CHARACTERIZATION OF UREA TRANSPORT MECHANISM ACROSS THE OMASUM EPITHELIUM IN SHEEP

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المستخلص

توجد عدة دراسات حول نقل اليوريا عبر أنسجة ظهارية عديدة من ضمنها ظهارة الكرش. أشارت هذه الدراسات بوضوح إلي أن نقل اليوريا عبر هذه الأنسجة الظهارية يتم بواسطة حامل. لاتوجد معلومات حول آلية نقل اليوريا عبر ظهارة الورقية. لهذا فإن هذه الدراسة قد صممت لتوضيح آلية نقل اليوريا عبر ظهارة الورقية.

أجريت هذه الدراسة على 42 نسيج ظهارة ورقية جمعت من 5 خراف، بإستخدام تقانة غرف أسنج Ussing chamber، حيث وضعت أنسجة ظهارة الورقية فى محاليل فسيولوجية تحتوي على خليط من الاملاح، مضادات حيوية ومثبط إنزيم اليوريز (Phenyl phosphorodiamidate).

اظهرت النتائج ارتباطاً معنوياً (r^2 = 0.85) بين توصيل الظهارة (G_t) ومعدل افراز اليوريا عبر ظهارة الورقية. بالإضافة لذلك فإن إضافة 10µM من مثبط الهيكل الخلوى الأكتين Actin cytoskeleton إلي المحلول فى الجانب المخاطي للظهارة أدي إلي زيادة معنوية ($p<0.05$) فى توصيل الظهارة (G_t) ومعدل افراز اليوريا عبر ظهارة الورقية. إلا أن خفض الأس اليدروجيني للمحلول فى الجانب المخاطي للظهارة من 7.4 إلي 6.4 لم يظهر اثراً معنوياً على معدل نقل اليوريا عبر ظهارة الورقية.

نتائج هذه الدراسة تشير إلي أن نقل اليوريا عبر ظهارة الورقية يتم بآلية سالبة عبر المسار بين الخلوي Passive paracellular pathway.

Key words: urea transport, omasum epithelium, Sheep.
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Abstract

The transport of urea has been studied in a variety of epithelial tissues including the rumen, where carrier-mediated transport mechanisms have been reported. No such information concerning the transport of urea in the omasum is known. We have therefore carried out this study to characterize the transport mechanism of urea across the isolated omasum epithelium in sheep. This study was conducted under short circuit condition using conventional Ussing-chamber technique on 42 pieces of omasal epithelial tissues isolated from 5 sheep. Tissues bathing buffer solutions contained a mixture of antibiotics and the urease inhibitor phenylphosphorodiamidate (PPD). Our results show significant correlation ($r^2 = 0.85$) between the transepithelial conductance (G_t) and the serosal to mucosal urea flux rates (J_{sm}). Moreover, mucosal application of the actin cytoskeleton inhibitor cytochalasin D (10µM) significantly ($p<0.05$) increased both the tissue conductance (G_t) and the serosal to mucosal (J_{sm}) flux rates of urea. However, reducing the mucosal pH from 7.4 to 6.4 did not induce a significant effect on the bidirectional urea flux rates across the omasum epithelium. The results indicate that transport of urea across the omasum epithelium involves a passive paracellular pathway mechanism.

Key words: Sheep; Omasum; Urea; Cytochalasin D

Introduction

In ruminants, the blood urea is recycled into the forestomach through its epithelia and with the saliva. In the forestomach urea is hydrolyzed into CO_2 and ammonia; the latter is partially fixed by the forestomach microorganisms into microbial protein. The transport of urea has been studied in a variety of tissues including the rumen (Ritzhaupt et al., 1997; Kato and Sands, 1998; Stewart et al., 2005), where carrier-mediated transport mechanisms have been reported. We have recently reported that urea transport across the rumen epithelium occurs by an electrically silent transcellular pathway, and that it could be modulated by extracellular and intracellular pH (Abdoun et al., 2007a, b). No such information concerning the transport of urea in the omasum is known. We have

therefore carried out this study to characterize the transport mechanism of urea across the isolated omasum epithelium in sheep.

