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Up-regulation of Na^+-dependent Mg^{2+} transport by nitric oxide and cyclic GMP pathway in renal epithelial cells

Akira Ikari *, Kumiko Nakajima, Sayuri Taki, Yasunobu Suketa

Department of Environmental Biochemistry and Toxicology, University of Shizuoka School of Pharmaceutical Sciences, 52-1 Yada, Shizuoka City, Shizuoka 422-8526, Japan

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Abstract

A putative, Na^+-dependent Mg^{2+} transport pathway controls the intracellular free Mg^{2+} concentration ($[Mg^{2+}]_i$) in various mammalian cells. The characteristics of this Mg^{2+} transport pathway have not been clarified. Herein, we examined the regulatory mechanism of Na^+-dependent Mg^{2+} efflux in renal epithelial NRK-52E cells. Mg^{2+} removal from the extracellular bathing solution induced an Na^+-dependent $[Mg^{2+}]_i$ decrease in Mg^{2+} (5 mM)-loaded cells but not in control cells. Amiloride inhibited the $[Mg^{2+}]_i$ decrease in a dose-dependent manner ($IC_{50} = 3$ μM). Similarly, atomic absorption spectrophotometry showed that Mg^{2+} removal decreased intracellular Mg^{2+} content, while it increased Na^+ content. Calphostin C (1 μM), a protein kinase C inhibitor, and genistein, a tyrosine kinase inhibitor (10 μM), blocked the $[Mg^{2+}]_i$ decrease. The $[Mg^{2+}]_i$ decrease was accompanied by an increase in intracellular nitric oxide (NO) and cyclic GMP contents. (*E*)-4-methyl-2-[(*E*)-hydoxyimino]-5-nitro-6-methoxy-3-hexenamide (0.1 mM), an NO donor, and 8-bromo-cyclic GMP (0.1 mM), a membrane-permeable cyclic GMP analogue, accelerated the $[Mg^{2+}]_i$ decrease. In contrast, N^G-monomethyl-L-arginine (L-NMMA, 0.1 mM), an NO competitive inhibitor, and 1*H*-[1,2,4]oxadiazolo[4,3-*a*]quinoxaline-1-one (ODQ, 10 μM), an NO-sensitive guanylate cyclase inhibitor, significantly blocked the $[Mg^{2+}]_i$ decrease. These results indicate that a decrease in extracellular Mg^{2+} concentration induces the production of NO and cyclic GMP, which leads to the up-regulation of Na^+-dependent Mg^{2+} efflux.

Keywords: Amiloride; Mg^{2+} concentration; Mag-fura 2; Kidney; Nitric oxide (NO)

1. Introduction

Regulation of the intracellular free Mg^{2+} concentration ($[Mg^{2+}]_i$) is important in maintaining the activity of many enzymes (Ebel and Gunther, 1980) and in changing the activity of various ion channels (Kelepouris et al., 1993; Petit-Jacques et al., 1999) and pumps (Dorup, 1996; Raftos and Lew, 1995). The plasma membrane Mg^{2+} transport pathway has been characterized into two classes according to their Na^+ dependence (Gunzel and Schlue, 2000): Na^+-dependent and Na^+-independent Mg^{2+} transport pathways. A putative, Na^+-dependent Mg^{2+} transporter seemed responsible for the efflux of Mg^{2+}, but this activity was mostly observed in high Mg^{2+}-loaded cells, indicating that the Na^+-dependent Mg^{2+} transporter is activated by an imbalance between extracellular and intracellular Mg^{2+} concentrations.

It has been reported that the Na^+-dependent Mg^{2+} transporter is inhibited by amiloride (Cefaratti et al., 2000; Gunther and Vormann, 1995; Yago et al., 2000). On the other hand, this Mg^{2+} transport pathway is activated by protein kinase C in rat erythrocytes (Gunther and Vormann, 1995) and vascular smooth muscle cells (Touyz and Schiffrin, 1996), or by protein kinase A in rat thymocytes (Gunther and Vormann, 1992) and mouse ascites cells (Wolf et al., 1996). However, other regulatory mechanisms are poorly understood.

