Expression and functional activity of the Na/Mg exchanger, TRPM7 and MagT1 are changed to regulate Mg homeostasis and transport in rumen epithelial cells

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Abstract. The present study was performed to show the molecular identity of functionally characterized Mg transport pathways in rumen epithelial cells (REC) and to investigate the effects of extracellular [Mg] changes on their expression and activity. By using RT-PCR, Western blot, flow cytometry and immunocytochemistry, TRPM7, MagT1 and a Na+/Mg2+ exchanger were found in REC. Compared with control conditions ([Mg]e = 1.2 mM), a decreased or increased MagT1 (-30%; 20%) and Na+/Mg2+ exchanger (-25%; 40%) protein abundance was observed after a 24-h incubation of REC in low (0.12 mM)- and high (5 mM)-Mg medium, respectively. To determine the Mg transport capacity, [Mg2+]i changes were measured by use of mag-fura 2. The basal [Mg2+]i (0.43 ± 0.03 mM) was not influenced by the [Mg] of the pre-incubation medium. However, compared to control cells, REC incubated in low- or high-Mg medium showed significantly reduced (50%) and elevated (57%) Mg extrusion rates, respectively. In addition, they were characterized by an increased influx capacity (30-40%). In low-Mg cells the latter results mainly from a strong TRPM7 related transport component whereas in high-Mg cells the imipramine-sensitive, the Na+/Mg2+ exchanger-mediated transport component causes this effect. In conclusion, TRPM7, MagT1 and a Na+/Mg2+ exchanger are shown to be the main Mg transport proteins in REC and their expression and functional activity is influenced by the cellular Mg status. The latter responses permit adaptation of epithelial Mg absorption and enable REC to maintain a physiological [Mg2+]i, which is a prerequisite for various cell functions.

Key words: magnesium transport, magnesium channel, mag-fura 2, epithelial cells, sheep

The existence of regulated magnesium (Mg) influx and efflux pathways in rumen epithelial cells (REC) and their importance for the regulation of the intracellular free magnesium concentration ([Mg2+]i) as well as for the directed transport of the ion across the rumen epithelium has been well established at a functional level [1, 2]. REC are characterized by a very high influx capacity and Mg uptake mechanisms are thought to include an ion channel [1] and an Mg-Cl-cotransport [3]. A Na+/Mg2+ exchanger was...
also shown to be the predominant route for Mg efflux, performing about 98% of the Mg extrusion [4, 5]. However, investigation of the modalities of REC Mg transporter operation and regulation is hindered by their unknown molecular identity. It was revealed that in many cells the TRPM7 channel of the melastatin-related transient receptor potential family is involved in the cellular Mg uptake [6-8]. Goytain and Quamme [9] described the magnesium transporter 1 (MagT1) as another ion channel very selectively mediating Mg transport in epithelial cells. This study was performed to identify the ruminal Mg influx pathways at a molecular level. In addition, the relationship between cellular Mg status and the expression and functional activity of TRPM7, MagT1 and the Na+/Mg2+ exchanger was investigated.

Material and methods

Primary cultures of ovine REC were prepared as described elsewhere [4]. Briefly, REC were isolated by fractional trypsinization and grown in Medium 199 containing 10% FCS, 1.36 mM glutamine, 20 mM HEPES, and antibiotics (gentamycin 50 mg/L, kanamycin 100 mg/L) in an atmosphere of humidified air-5% CO2 at 38°C. On day four, cells were switched to 24 h to totally Mg-free media (costume-made by Biochrom, Berlin, Germany) supplemented with either 1.2 (control), 0.12 (low-Mg) or 5 (high-Mg) mM Mg (pre-incubation period).

Thereafter, REC from each Mg group were loaded with 5 μM mag-fura 2-AM for the measurement of [Mg2+]i, [4]. Mg efflux and influx capacity were determined from the [Mg2+]i changes observed during a 20-min incubation in totally Mg-free, Na-containing or Na-free, Mg (5 mM)-containing media. Known inhibitors of the Na+/Mg2+ exchanger (imipramin) [4] and of channel-mediated Mg influx (cobalt(II)-hexaammine, nitrendipine) [8-9] were used to differentiate between transport components. In parallel, the expression of TRPM7, MagT1 and Na+/Mg2+ exchanger was investigated by qRT-PCR, Western blot, flow cytometry and immunocytochemistry [5]. RNA and protein samples of REC were prepared using commercial kits (M-PER Mammalian Protein Extraction Reagent, Pierce, Bonn, Germany; NucleoSpin RNA II, Macherey-Nagel, Düren, Germany). For flow cytometry, 1 x 10⁷ cells per condition were fixed in 20 ml methanol. The forward and reverse primer sets of TRPM7 and MagT1 were 5′-gcgatgctcgctgtgg-3′ / 5′-ttccagctgtgct-3′ and 5′-tggtactgcagaaggatc-3′ / 5′-gtaaccccaccgataga-3′, respectively. The monoclonal mouse anti-Na+/Mg2+ exchanger antibody (mab) was raised against the porcine erythrocyte Na+/Mg2+ exchanger in our laboratory [10] and has been shown to detect the protein in REC [5]. A rabbit anti-TRPM7 antibody (ACC-047, lot AN-01) was obtained from Alamone Labs (Jerusalem, Israel), the rabbit anti-MagT1 antibody was a kind gift from Dr. G. Quamme (University of British Columbia, Vancouver, Canada).