Materials and methods

Ussing-chamber experiments

Experiments were conducted on 42 pieces of omasum epithelial tissues isolated from 5 sheep of different age and sex, using conventional Ussing-chamber technique to determine short circuit current (I_{sc}), tissue conductance (G_t) and the bidirectional flux rates of urea. The incubation of forestomach epithelium has been described in detail by Martens et al. (1987). Briefly, sheep were killed in a local slaughterhouse, and the omasum was removed from the abdominal cavity within 2–3 min. The omasum laminae were isolated and cleaned in a buffer solution, stripped from the muscle layer, and taken (some 20 min) to the laboratory in a buffer solution containing a mixture of antibiotics (1%), and maintained at 38 ^{o}C. The buffer was gassed with 95% O_2 - 5% CO_2. Pieces of the omasum epithelium (3 × 3 cm) were mounted between the two halves of an Ussing chamber to give an exposed area of 3.14 cm^2. The mounted tissues were bathed on each side with 1% antibiotics (penicillin + chloramphenicol + 5-fluorocytosine) containing buffer solution, and were gassed with 100% O_2 at 38^{o}C. The standard electrolyte solution contained (in mM) 140 Na^+, 5 K^+, 1 Ca^{+2}, 2 Mg^{+2}, 8 MOPS, 100 Cl^-, 1 $H_2PO_4^-$, 2 HPO_4^{-2}, 25 acetate, 10 propionate, 5 butyrate, 10 glucose, 1 urea and 1 Phenyl phosphorodiamidate (urease inhibitor).

The isolated omasum epithelial tissues from each animal were divided randomly into four groups. Each group was incubated under different experimental condition (mucosal pH 7.4, mucosal pH 7.4 + cytochalasin D, mucosal pH 6.4 or mucosal pH 6.4 + cytochalasin D). Three flux periods of 20 min for ^{14}C-labelled urea were assayed using Liquid Scintillation Counter (Wallace-Perkin-Elmer).

RT-PCR experiments

We used primers against the human erythrocytes UT, HUT11 designed by Ritzhaupt et al. (1998), in a reverse transcription polymerase chain reaction (RT-PCR). The RT-PCR reaction was performed in a single tube containing 100 ng totals RNA isolated from sheep rumen or omasum. The antisense primer, nt 763-782 (5'– GCAGCATGCAGGCACATGAG-3')

was used for the reverse transcription and the sense primer, nt 193-212 (5'-GGCATATCCCAAGTGGTGTT-3') was used for DNA amplification. 10µl of the RT-PCR reaction mixture was analysed on a 1.5% agarose gel.

Statistical analysis

Statistical evaluations were carried out by using the Sigma Plot program version 8.0 for Windows. Results are given as means ± SE. The comparison between the groups (mucosal pH 7.4, mucosal pH 7.4 + cytochalasin D, mucosal pH 6.4 or mucosal pH 6.4 + cytochalasin D) was carried out in the form of Student's t-test or paired t-test. P values < 0.05 were considered significant. N refers to the number of experimental animals, and n refers to the number of tissues.

Results

Correlation between the tissue conductance and the serosal to mucosal urea flux rate

Our results show significant correlation (r^2 = 0.85) between the transepithelial conductance (G_t) and the serosal to mucosal urea flux rate (J_{sm}) (Fig. 1).

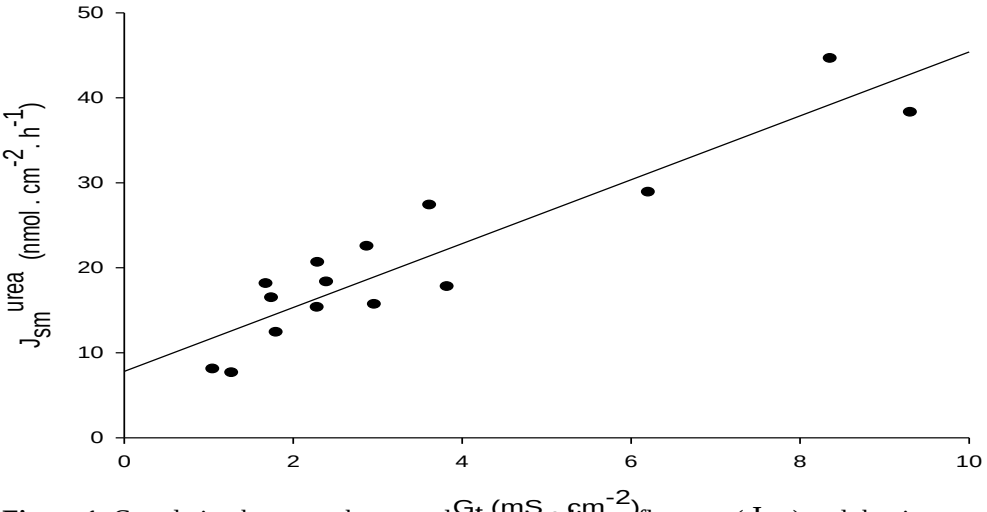

Figure 1. Correlation between the serosal to mucosal urea flux rate (J_{sm}) and the tissue conductance (G_t) (N = 5; n= 15).

Effect of cytochalasin D

Mucosal application of the actin cytoskeleton inhibitor cytochalasin D (10µM) significantly ($p<0.05$) increased both the tissue conductance (G_t) (Fig. 2) and the serosal to mucosal (J_{sm}) flux rates of urea (Fig. 3).