Mg^{2+} reabsorption in the nephron occurs in the loop of Henle and the distal tubule (Dai et al., 2001), and the epithelial cells are frequently exposed to conditions of Mg^{2+} excess or deficiency. Dietary Mg^{2+} deficiency increased plasma nitric oxide (NO) production in rats (Mak et al., 1996; Rock et al., 1995), and NO stimulated

* Corresponding author. Tel.: +81-54-264-5674; fax: +81-54-264-5672.

E-mail address: ikari@u-shizuoka-ken.ac.jp (A. Ikari).

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soluble guanylate cyclase in proximal tubule cells (Roczniak and Burns, 1996). NO and the cyclic GMP pathway regulate the transporters of the plasma membrane such as the Na^+–HCO_3^- cotransporter (Ruiz et al., 1999), Na^+–K^+–$2Cl^-$ cotransporter (Ortiz et al., 2001), and Na^+/K^+-ATPase (Zhang and Mayeux, 2001); so, these may play important roles in controlling renal function under the physiological and pathological conditions.

In the present study, we examined the regulatory mechanisms of the Na^+-dependent Mg^{2+} transport pathway. We found that intracellular NO and cyclic GMP are involved in the up-regulation of Na^+-dependent Mg^{2+} efflux in renal epithelial cells.

2. Materials and methods

2.1. Chemicals

Dulbecco's minimal essential medium (DMEM) and 1*H*-[1,2,4]oxadiazolo[4,3-*a*]quinoxaline-1-one (ODQ) were purchased from Sigma (St. Louis, MO, USA). Mag-fura 2/acetoxymethyl ester (AM), fura 2/AM and Pluronic F-127 were obtained from Molecular Probes (Eugene, OR, USA). (*E*)-4-methyl-2-[(*E*)-hydroxyimino]-5-nitro-6-methoxy-3-hexenamide (NOR1) was from Dojindo Laboratories (Kumamoto, Japan). N^G-monomethyl-L-arginine (L-NMMA) and 8-bromo-cyclic GMP were from Research Biochemicals International (Natick, MA, USA). Diaminofluorescein-2 diacetate was from Daiichi Chemistry (Osaka, Japan).

2.2. Cell culture

Rat renal epithelial cell line, NRK-52E, was obtained from American Type Culture Collection (Manassas, USA). The cells were grown on 100-mm plastic culture dishes (Corning, NY, USA) in DMEM supplemented with 5% fetal bovine serum, 0.14 mg ml^{-1} streptomycin sulfate, and 0.07 mg ml^{-1} penicillin G potassium in a humidified environment of 5% CO_2–95% air at 37 °C. All cells used in experiments were cultured in 5 mM Mg^{2+}-containing DMEM for 1 or 2 days before use, unless described otherwise.

2.3. Measurement of intracellular free Mg^{2+} concentration

Cells were grown on glass slides to sub-confluence. Intracellular free Mg^{2+} concentration ($[Mg^{2+}]_i$) was determined using an Mg^{2+}-sensitive fluorescent dye, mag-fura 2. The cells were preincubated with Mg^{2+}-loading solution for 30 min, followed by the Mg^{2+}-loading solution containing 2 μM mag-fura 2/AM and a detergent Pluronic F127 (0.025%, w/v) for 30 min. The Mg^{2+}-loading solution contained (in mM): choline-Cl (140), HEPES (20), KCl (5.4), $MgSO_4$ (0.8), $MgCl_2$ (4.2), NaH_2PO_4 (0.33), KH_2PO_4 (0.4), and glucose (10). The pH was adjusted to 7.4 with Tris. Then, the mag-fura 2-loaded cells were washed twice with dye-free Mg^{2+}-loading solution and placed in a glass cuvette. The mag-fura 2 fluorescence was monitored at 1-s intervals using a dual-excitation wavelength spectrofluorometer (Hitachi F-2000, Tokyo, Japan) with excitation at 340 and 380 nm and emission at 495 nm. Bathing solution was perfused continuously at a speed of 1.5 ml min^{-1}. The $[Mg^{2+}]_i$ was calculated, as previously described (Grynkiewicz et al., 1985), using a dissociation constant (K_d) of 1.45 mM for the Mg^{2+}–mag-fura 2 complex. The change in $[Mg^{2+}]_i$ was estimated in the first 200 s following perfusion of Mg^{2+}-free or Na^+/Mg^{2+}-free solutions. The Mg^{2+}-free solution contained (in mM): NaCl (140), HEPES (20), KCl (5.4), NaH_2PO_4 (0.33), KH_2PO_4 (0.4), EDTA (1), and glucose (10). The pH was adjusted to 7.4 with NaOH. The Na^+/Mg^{2+}-free solution contained (in mM): choline-Cl (140), HEPES (20), KCl (5.4), KH_2PO_4 (0.73), EDTA (1), and glucose (10). The pH was adjusted to 7.4 with Tris.

