CHARACTERIZATION OF UREA TRANSPORT MECHANISM ACROSS THE OMASUM EPITHELIUM IN SHEEP

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The abstract

There have been several studies on urea transport. These studies have shown that urea transport is a complex process involving various mechanisms. This study aimed to characterize the urea transport mechanism across the omasum epithelium in sheep.

The results showed that the urea transport mechanism is mediated by passive paracellular and transcellular pathways. The passive paracellular pathway is the major route for urea transport, while the transcellular pathway plays a minor role.

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 °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°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 ^14C-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).](image-url)
### 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.

<table>
<thead>
<tr>
<th>Mucosal pH</th>
<th>$J_{m}^{urea}$</th>
<th>$J_{m}^{urea}$</th>
<th>$J_{net}^{urea}$</th>
<th>N/n</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.4</td>
<td>25.06 ± 3.52</td>
<td>20.78 ± 2.66</td>
<td>04.28 ± 1.76</td>
<td>5/15</td>
</tr>
<tr>
<td>6.4</td>
<td>27.01 ± 4.06</td>
<td>24.61 ± 3.12</td>
<td>02.40b ± 2.49</td>
<td>5/15</td>
</tr>
</tbody>
</table>

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:

$$\text{(NH}_2\text{)}_2\text{CO} + \text{CO}_2 + \text{3H}_2\text{O} \rightarrow \text{2NH}_4^+ + \text{2HCO}_3^-$$

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