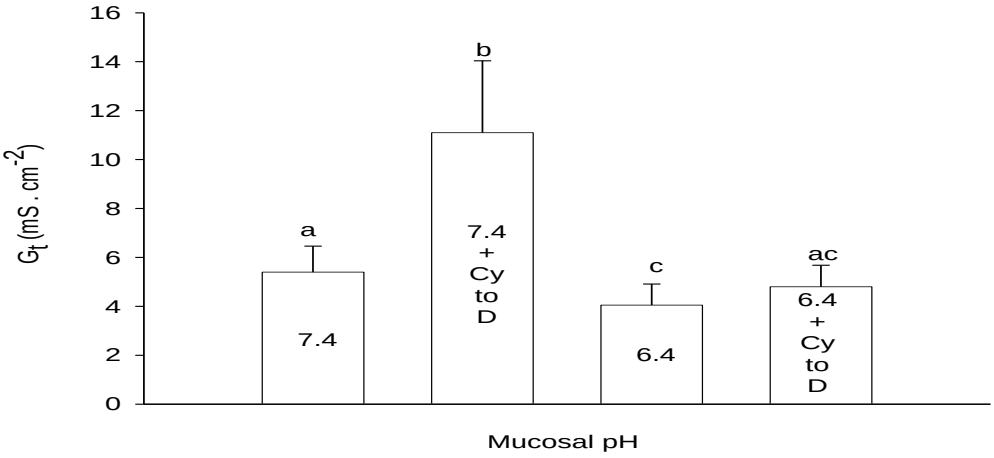

Figure 2. Effect of cytochalasin D on tissue conductance (G_t) at mucosal pH 7.4 and 6.4 [bars with different superscript letters are significantly different at $p<0.05$; (N = 5; n = 12)].

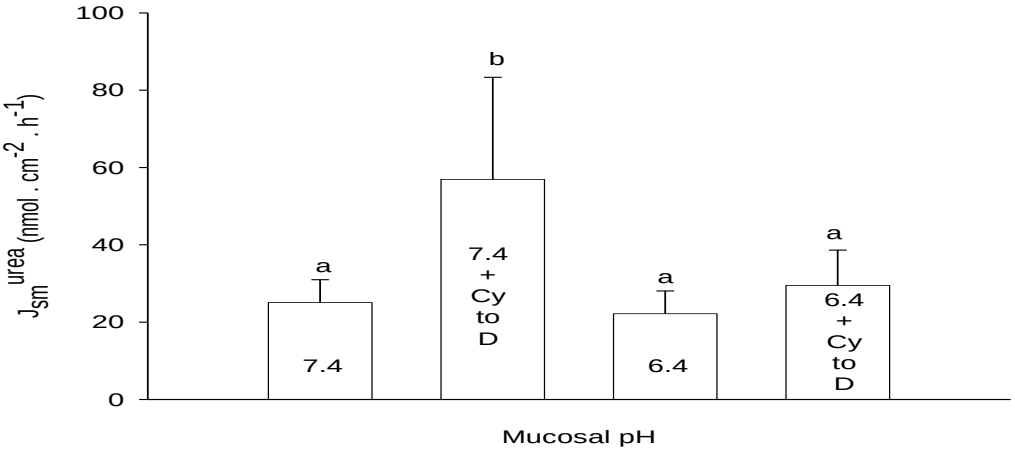

Figure 3. Effect of cytochalasin D on serosal to mucosal (J_{sm}) urea flux rates at mucosal pH 7.4 and 6.4 [bars with different superscript letters are significantly different at $p<0.05$; (N = 5; n = 6)].

Effect of mucosal pH

Reducing the mucosal pH from 7.4 to 6.4 did not show significant effect on the unidirectional urea flux rates across the omasum epithelium (Table 1 and Fig. 3).

Table 1. Effect of mucosal pH on unidirectional urea flux rates across the omasum epithelium**.**

Mucosal pH	J_{ms}^{urea}	J_{sm}^{urea}	J_{net}^{urea}	N/n
	$(nmol \cdot cm^{-2} \cdot h^{-1})$			
7.4	25.06 ± 3.52	20.78 ± 2.66	04.28 ± 1.76	5/15
6.4	27.01 ± 4.06	24.61 ± 3.12	02.40b ± 2.49	5/15

Investigation for mRNA expressing urea transport protein (UT) in RNA samples isolated from the omasum epithelium

Using primers against the human erythrocytes UT, HUT11, in a reverse transcription polymerase chain reaction (RT-PCR), we identified a product of 600bp molecular size in RNA sample isolated from the rumen epithelium. However, we couldn't identify the same product in RNA samples isolated from the omasum epithelium (Fig. 4).