2.4. Measurement of intracellular Mg^{2+} and Na^+ contents

For measuring intracellular Mg^{2+} and Na^+ contents, the cells grown on 24-well plates were incubated in the Mg^{2+}-loading solution or Mg^{2+}-free solution for the indicated time at 37 °C. Then, the cells were washed twice with 150 mM choline-Cl and deproteinized with 5% trichloroacetic acid/0.175% $LaCl_3$. Mg^{2+} and Na^+ contents of the supernatant were measured by atomic absorption spectrophotometry (Model AA 880 Mark II, Nippon Jarrell-Ash, Kyoto, Japan).

2.5. Measurement of intracellular NO content

The cells cultured on 35-mm glass-bottom culture dishes were incubated at 37 °C for 1 h in the Mg^{2+}-loading solution containing 10 μM diaminofluorescein-2 diacetate and 0.025% (w/v) Pluronic F127. The fluorescence intensity of diaminofluorescein-2 increases with the binding of NO (Kojima et al., 1998). The diaminofluorescein-2 diacetate-loaded cells were mounted on an inverted fluorescence microscope. A confocal laser scanning microscope (LSM510, Zeiss) equipped with an objective lens (×40) and a long-pass emission filter (505 nm) was used for scanning cells. Images were taken every 1 min. The fluorescence intensities obtained from each experiment were normalized using a reference image measured in the Mg^{2+}-loading solution.

2.6. Measurement of intracellular cyclic GMP content

Intracellular cyclic GMP content was determined in sub-confluent cells cultured in 96-well plates. The cells were incubated in the Mg^{2+}-loading solution, Mg^{2+}-free solution, or Na^+/Mg^{2+}-free solution for the indicated time at 37 °C. The cyclic GMP content was measured using an

enzyme immunoassay kit (Amersham Pharmacia Biotec, Piscataway, USA). Protein concentration was measured using the protein assay kit (Bio-Rad Laboratories, CA, USA).

2.7. Statistics

Results are presented as the means ± S.E.M. of a number of observations. Differences between groups were analyzed by one-way analysis of variance (ANOVA), and correction for multiple comparison was made using Tukey's and Dunnett's multiple comparison tests. Statistically significant differences were assumed at $P<0.05$.

3. Results

3.1. Effect of extracellular high Mg^{2+} load on $[Mg^{2+}]_i$

The $[Mg^{2+}]_i$ of NRK-52E cells cultured in normal medium was 0.32 ± 0.04 mM ($n=12$). Short (1 h) exposure of the cells to 5 mM Mg^{2+}-containing medium did not significantly change the basal $[Mg^{2+}]_i$ (0.35 ± 0.02 mM, $n=12$). These data are coincident with the case of neuropile glial cells (Hintz et al., 1999). Removal of Mg^{2+} from the extracellular bathing solution did not significantly decrease $[Mg^{2+}]_i$ in normal cells ($n=18$). When the cells were cultured in 5 mM Mg^{2+}-containing medium for 1–2 days, $[Mg^{2+}]_i$ was significantly higher (1.00 ± 0.04 mM, $P<0.01$, $n=12$) than in normal cells. Furthermore, Mg^{2+} removal significantly decreased $[Mg^{2+}]_i$ from 1.02 ± 0.05 to 0.85 ± 0.05 mM ($n=15$). In the present study, we used Mg^{2+}-loaded cells in order to examine the regulatory mechanisms of Mg^{2+} efflux.

