Journal of Animal and Feed Sciences, 13, Suppl. 1, 2004, 277–280

Magnesium increases calcium absorption mediated by transcellular transport in small intestine of goats and rats*

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ABSTRACT

The effect of an increase in mucosal Mg concentration on Ca absorption mediated by transcellular transport in the stripped epithelia of caprine ileum and rat jejunum was studied. In Ussing chamber experiments, mucosal to serosal flux of Ca (Jms) at 60 mM $MgCl_2$ was significantly ($P<0.05$) greater than that of control (1.2 mM) in the absence of electrochemical gradient in goats and rats. Furthermore, Mg-induced Jms was significantly decreased ($P<0.05$) after pre-incubation with thapsigargin. It is concluded that Mg stimulates Ca absorption mediated by transcellular transport, which contributed to the Ca store of endoplasmic reticulum.

KEY WORDS: magnesium, calcium absorption, intestinal epithelia, transcellular pathway

INTRODUCTION

In goats, the Mg concentrations in the supernatant of the digesta sampled from the duodenum to ileum increased from 3.9 to 8.2 mM (mean value was 5.8 mM). Increasing the Mg concentration from 1.2 to 60.0 mM in the mucosal solution increased net Ca absorption in a concentration-dependent manner in the everted sacs of the caprine ileum (Kozakai et al., 1999). The action was not dependent on the potential difference between mucosal and serosal sides in the everted sacs of the ovine ileum (Kozakai et al., 2000). Further, we recently observed that mucosal Mg increase Ca absorption in the gastrointestinal tract in sheep when the dietary Mg level was raised *in vivo* (Kozakai et al., 2002). The purpose of this study

* Supported by Mercian Corporation Co. LTD, Japan

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was to elucidate if an increase in mucosal Mg concentration raises Ca absorption mediated by transcellular transport in the small intestine of goats and rats, and which mechanism of Mg-induced transcellular Ca transport is involved.

MATERIAL AND METHODS

Unidirectional flux rates of $^{45}Ca^{2+}$ across stripped epithelia of caprine ileum and rat jejunum were measured in Ussing chambers (exposed area; 1 or 0.5 cm^2) under open or short circuit conditions. After 30 min pre-incubation, the tissue was bathed with 5 ml of HEPES-buffered solution (HBS) at 37°C. The control HBS contained (mM): 135.0 NaCl, 4.7 KCl, 1.2 $MgCl_2$, 1.0 $CaCl_2$, 10.0 glucose, 20.0 HEPES; pH 7.4. High Mg-solutions were made by raising the $MgCl_2$ concentration from 1.2 to 60 mM, of which osmolality was adjusted by reducing NaCl concentration from 135 to 55 mM. All HBS solutions were finally adjusted to 300-310 mOsm/Kg H_2O by 0-10 mM mannitol. In the experiments with an electrochemical gradient (Na^+, Cl^-, Mg^{2+} and H^+), the mucosal solutions were adjusted to pH 6.5.

After an addition of 185 kBq $^{45}Ca^{2+}$ on one side in each chamber, solution of 100 µl was sampled three times at 30 min intervals to measure ^{45}Ca radioactivity. Ca^{2+} flux rates of mucosal to serosal (Jms), serosal to mucosal (Jsm) and Jnet (Jms-Jsm) were calculated by using standard equations (Schults and Zalusky, 1964). Tissue conductance (G_T), short circuit current (I_{SC}) and PD were measured by automatic voltage clamp with calomel electrodes in 3 M KCl / 2% agar.

The chemical compounds were dissolved in DMSO to make highconcentration stock solutions. Stock solution (10 µl) was added to HBS. The mean values were analysed by Student's *t*-test or ANOVA followed by Duncan's multiple range test.

RESULTS

Experiment 1. Effect of Mg on transepithelial Ca^{2+} flux rates in ileum of goats

In the presence of an electrochemical gradient, Jnet at 60 mM of mucosal $MgCl_2$ concentration (serosal; 1.2 mM Mg) was 64.0±17.2 nmol/cm^2/h, which was significantly ($P<0.05$) greater than that of control (both sides; 1.2 mM Mg, -3.2±7.8 nmol/cm^2/h). This was due to an increased Jms and a reduced Jsm. In the absence of electrochemical gradient, Jms at 60 mM of $MgCl_2$ concentration was significantly ($P<0.05$) greater than that of control, but there was no significant difference between open and short circuit conditions.

