs.

$170.41 \pm 10.76 \mu\text{mol}\cdot\text{min}^{-1}$, $P < 0.05$) were significantly lower than the control values while the renal urea reabsorption ($\text{Reab}_{\text{urea}}$, 239.72 ± 26.16 vs. $390.53 \pm 33.25 \mu\text{mol}\cdot\text{min}^{-1}$, $P < 0.01$) was significantly larger in vasopressin-treated animals (Fig. 1).

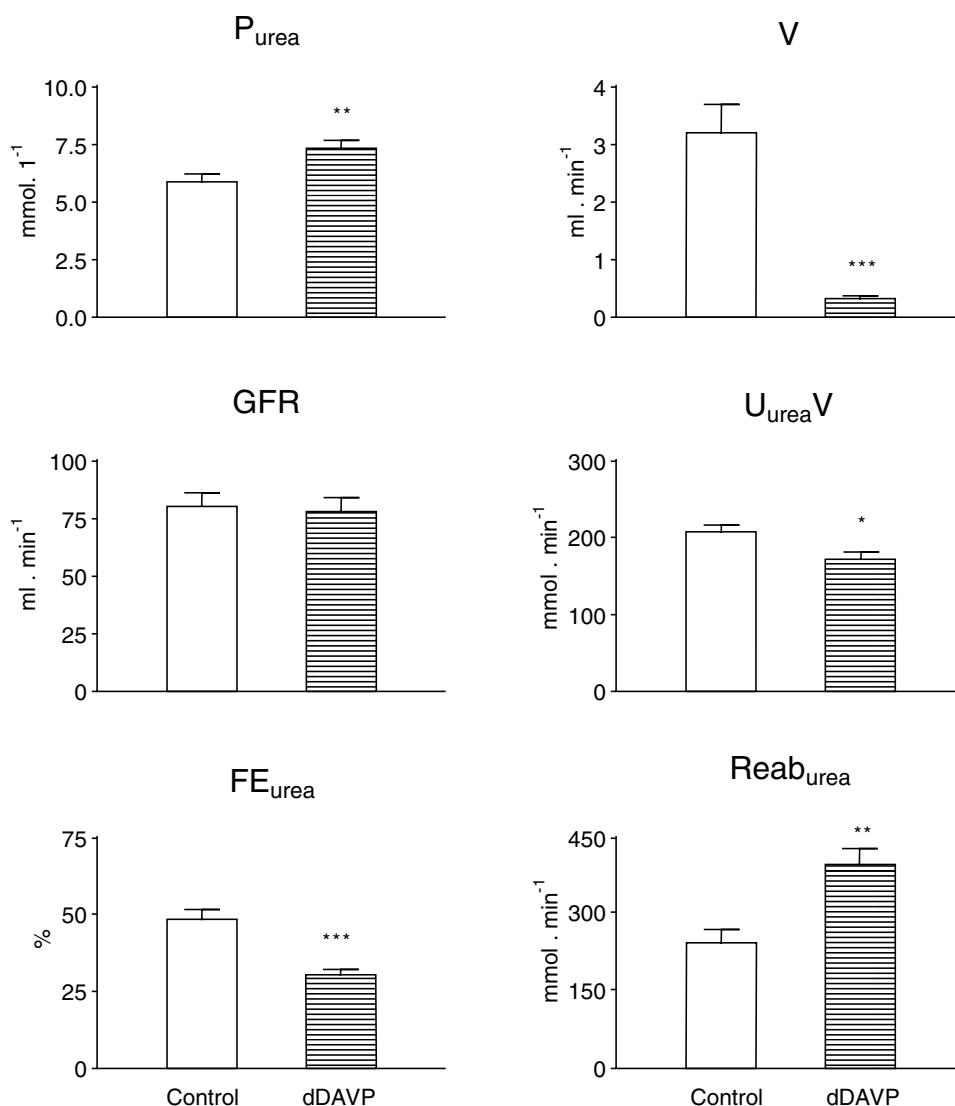


Fig. 1. The effects of one week-lasting administration of vasopressin analogue (dDAVP) on the urine flow rate (V), the glomerular filtration rate (GFR), the plasma urea level (P_{urea}), the amount of urea excreted ($U_{\text{urea}} V$), the fractional urea excretion (FE_{urea}) and on the renal urea reabsorption ($\text{Reab}_{\text{urea}}$) in sheep fed a high protein diet. Values are means \pm SEM, $n = 11$ for each group. Significance levels: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

The plasma levels of sodium and calcium were stable, with no significant differences between the control and experimental group (148.32 ± 1.05 vs. $146.36 \pm 0.80 \text{ mmol}\cdot\text{l}^{-1}$ for Na and 2.48 ± 0.05 vs. $2.43 \pm 0.03 \text{ mmol}\cdot\text{l}^{-1}$ for Ca). The significant decrease in the

potassium and magnesium levels in plasma was recorded in dDAVP treated sheep (4.01 ± 0.08 vs. $3.66 \pm 0.10 \text{ mmol}\cdot\text{l}^{-1}$ for K, $P < 0.05$ and 0.91 ± 0.02 vs. $0.80 \pm 0.01 \text{ mmol}\cdot\text{l}^{-1}$ for Mg, $P < 0.001$).