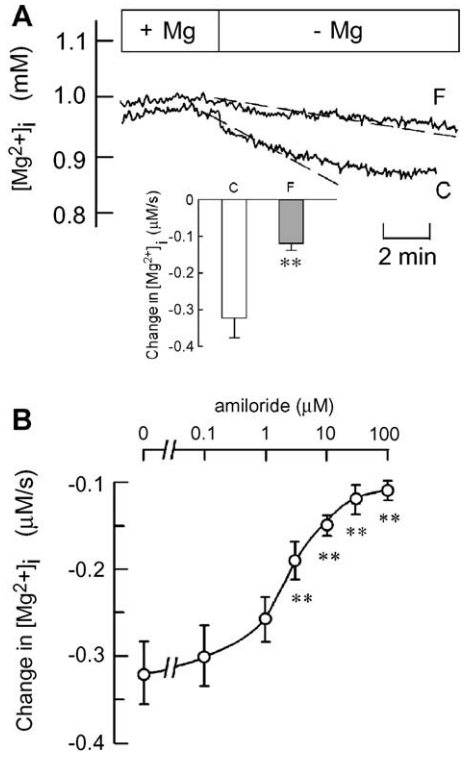

Fig. 1. Removal of extracellular Mg^{2+} induces Na^+-dependent $[Mg^{2+}]_i$ decrease. (A) Typical traces of $[Mg^{2+}]_i$ change in NRK-52E cells. The cells were perfused with Mg^{2+}-loading solution (+Mg), and then with Mg^{2+}-free solution (−Mg) in the presence (C) and absence (F) of Na^+. Inset: the change in $[Mg^{2+}]_i$ with time was estimated in the first 200 s following perfusion of Mg^{2+}-free (□) or Na^+/Mg^{2+}-free solutions (■); **, significantly different from the value of Na^+-containing solution ($P<0.01$). (B) Amiloride was preincubated with each concentration for 5 min before measurements; **, significantly different from the value in the absence of amiloride ($P<0.01$).

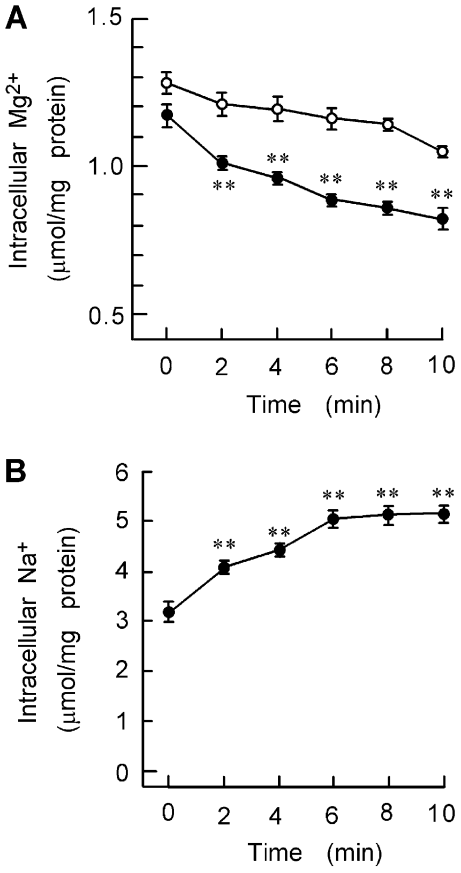

Fig. 2. Effect of Mg^{2+} removal on intracellular Mg^{2+} and Na^+ contents. (A) The bathing solution was changed from Mg^{2+}-loading solution to the Na^+-containing Mg^{2+}-free solution (●) or the Na^+/Mg^{2+}-free solution (○) at 0 min; **, significantly different from the value for the Na^+/Mg^{2+}-free solution ($P<0.01$). (B) The bathing solution was changed from the Mg^{2+}-loading solution to the Mg^{2+}-free solution at 0 min; **, significantly different from the value for 0 min ($n=4-5$).

3.2. Inhibition of Na^+-dependent $[Mg^{2+}]_i$ decrease by amiloride

The $[Mg^{2+}]_i$ decrease was potently inhibited by removal of extracellular Na^+ (Fig. 1A), suggesting that the $[Mg^{2+}]_i$ decrease is induced through a Na^+-depend-

