Mg^{2+}-Uptake of Cultured Ruminal Epithelial Cells: Evidence for an Electrodiffusive Transport

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Introduction

In ruminants the primary site of magnesium absorption is the forestomach. Based on in vivo- and in vitro studies it was demonstrated that magnesium absorption from the rumen is mainly mediated by a transcellular active process, but the mechanisms of luminal uptake and basolateral extrusion are not well understood. Two distinct transport mechanisms are thought to mediate apical magnesium entry in ruminal epithelial cells (REC). The hypothesis is that Mg^{2+} enters the REC by an electrodiffusive (sensitive to potassium) and an electroneutral $Mg^{2+}/2H^+$ exchange mechanism (*Leonhard* (1990); *Leonhard-Marek* and *Martens* (1995); *Leonhard-Marek* and *Martens* (1997)). The aim of the studie was to get first informations about the electrogenic component of Mg^{2+}-uptake at the cell level.

Methods

Primary cultures of ruminal epithelial cells (REC) were prepared as described by *Galfi* et al. (1981). Briefly, REC were isolated by fractional trypsination and grown in Medium 199 containing 10% fetal calf serum, 6,8 ml · l^{-1} glutamine, 20 ml · l^{-1} Hepes and antibotics (gentamycine 50 mg · l^{-1}, kanamycine 100 mg · l^{-1}) in an atmosphere of humidified air-5% CO_2 at 38°C. Experiments were performed between 6 and 12 days after seeding. Cells were loaded with either 5 μM Mag-fura-2-AM, 10 μM Fura-2-AM or 0,5 μM BCECF-AM for determination of $[Mg^{2+}]_i$, $[Ca^{2+}]_i$ and $[pH]_i$, respectively. Intracellular ion concentrations were determined by measuring the fluorescence of the probe-loaded REC in the fluorescence spectrometer LS-50 B (Perkin-Elmer) at 37°C under stirring using the fast filter accessory (FFA). After in vivo calibration of the signals, $[Mg^{2+}]_i$ and $[Ca^{2+}]_i$ were calculated according to the formula of *Grynkiewicz* et al. (1985).

Results

Mg^{2+}-depleted and normal (with physiological Mg^{2+}-content) REC show a rapid, concentration-dependend increase in $[Mg^{2+}]_i$ when placed in bathing solutions with Mg^{2+} concentrations between 0.5 and 2 mM. Because total magnesium, determined by atomic absorption spectrophotometrie, also increased this elevation of $[Mg^{2+}]_i$ could be interpreted to reflect a Mg^{2+}-influx.

To identify the driving force for this Mg^{2+}-uptake we change the membrane potential (E_m) of REC. A reduction of K^+ secretion, by high extracellular K^+ (40, 80, 140 mM potassium) decreased $[Mg^{2+}]_i$ significantly ($P < 0,01$) by 7, 11 and 42%, respectively. An increase of extracellular K^+ led to membrane depolarization, as it was shown in REC by whole cell patch clamp recordings. Application of quinidine, a blocker of K^+ channels, reduced $[Mg^{2+}]_i$ significantly ($P < 0.05$) by approximately 17% (50 μM quinidine;) or 29% (100 μM quinidine). On the other hand, hyperpolarization created by K^+-diffusion ($K_i > K_o$) in presence

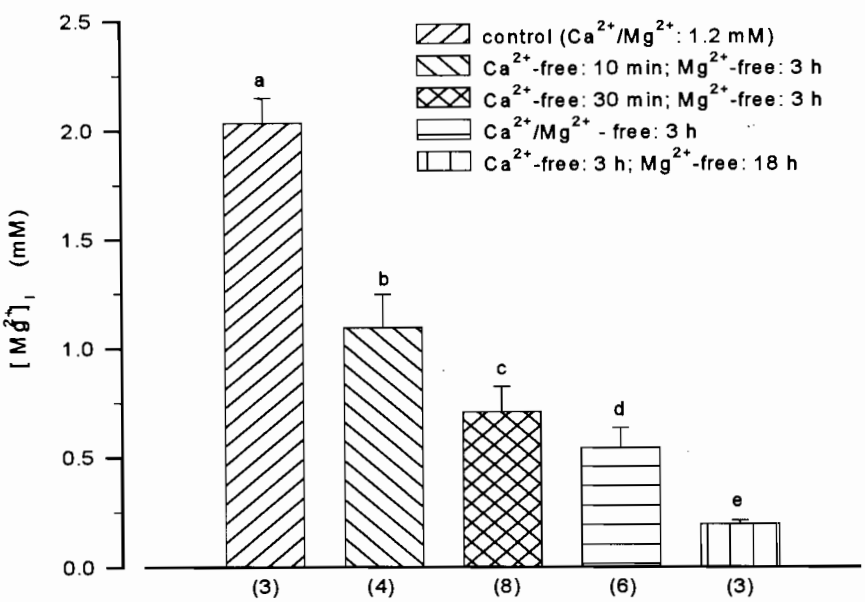

Fig. 1: Effects of changing extracellular Mg^{2+}- and Ca^{2+}-concentrations on $[Mg^{2+}]_i$ of REC. Values are mean ± SD. Number of experiments is given in parentheses. Different superscripts denote significant differences, $P < 0,05$.

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of valinomycine and 2 mM Mg^{2+} induced a rapid 15 % increase in $[Mg^{2+}]_i$ (significant with $P > 0{,}01$). If there is no K^+-gradient ($K_i = K_o$) valinomycine application resulted in a slow, not significant elevation of $[Mg^{2+}]_i$ by only 5 %.

With microelectrode techniques it has been shown that incubation in Ca^{2+}- and Mg^{2+}-free media depolarised REC by approximately 20 mV (*Lang*, 1996). As Figure 1 shows this depolarization is accompanied by a time dependend decrease in $[Mg^{2+}]_i$. Repolarization by solutions with Mg^{2+} or Mg^{2+} and valinomycine reversed this effect. All manipulations had no effects on $[Ca^{2+}]_i$ and $[pH]_i$.

Discussion

In the present experiments we have demonstrated $[Mg^{2+}]_i$-changes induced by manipulations which are known to depolarize or hyperpolarize E_m of REC. *Leonhard-Marek* and *Martens* (1997) previously reported that depolarization of the apical membrane potential by increasing the transepithelial potential, elevating luminal K concentration or adding verapamil (which has been shown to block apical K conductances in rumen epithelium) to the mukosal side of the epithel depresses Mg ms flux across the sheep rumen epithelium. This effects are interpreted to be the result of a decreased driving force for an electrogenic uptake of Mg^{2+}. If this assumption is correct then decreasing the driving force for Mg^{2+} entry should result in a decrease in intracellular $[Mg^{2+}]$. Conversely, increasing the driving force for Mg^{2+} entry by membrane hyperpolarization would be predicted to increase $[Mg^{2+}]_i$. The results presented are consistent with this hypothesis. The study has shown that $[Mg^{2+}]_i$ is decreased with E_m depolarization and increased with E_m hyperpolarization. The results support the assumption that the membran potential acts as a principal driving force for Mg^{2+}-entry in REC. The pathway for this electrodiffusive Mg^{2+}-uptake may be a channel or a carrier.

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