The data dealing with the excretion of electrolytes and osmolality parameters is summarized in Table 1. The treatment with a vasopressin analogue resulted in the significant

Table 1
The effects of the week lasting-administration of vasopressin analogue (dDAVP) on the amount of electrolytes excreted ($U_{\text{X}}V$), the fractional excretion of electrolytes (FEx), plasma (P_{osm}) and urine (U_{osm}) osmolality and on the osmotic clearance (C_{osm}) in sheep fed a high protein diet. Values are means \pm SEM.

	Control (n = 11)	dDAVP (n = 11)	
$U_{\text{Na}}V$ ($\mu\text{mol}\cdot\text{min}^{-1}$)	6.21 ± 1.65	2.72 ± 0.54	NS
$U_{\text{K}}V$ ($\mu\text{mol}\cdot\text{min}^{-1}$)	170.15 ± 7.27	112.01 ± 8.60	$P < 0.001$
$U_{\text{Ca}}V$ ($\mu\text{mol}\cdot\text{min}^{-1}$)	2.18 ± 0.39	1.98 ± 0.98	NS
$U_{\text{Mg}}V$ ($\mu\text{mol}\cdot\text{min}^{-1}$)	14.17 ± 1.17	14.35 ± 1.06	NS
FE_{Na} (%)	0.06 ± 0.02	0.03 ± 0.01	NS
FE_{K} (%)	55.90 ± 3.66	43.44 ± 4.88	NS
FE_{Ca} (%)	0.91 ± 0.15	0.86 ± 0.38	NS
FE_{Mg} (%)	20.68 ± 2.05	23.99 ± 1.59	NS
P_{osm} ($\text{mosm}\cdot\text{kg}^{-1} \text{H}_2\text{O}$)	300.07 ± 0.75	296.55 ± 0.86	$P < 0.05$
U_{osm} ($\text{mosm}\cdot\text{kg}^{-1} \text{H}_2\text{O}$)	254.18 ± 61.71	1491.36 ± 67.75	$P < 0.001$
C_{osm} ($\text{ml}\cdot\text{min}^{-1}$)	1.77 ± 0.10	1.62 ± 0.08	NS

decrease of the amount of potassium excreted. No differences were found in the excreted amounts of sodium, calcium and magnesium between control and experimental group. Vasopressin had no effect on the fractional excretions of electrolytes.

The osmolality of plasma was slightly but significantly decreased in dDAVP group. Obviously, the osmolality of urine was highly significantly elevated in vasopressin given animals. Vasopressin had no effect on the clearance of osmotically active substances.

Discussion

The results of this study show that a one-week-lasting formation of highly concentrated urine induced by vasopressin had no effect on GFR in sheep fed a high protein diet. A small but significant increase in plasma urea level confirms the efficiency of dDAVP treatment in our experiment. This was a consequence of the higher renal urea reabsorption due to administration of exogenous vasopressin. The effects of vasopressin on renal urea reabsorption have been known for a long time. To date, the vasopressin-induced urea transport is well described as a facilitated process acting via adenylate cyclase-dependent pathway and activation of the specialized urea transporters like UT1, UT2 and UT3 which are located in the various structures of kidney medulla (Tsukaguchi et al. 1998). Although these specific urea transporting proteins or channels were determined in rats or rabbits only, their appearance in sheep or in other ruminants could be supposed.

The missing response of GFR in our sheep is a completely different result to that achieved in animals with the simple stomachs. Vasopressin and the ensuing concentrating activity

developed by the kidney were reported to be responsible for an increase in GFR in rats and probably also in humans (Bankir et al. 1996). There are strong similarities between the protein- and vasopressin-induced changes in renal function of simple stomach animals. It seems to be a typical feature of simple stomach animals that a rise in plasma urea level induced by protein intake or vasopressin is accompanied by the increase in GFR in order to provide a larger delivery of urea to nephron to increase its subsequent excretion (Bankir 1996). It is obvious because the urea accumulation in blood of simple stomach animals means the serious toxic consequences. But it was not the case in our experiment on sheep which were yet on high protein diet. One could expect that urea reabsorption increased by 63 % in our dDAVP sheep will result in a considerable higher plasma urea level as it was actually found. The real increment was approximately $1.5 \text{ mmol}\cdot\text{l}^{-1}$ (26 %) only. The explanation is that urea recycling through digestive tract of ruminants is of an order of magnitude larger than that in simple stomach animals (Bodá 1980). This fact together with a somewhat higher resistance of ruminants to uraemia could be an explanation why the GFR of sheep kidneys did not respond to the long-term formation of highly concentrated urine by the same way as it is known to be in animals with simple stomachs.