ent Mg^{2+} efflux pathway. Amiloride, which inhibits Na^+-dependent Mg^{2+} transport (Cefaratti et al., 2000; Gunther and Vormann, 1995; Yago et al., 2000), dose dependently inhibited the $[Mg^{2+}]_i$ decrease (Fig. 1B). The IC_{50} was about 3 μM. However, the inhibitory effect was not complete at maximal concentration (100 μM). The remaining component may be Na^+-independent transport and passive leak because it was not inhibited by Na^+ removal and amiloride. These results indicated that the extracellular Mg^{2+} removal-induced decrease in $[Mg^{2+}]_i$ is mainly mediated via an Na^+-dependent pathway that is sensitive to amiloride. Using atomic absorption spectrophotometry, we measured the intracellular Mg^{2+} and Na^+ contents. The resting Mg^{2+} content was 0.65 ± 0.08 μmol/mg protein in normal cells and 1.28 ± 0.04 μmol/mg protein in 5 mM Mg^{2+}-loaded cells ($n = 4$). The Na^+-dependent Mg^{2+} decrease was observed in the Mg^{2+}-loaded cells (Fig. 2A). The intracellular Na^+ content was increased by Mg^{2+} removal (Fig. 2B). Both Na^+ and Mg^{2+} contents changed within 2 min after Mg^{2+} removal, same as that of $[Mg^{2+}]_i$. These results indicated that the Na^+-dependent Mg^{2+} transport pathway operated