If not otherwise stated, data are presented as means ± standard error (SE). Significance was determined by Student’s t-test or paired t-test as appropriate. P < 0.05 was considered to be significant. All statistical calculations were performed by using SigmaStat (Jandel Scientific).

Results

The mRNA transcripts and proteins of TRPM7 and of MagT1 were found in RNA and protein lysates from REC. By using a monoclonal antibody (mAb) prepared in our laboratory, a protein with a molecular mass of ~70-kDa was identified as candidate Na+/Mg2+ exchanger. Immunostaining and flow cytometry analysis confirmed the presence of these proteins in REC with an average of 93 ± 2%, 80 ± 10% and 80 ± 3% of TRPM7-, MagT1- and Na+/Mg2+ exchanger positive cells, respectively. In contrast, TRPM6, another ion channel of the melastatin-related transient receptor potential family involved in Mg transport [7], is not existent in REC.

[Mg2+]i and Mg transport capacity of differently pre-incubated REC

Independent of the [Mg] of the pre-incubation medium, the basal [Mg2+]i of REC amounted to about 0.43 ± 0.03 mM (figure 1). This result points to mechanisms enabling cells to maintain an optimal [Mg2+]i, under various conditions.

As shown by the data presented in figure 2A and table 1, the Mg transport capacity of REC has indeed been modulated by pre-incubation in low- and high-Mg media. figure 2A shows typical original traces of [Mg2+]i, changes observed under efflux ([Mg]o = 0 mM, [Na]o = 125 mM) and influx ([Mg]o = 5 mM, [Na]o = 0 mM) conditions. After omission of extracellular Na, part of the Mg uptake is mediated via the Na+/Mg2+ exchanger working in reverse mode [4, 5]. Clearly, REC incubated in low- or high-Mg medium showed reduced (59%) and
elevated (57%) Mg extrusion rates, respectively, when compared to control REC (figure 2A, top; table 1). In addition, low- and high-Mg pre-incubated REC are both characterized by an increased Mg influx capacity (figure 2A, bottom; table 1).

As shown in figure 2B, Co(III)hex (1 mM) and imipramine (250 μM) both effectively reduced the Mg influx rate in control and treatment groups, thereby reflecting the existence of channel-mediated as well as Na-dependent components of Mg transport. However, Co(III)hex was most effective in REC pre-incubated in Mg-deficient media, and high-Mg cells were characterized by a 2- to 3-fold increase of the imipramin-sensitive transport component when compared to control and low-Mg REC (figure 2B).

Recently, we showed that Co(III)hex, a known Mg channel inhibitor in bacteria and mitochondria [11, 12], is able to block the mammalian Mg channel TRPM7 [8]. To get more information on MagT1-related transport, we performed influx experiments with nitrendipin (50 μM), a substance that blocked the channel very effectively in the study of Goytain and Quamme [9]. There was no difference in the nitrendipine effect between groups and the observed reduction amounted to 51.2 ± 3.0%.

Expression of Na+/Mg2+ exchanger, TRPM7 and MagT1 in differently pre-incubated REC

Next we wanted to investigate whether a distinct Na+/Mg2+ exchanger, TRPM7 or MagT1 protein expression could cause the changed efflux and influx capacity. Typical results from Western blot experiments are given in figure 3A showing a decreased or increased Na+/Mg2+ exchanger and MagT1 protein abundance after a 24-h incubation of REC in low- and high-Mg medium, respectively. These results were confirmed by flow cytometric analysis allowing quantification of the protein abundance per single cell. Compared with control conditions, it reveals a decreased (25.3 ± 3.0% vs 30 ± 10%) or increased (40.0 ± 6.7% vs 20.1 ± 9.5%) Na+/Mg2+ exchanger and MagT1 protein amount per cell after exposing them to Mg deficient or high-Mg media (figure 3B). In contrast, pre-incubation in media with different [Mg2+]o had only a slight influence on the expression of TRPM7 (figure 3A and B).

Discussion

The modulation of the extracellular [Mg] induced no significant change of the REC basal [Mg2+]i (0.43 ± 0.02 mM) measured in Ca2+/Mg2+-free medium. Moreover, it is consistent with the values of 0.37 ± 0.05 and 0.54 ± 0.08 mM seen in our previous studies [1, 5]. This led us to hypothesize that transmembrane Mg transport is adapted to maintain the physiological [Mg2+]i.