The lower plasma levels of both potassium and magnesium in dDAVP treated group could be a result of a week-lasting administration of hormone only because the mineral intake was the same as in the control group. These changes seem to be a consequence of the setting of renal handling of minerals yet during the first hours or days of administration of vasopressin but our measurements were done on day 8 of dDAVP treatment. The effects of vasopressin on the transepithelial fluxes of sodium, potassium, calcium and magnesium in the renal tubules are well documented (De Rouffignac 1991). Surprisingly, we did not find any natriuretic effects of vasopressin which were usually described in the acute experiments on sheep (Gans 1964; Park et al. 1985). A lack of this phenomenon in our experiment could be caused also by the time delay as mentioned above. The reduction in the amount of potassium excreted appears to be a result of its decreased plasma level.

The weak effects of long-term dDAVP treatment on the electrolyte excretion are reflected also by non-significant change in the clearance of osmotically active substances. It is obvious that the slightly but significantly smaller osmolality of plasma in dDAVP sheep was due to considerably larger reabsorption of water from collecting ducts than in the control animals.

In summary, results of this experiment demonstrate that a vasopressin induced long-term formation of highly concentrated urine in sheep fed a high protein diet does not result in a rise of GFR. The explanation for such a different renal response of sheep compared to the simple stomach animals could be a well known fact the recycling of urea nitrogen through digestive tract of ruminants is of an order of magnitude larger.

Renálna odpoveď oviec kŕmených vysokobielkovinovou diétou na analóg vazopresínu

Cieľom tohto pokusu bolo overiť, či dlhodobá tvorba vysoko koncentrovaného moča má za následok vzostup glomerulárnej filtračnej rýchlosti aj u oviec, podobne ako je tomu u zvierat s jednoduchým žalúdkom. Pokus bol robený na mladých ovciach kŕmených tri týždne vysokobielkovinovou diétou. Pre získanie zvierat s vysoko koncentrujúcimi obličkami sme podávali pokusnej skupine 1-desamino-8-D-arginin vazopresín (dDAVP, Adiuretin-SD, Ferring, Praha) subkutánne v dávke $12,5 \mu\text{g}$ dDAVP dvakrát denne po dobu jedného týždňa pred meraniami renálnych funkcií. Clearance protokol ukázal znížené vylučovanie moča (kontrolná skupina $3,19 \pm 0,50$, dDAVP skupina $0,33 \pm 0,03 \text{ ml}\cdot\text{min}^{-1}$, $P < 0,001$), avšak zmeny v rýchlosti glomerulárnej filtrácie neboli zaznamenané ($80,18 \pm 6,36$ vs. $77,86 \pm 6,26 \text{ ml}\cdot\text{min}^{-1}$, NS). Hladina močoviny v plazme signifikantne stúpla (z $5,76 \pm 0,39$ na $7,28 \pm 0,25 \text{ mmol}\cdot\text{l}^{-1}$, $P < 0,01$) a taktiež bola zvýšená renálna reabsorpcia močoviny (z $239,72 \pm 26,16$ na $390,53 \pm 33,25 \mu\text{mol}\cdot\text{min}^{-1}$, $P < 0,01$) u zvierat

dostávajújúcich dDAVP. Frakčná exkrécia močoviny ($48,44 \pm 3,12$ vs. $30,33 \pm 1,94$ %, $P < 0,001$), ako aj množstvo vylúčenej močoviny ($206,22 \pm 9,04$ vs. $170,41 \pm 10,76$ $\mu\text{mol}\cdot\text{min}^{-1}$, $P < 0,05$) boli významne redukované. Týždenné podávanie analógu vazopresínu malo za následok zníženie clearance močoviny (z $36,95 \pm 2,07$ na $23,76 \pm 1,97$ $\text{ml}\cdot\text{min}^{-1}$, $P < 0,001$), avšak neovplyvnilo clearance osmoticky aktívnych látok ($1,77 \pm 0,10$ vs. $1,62 \pm 0,08$ $\text{ml}\cdot\text{min}^{-1}$, NS). Prezentované výsledky ukazujú, že obličky oviec nereagujú na samotnú dlhodobú tvorbu vysoko koncentrovaného moča zvýšením glomerulárnej filtračnej rýchlosti. Možným vysvetlením je rádovo väčšia recyklizácia močoviny cez tráviaci trakt prežúvavcov, než u zvierat s jednoduchým žalúdkom.

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