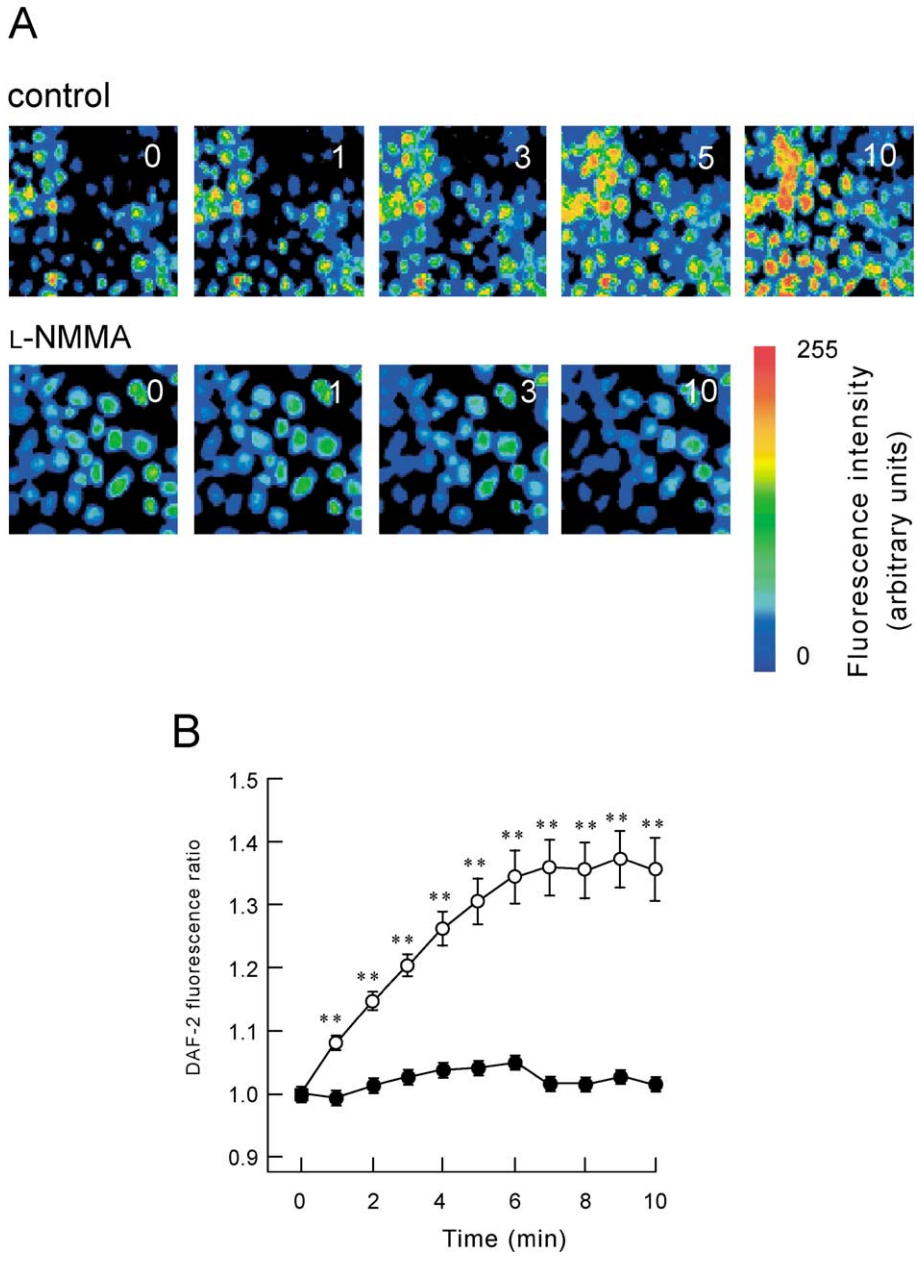

Fig. 3. Effect of Mg^{2+} removal on intracellular NO content. (A) Typical images of intracellular NO content in NRK-52E cells. The fluorescence intensity of DAF-2 was measured every 1 min. The cells were preincubated for 30 min in the absence (○) and presence (●) of 0.1 mM L-NMMA. Then, the cells were exposed to the Mg^{2+}-free solution just after the measurement at 0 min. (B) The DAF-2 fluorescence ratio was normalized to that of a reference image recorded before Mg^{2+} removal ($n = 4–5$); **, significantly different from the value in the L-NMMA treated cells ($P < 0.01$).

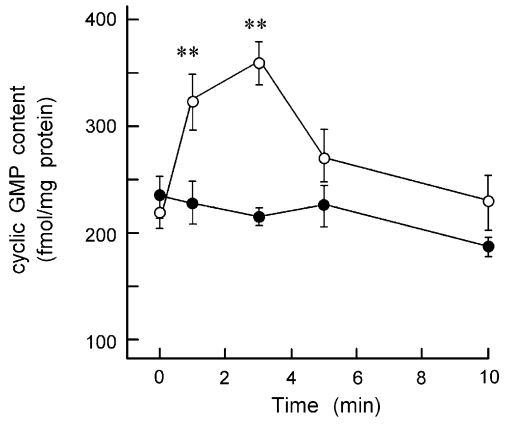

Fig. 4. Effect of Mg^{2+} removal on intracellular cyclic GMP content. Intracellular cyclic GMP content was measured at the indicated times. The cells were preincubated for 30 min in the absence (○) and presence (●) of 0.1 mM L-NMMA. Then, the cells were exposed to Mg^{2+}-free solution just after the measurement at 0 min ($n=4-5$); **, significantly different from the value for the L-NMMA treated cells ($P<0.01$).

in Na^+ influx and Mg^{2+} efflux mode under these experimental conditions, and that the $[Mg^{2+}]_i$ decrease induced by Mg^{2+} removal (Fig. 1A) was not caused by a change in buffering capacity, but due to Na^+-dependent Mg^{2+} efflux from the intracellular compartment to the extracellular compartment.

3.3. Regulation of $[Mg^{2+}]_i$ decrease by protein kinase C and tyrosine kinase

So far, it is understood that Na^+-dependent Mg^{2+} transport activity is up-regulated by protein kinase C in rat erythrocytes (Gunther and Vormann, 1995) and vascular smooth muscle cells (Touyz and Schiffrin, 1996). However, it is up-regulated by protein kinase A in rat thymocytes (Gunther and Vormann, 1992) and ascites cells (Wolf et al., 1996). We examined the effect of the three types of protein kinase inhibitors on the $[Mg^{2+}]_i$ decrease because the sensitivity of protein kinases may be different among cell types. Calphostin C (1 μM), a protein kinase C inhibitor, and genistein (10 μM), a tyrosine kinase inhibitor, significantly blocked the $[Mg^{2+}]_i$ decrease. The change in $[Mg^{2+}]_i$ was 0.30 ± 0.03 μM/s (control), 0.13 ± 0.01 μM/s (calphostin C), and 0.14 ± 0.02 μM/s (genistein), respectively ($P<0.01$). Furthermore, phorbol 12,13-dibutyrate (1 μM), a protein kinase C activator, increased the $[Mg^{2+}]_i$ decrease (0.37 ± 0.01 μM/s, $P<0.05$). But *N*-(2-[*p*-bromocinnamylamino]ethyl)-5-isoquinolinesulfonamide hydrochloride (10 μM), a protein kinase A inhibitor, did not affect the $[Mg^{2+}]_i$ decrease (0.33 ± 0.05 μM/s, $P>0.05$). These results indicate that the Na^+-dependent Mg^{2+} transporter in NRK-52E cells is positively regulated by protein kinase C, just as reported in rat erythrocytes and vascular smooth muscle cells, and by tyrosine kinase, which has not been examined in other cells.