Indeed, REC pre-incubated in low- and high-Mg medium were characterized by a reduced (59%) and accelerated (56%) Mg efflux rate, respectively, when compared to controls. In addition, both low- and high-Mg REC show an increased Mg influx capacity. Goytain and Quamme [9, 13] suggested that epithelial cells can sense environmental Mg and alter Mg transport through transcription- and translation-dependent processes to maintain Mg balance. They showed such adaptation responses for Mg influx pathways reacting to reduced [Mg]e, with increased expression and/or translocation to the cell membrane. Our results from Western blot and flow cytometric measurements clearly show that Na+/Mg2+ exchanger and MagT1 proteins are quantitatively altered with changes in extracellular [Mg]. It can be assumed that excess extracellular Mg induce an increased Mg efflux via increased abundance and activity of the Na+/Mg2+ exchanger. First, this protects cells against Mg overload; and second it generates a driving force to stimulate the Mg uptake in this absorbing cell [14]. Sahni et al. [15] proposed that TRPM7 could have a dominant role in physiological situations where receptor-regulated Mg influx is required, such as Mg uptake needed for organismal Mg homeostasis, cell growth or neuronal functions. This corresponds to our findings that the expression
of TRPM7 protein was not changed in dependence on extracellular Mg and that its functional activity is very low in non-stimulated control cells being in Mg equilibrium. In high-Mg and, in particular in low-Mg REC, however, the Co(III)Hex-sensitive and thus, TRPM7-related [8] transport component, was markedly increased. Thus, our findings suggest that an ion channel-mediated mechanism, most probably TRPM7, facilitates Mg influx into previously Mg deprived REC. TRPM7 is thought to be a main regul-

**Figure 2.** Mg transport capacity of differently pre-incubated sheep REC and inhibitory effect of imipramine and cobalt(III)hexaammine. A: Representative original recordings of [Mg$^{2+}$], changes after re-suspension in Mg-free, Na-containing medium (efflux conditions) or Mg-containing (5mM), Na-free medium (influx conditions). B: Decrease of the Mg uptake rate after application of the Na$^+$/Mg$^{2+}$ exchanger inhibitor imipramine or the Mg channel blocker cobalt(III)hexaammine. Values are means ± SE from 8 single experiments; * p < 0.05 vs control (without inhibitor).

**Table 1.** Effect of Mg deficiency and oversupply on efflux and influx capacity of ruminal epithelial cells.

<table>
<thead>
<tr>
<th>Change of [Mg$^{2+}$], (µM/min)</th>
<th>Efflux conditions</th>
<th>Influx conditions</th>
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<tbody>
<tr>
<td>Control (1.2 mM)</td>
<td>-4.1 ± 0.6 (n = 17)</td>
<td>19.8 ± 1.4 (n = 12)</td>
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<tr>
<td>Pre-incubation in:</td>
<td></td>
<td></td>
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<tr>
<td>Low (0.12 mM)-Mg$^{2+}$ medium</td>
<td>-1.7 ± 0.9* (n = 12)</td>
<td>25.8 ± 1.7* (n = 12)</td>
</tr>
<tr>
<td>High (5.0 mM)-Mg$^{2+}$ medium</td>
<td>-6.4 ± 0.9* (n = 11)</td>
<td>26.4 ± 2.8* (n = 12)</td>
</tr>
</tbody>
</table>

Values are means ± SD; n = number of single experiments; * p < 0.05 vs control.

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tor of the cellular Mg homeostasis, and destruction of the TRPM7 gene or its down-regulation markedly decreased Mg accumulation, reduced the basal \([\text{Mg}^2+]_i\) and is followed by growth arrest and/or reduced viability of cells [7, 16-18]. An elevated TRPM7 activity after pre-incubation of REC in Mg-deficient media will promote the replenishment of intracellular Mg stores and thus, help to avoid such negative consequences of intracellular Mg deficiency. Other proteins, most probably MagT1 might provide a background level of Mg uptake required to maintain Mg homeostasis under static conditions. This is supported by a general high expression level of MagT1 protein observed in our flow cytometry studies and by the constant 50% inhibitory effect observed after application of nitrendipin.

**Conclusion**

TRPM7, MagT1 and a Na+/Mg2+ exchanger are present in REC and represent their main influx and efflux mechanisms. As expected for proteins, relevant to regulation of Mg homeostasis and directed epithelial transport of the ion, their functional activity and/or expression is quantitatively altered by extracellular Mg deficiency or overload. Our data demonstrate an intrinsic regulation of REC transmembrane Mg transport involving changes of the Na+/Mg2+ exchanger and MagT1 protein expression. TRPM7 seems to play an important role for the re-uptake of Mg and thus replenishment of intracellular Mg stores after exposure to Mg deficient conditions.

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**References**