Experiment 2. Effect of Mg on transepithelial Ca^{2+} flux rates in jejunum of rats

In the absence of electrochemical gradient, Jnet at 60 mM of $MgCl_2$ concentration was positive (0.7±1.9 nmol/cm^2/h) (not significantly different from zero), while that of control was negative (-6.7±3.5 nmol/cm^2/h). Jms at 60 mM

of $MgCl_2$ concentration was significantly ($P<0.05$) greater than that of control, but there was no significant difference between open and short circuit conditions.

Experiment 3. Electrical properties of intestinal epithelia of goats and rats

In the absence of electrochemical gradient, an increase in $MgCl_2$ concentration from 1.2 mM to 60.0 mM significantly increased G_T in caprine ileum. On the other hand, an increase in Mg concentration significantly decreased I_{SC} and PD in rat jejunum.

Experiment 4. The mechanism for intracellular Ca metabolism in Mg-induced Jms

When epithelial tissue was incubated in control HBS and high Mg HBS for 90 min after pre-loading of $^{45}CaCl_2$ for 60 min, $^{45}Ca^{2+}$ efflux from internally store to extracellular medium at 60 mM Mg was significantly increased ($P<0.05$). Furthermore, Mg-induced Jms was significantly decreased ($P<0.05$) after pre-incubation with thapsigargin (Figure 1) which depletes Ca store in endoplasmic reticulum, as inhibitor of Ca^{2+}-ATPase.

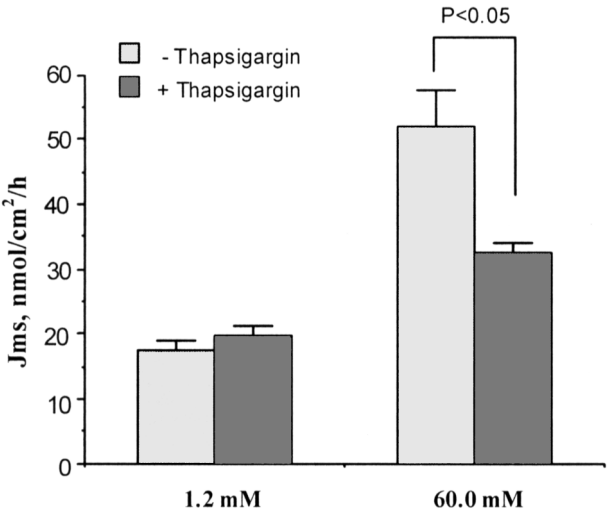

Figure 1. The effect of pre-treatment of Thapsigargin on Mg-induced Jms of intestinal epithelia in rats

DISCUSSION

We have reported that an increased in Mg concentration but not Na or Cl concentration in the mucosal solution increased net Ca absorption in a concentration-dependent manner in the everted sacs of the caprine ileum (Kozakai et al., 1999). From the results of Experiment 1, it was shown that an increase in the mucosal Mg concentration enhances Jnet as a result of an increase in Jms in the stripped epithelia of caprine ileum. In addition, Mg-induced Jms is mainly mediated by transcellular

pathway including active transport mechanism because there are no difference in Jms between open and short circuit conditions.

From the results of Experiment 2, it was suggested that rat intestine can be used as model to research Mg-induced Jms of Ca of the intestine in goat because Mg-induced Jms mediated by transcellular transport of the small intestine in rat was similar to that in goats.

However, the results of Experiment 3 show that comparisons of the effect of Mg on permeability of paracellular pathway between goats and rats must be interpreted with caution because the reactions of G_T, I_{SC} and PD for high Mg concentration differ between goats and rats. In rat jejunum, high Mg concentration did not decrease I_{SC} and PD. This may be because the jejunum of rats has a large capacity for active transport depended on Na. But the reason why G_T was increased by high Mg concentration in goats is not clear.

The results of Experiment 4 show that the mechanism for Mg-induced Jms mediated by transcellular transport contribute to intracellular Ca metabolism regulated by the Ca pool size of endoplasmic reticulum. It is known that the exhaustion of stored Ca in endoplasmic reticulum stimulates the capacitative Ca entry. In addition, high Mg concentration stimulated Ca efflux in the results of Experiment 4.

CONCLUSIONS

An increase in mucosal Mg concentration stimulates Ca absorption mediated by transcellular transport, which contributed to the intracellular Ca metabolism in endoplasmic reticulum, in small intestine of goats and rats.

In the absence of electrochemical gradient, 60 mM Mg-induced Jms was significantly decreased ($P<0.05$) by pre-treatment of thapsigargin in control HBS for 1 h.

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