3.4. Effect of Mg^{2+} removal on intracellular NO and cyclic GMP contents

Intracellular NO content was measured with an NO-sensitive fluorescent indicator, diaminofluorescein-2 (Kojima et al., 1998), and a confocal laser scanning microscope. Removal of extracellular Mg^{2+} induced an increase in NO content in a time-dependent manner (Fig. 3). Preincubation with L-NMMA (0.1 mM), an inhibitor of NO production, completely inhibited the increase in NO content. Similarly, extracellular Mg^{2+} removal induced an increase in cyclic GMP content (Fig. 4). This increase peaked at 3 min and then returned to the basal level. Preincubation with L-NMMA (0.1 mM) did not affect the basal cyclic GMP content, but completely inhibited the increase induced by Mg^{2+} removal.

3.5. Effect of intracellular NO and cyclic GMP levels on $[Mg^{2+}]_i$ decrease

We examined whether intracellular NO and cyclic GMP levels change the $[Mg^{2+}]_i$ decrease induced by Mg^{2+} removal. NOR1 (0.1 mM), an NO donor, and 8-bromo-cyclic GMP (0.1 mM), a membrane-permeable cyclic GMP analogue, accelerated the $[Mg^{2+}]_i$ decrease (Fig. 5A). These NO- and cyclic GMP-elicited $[Mg^{2+}]_i$ decreases were

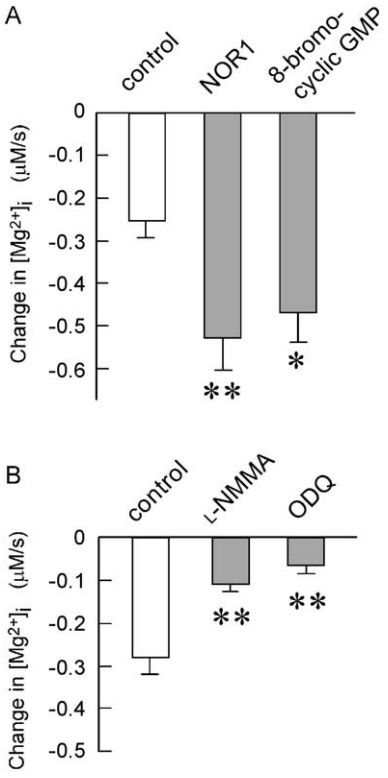

Fig. 5. NO and cyclic GMP accelerate $[Mg^{2+}]_i$ decrease. (A) NOR1 (0.1 mM) and 8-bromo-cyclic GMP (0.1 mM) were added 3 min before the perfusion of Mg^{2+}-free solution. (B) L-NMMA (0.1 mM) and ODQ (10 μM) were preincubated for 30 min ($n=6-8$); * and **, significantly different from the control value ($P<0.05$ and <0.01, respectively).

inhibited by amiloride (change in $[Mg^{2+}]_i$: -0.13 ± 0.02 μM/s in NOR1-treated cells and -0.14 ± 0.01 μM/s in 8-bromo-cGMP-treated cells, $n=4$). L-NMMA (0.1 mM) and ODQ (10 μM), an NO-sensitive guanylate cyclase inhibitor, blocked the $[Mg^{2+}]_i$ decrease (Fig. 5B). The remaining $[Mg^{2+}]_i$ decrease, which was not inhibited by L-NMMA or ODQ, was the same as those seen in amiloride-treated cells. These results indicate that the generation of intracellular NO and cyclic GMP leads to an up-regulation of the Na^+-dependent $[Mg^{2+}]_i$ decrease.

4. Discussion

Several Mg^{2+} transporters, such as passive Mg^{2+} transport, Na^+/Mg^{2+} exchange, and an energy-dependent Mg^{2+} pump, are thought to exist in mammalian cells (Quamme, 1989; Yago et al., 2000). The regulatory mechanisms of these putative transporters have not been characterized well because (1) the cDNAs are not cloned; (2) the measurement of Mg^{2+} transport activity is difficult under physiological conditions; and (3) the regulatory mechanisms differ in different types of cells. We needed to establish the proper conditions for Mg^{2+} measurement and examine the regulatory mechanisms in detail.