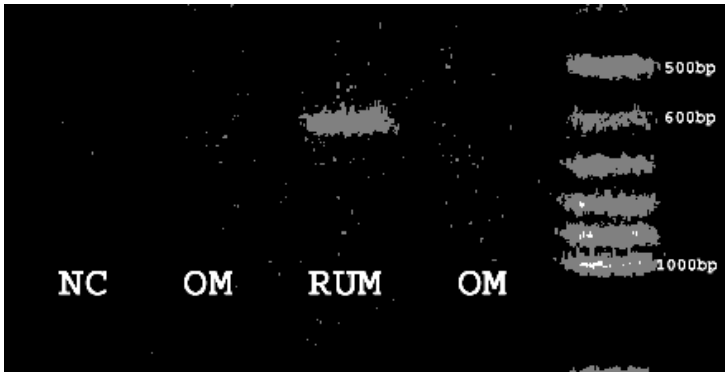

Figure 4. Electrophoretic analysis of RT-PCR products. [NC = negative control; OM = omasum; RUM = rumen].

Discussion

In this study, the serosal to mucosal flux rates of urea across the omasum epithelium showed significant correlation ($r^2 = 0.85$) to the changes in the transepithelial conductance. This indicates the importance of the paracellular pathway in the serosal to mucosal urea flux across the omasum epithelium.

Cytochalasin D is a fungal metabolite that disturbs the ongoing cycle of F-actin polymerization / depolymerization by binding to fast growing end of the filament, thereby preventing the addition of further monomers (MacLean-Fletcher and Pollard, 1980). This results in gradual disappearance of normal actin stress fibres and marked alteration of cell morphology. We found this drug to induce a profound increase in the tissue conductance (G_t), with a consequent enhancement of the serosal to mucosal urea transport across the omasum epithelium. Since cytochalasin D was reported to alter the cell morphology (Fletcher and Pollard, 1980), this could lead to an elevated shunt pathway conductance (G_s) and consequently increase the paracellular urea transport. Moreover, we couldn't demonstrate UT-mRNA in the RNA samples isolated from the omasum epithelium.

Unlike the rumen epithelium (Abdoun et al 2007a,b), reducing the mucosal pH from 7.4 to 6.4 didn't enhance the bidirectional urea transport across the omasum epithelium suggesting different mechanisms of urea transport across the rumen and omasum epithelium. If the ammonia arises from urea, the overall reaction in the presence of bicarbonate buffers would be:

$$(NH_2)_2CO + CO_2 + 3H_2O \longrightarrow 2NH_4^+ + 2HCO_3^-$$

For every mole of ammonia produced, a mole of bicarbonate is formed. In this context, the low urea secretion into the omasum (compared to the rumen) is an important physiological function reducing the production of ammonia and bicarbonate in the omasum, thus protecting the abomasum against their alkalinizing effect.

Conclusion

The results indicate that transport of urea across the omasum epithelium involves a passive paracellular pathway mechanism, which is obviously different from that existing in the rumen epithelium.

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References

Abdoun, K., Stumff, F., and Martens, H. (2007a). Short chain fatty acids (SCFAs) and carbon dioxide (CO_2) enhance transepithelial urea transport across the rumen epithelium in sheep. Proceedings of the Society of Nutrition Physiology, **16**: 52.

Abdoun, K., Stumpff, F., and Martens, H. (2007b). Urea transport across the rumen epithelium is electrically silent and modulated by changes in pH. Journal of Physiology and Biochemistry, 63 (1): 33.

Kato, A. and Sands, J.M. (1998). Evidence for Sodium-dependent active urea secretion in the deepest subsegment of the rat inner medullary collecting duct. Journal of Clinical Investigation, **101** (2): 423-428.

MacLean-Fletcher, S. and Pollard, T.D. (1980). Mechanism of action of cytochlasin B on actin. Cell, 20: 329-341.

Martens, H., Gaebel, G. and Strozyk, H. (1987). The effect of potassium and the transmural potential difference on magnesium transport across an isolated preparation of sheep rumen epithelium. Quaternary Journal of Experimental Physiology, **72**: 181–188.

Ritzhaupt A., Breves G., Schröder, B., Winckler, C.G. and Shirazi-Beechey, S.P. (1997). Urea transport in gastrointestinal tract of ruminants: effect of dietary nitrogen. Biochemical Society Transaction, **25**: S490 (Abstract).

Ritzhaupt, A., Wood, I.S., Jackson, A.A., Moran, J.B. and Shirazi-Beechey, P.S. (1998). Isolation of a RT-PCR fragment from human colon and sheep rumen RNA with nucleotide sequence similarity to human and rat urea transporter isoforms. Biochemical Society Transaction, **26**: S122.

Stewart, G.S., Graham, C., Cattell, S., Smith, T.P.L., Simmons, N.L. and Smith, C.P. (2005). UT-B is expressed in bovine rumen: potential role in ruminal urea transport. American Journal of Physiology, **289**: R605–R612.