In the present study, we used renal NRK-52E cells cultured in high Mg^{2+} (5 mM) medium for 1 or 2 days beforehand. The resting $[Mg^{2+}]_i$ was higher than in normal cells, and extracellular Mg^{2+} removal induced an $[Mg^{2+}]_i$ decrease that was not observed in normal cells (Fig. 1A). As shown in Fig. 2A, we confirmed that Mg^{2+} removal caused Mg^{2+} efflux, using atomic absorption spectrophotometry. We suggest that the Mg^{2+} transport pathway is silent under physiological conditions and is activated by increasing the Mg^{2+} gradient.

A putative Na^+-dependent Mg^{2+} transport system has been reported in various mammalian cells, such as mouse ascites cells (Wolf et al., 1996), human, rat, and chicken erythrocytes (Gunther and Vormann, 1989), rat thymocytes (Gunther and Vormann, 1992), sublingual acini (Zhang and Melvin, 1995), and hepatocytes (Cefaratti et al., 2000). This transport system is positively regulated by protein kinase C or protein kinase A, respectively. Amiloride commonly inhibits Na^+-dependent Mg^{2+} transport, whereas it does not inhibit Na^+-independent Mg^{2+} transport (Cefaratti et al., 2000; Jutter and Ebel, 1998). Our results also showed that the $[Mg^{2+}]_i$ decrease was mostly sensitive to extracellular Na^+ concentration (Fig. 1A) and inhibited by amiloride in a dose-dependent manner (Fig. 1B). Furthermore, protein kinase C and tyrosine kinase regulated the $[Mg^{2+}]_i$ decrease, but protein kinase A had no effect. We suggest that an amiloride-sensitive and Na^+-dependent Mg^{2+} efflux pathway is present in NRK-52E cells, and that this pathway is positively regulated by protein kinase C as reported in rat erythrocytes (Gunther and Vormann, 1995) and vascular smooth muscle cells (Touyz and Schiffrin, 1996).

NO plays prominent roles in the homeostatic regulation of glomerular, vascular, and tubular function (Kone and Baylis, 1997). Interestingly, the plasma NO level in Mg^{2+}-deficient rats is higher than in normal rat (Mak et al., 1996; Rock et al., 1995). We found that Mg^{2+} removal increased the NO content in these cells (Fig. 3). Morrill et al. (1998) reported that a low extracellular Mg^{2+} concentration changed the membrane phospholipid composition and down-regulated sphingomyelin synthase in vascular smooth muscle cells. It may be possible that Mg^{2+} removal disrupts the stability of the plasma membrane and liberates ceramide, which is reported to increase NO production in bovine aortic endothelial cells (Igarashi et al., 1999).

The increase in NO and cyclic GMP contents was observed within 1 min after Mg^{2+} removal, but the time course was different: the NO content increased continuously over 6 min, while the cyclic GMP content increased transiently and returned to the basal level (Figs. 3 and 4). We suggest that cyclic GMP acts downstream of NO because (1) cyclic GMP production was inhibited by L-NMMA, and (2) the decrease in $[Mg^{2+}]_i$ and Mg^{2+} content slowed within 4 min. We also found that NOR1 and 8-bromo-cyclic GMP accelerated the $[Mg^{2+}]_i$ decrease (Fig. 5A). In contrast, L-NMMA and ODQ inhibited this decrease (Fig. 5B). These results indicate for the first time that intracellular NO and cyclic GMP up-regulate the putative, Na^+-dependent Mg^{2+} transport pathway. The target of cyclic GMP is proposed to be phosphodiesterases, cyclic GMP-gated cation channels, and cyclic GMP-dependent protein kinases (Vaandrager and Jonge, 1996). Further studies are needed to investigate how the NO and cyclic GMP pathway up-regulate the Mg^{2+} transport pathway.

In conclusion, NRK-52E cells have a Na^+-dependent Mg^{2+} transport pathway that is sensitive to amiloride, protein kinase C, and tyrosine kinase. Furthermore, NO and cyclic GMP up-regulate this Na^+-dependent Mg^{2+} transport pathway. We suggest that the Mg^{2+} transport pathway of the plasma membrane may be silent under physiological conditions but is activated by an increase in the Mg^{2+} gradient accompanied by an increase in NO and cyclic GMP concentrations under pathological conditions.

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