Regulation by extracellular Na^+ of cytosolic Mg^{2+} concentration in Mg^{2+}-loaded rat sublingual acini

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Abstract The regulation of cytosolic free Mg^{2+} concentration ($[Mg^{2+}]_i$) in Mg^{2+}-loaded rat sublingual mucous acini was examined using the Mg^{2+}-sensitive fluorescent indicator mag-fura-2. Loading sublingual acini with 5 mM Mg^{2+} elevated the $[Mg^{2+}]_i$ from 0.35 ± 0.01 mM to 0.66 ± 0.01 mM. Removal of extracellular Mg^{2+} resulted in a significantly faster $[Mg^{2+}]_i$ decrease in Mg^{2+}-loaded acini than in unloaded acini. Membrane depolarization with high extracellular $[K^+]$ and inhibition of P-type ATPases by vanadate did not alter the $[Mg^{2+}]_i$ decrease, indicating that the Mg^{2+} efflux mechanism is not electrogenic. Na^+-free medium inhibited 80% of the $[Mg^{2+}]_i$ decrease suggesting that a Na^+-dependent Mg^{2+} efflux pathway mediates the $[Mg^{2+}]_i$ decrease. Accordingly, the Na^+-dependent antiport inhibitor quinidine reduced > 80% of the $[Mg^{2+}]_i$ decrease, suggesting that the Na^+-dependent Mg^{2+} efflux is mediated by the Na^+/Mg^{2+} antiport system. Mg^{2+} efflux was also partly driven by K^+. The $[Mg^{2+}]_i$ decrease was significantly inhibited by carbachol, a muscarinic agonist, but not by cAMP. These results indicate that in sublingual acinar cells a Na^+-dependent pathway mediates Mg^{2+} efflux and that muscarinic stimulation may regulate Mg^{2+} extrusion.

Key words: Salivary acini; Mg^{2+} loading; $[Mg^{2+}]_i$ decrease; Na^+/Mg^{2+} antiport

1. Introduction

Mg^{2+} is necessary for many cell functions [1,2], such as activating enzymes, stabilizing the structure of membranes and ribosomes, and modifying ion channel and cotransporter activities. However, the mechanisms regulating the cytosolic free Mg^{2+} concentration ($[Mg^{2+}]_i$) and Mg^{2+} transport are still poorly understood. $[Mg^{2+}]_i$ is controlled within a narrow range, although the extracellular $[Mg^{2+}]$ varies widely [2]. Since the physiological $[Mg^{2+}]_i$ is much (~100×) lower than predicted from the electrochemical equilibrium, Mg^{2+} extrusion is required for maintaining the low $[Mg^{2+}]_i$. A Mg^{2+} pump is a possible mechanism to extrude Mg^{2+} [1,3]. Mg^{2+} transporter genes have been cloned from bacterium, *Salmonella typhimurium* [4,5]. However, no evidence shows that the similar Mg^{2+} transport systems appear to exist in mammalian cells. Many mammalian cell types, including parotid gland cells [6], have Mg^{2+}-ATPase, however, this activity is unrelated to Mg^{2+} transport. Na^+/Mg^{2+} antiport is found in several cell types [3,7–12]. This Mg^{2+} efflux system is sensitive to quinidine [10,13], Mn^{2+} and tricyclic antidepressant drugs [14]. The inwardly directed Na^+ gradient is the driving force for Mg^{2+} efflux mediated by this pathway [15]. In addition, a Na^+-independent Mg^{2+} efflux pathway is present in red blood cells [13,16].

The Mg^{2+} extrusion mechanism and its regulation in salivary acinar cells are unknown. In a previous study [17], we found that under physiological conditions the plasma membrane of sublingual acinar cells has a low permeability to Mg^{2+}. The muscarinic agonist-stimulated $[Mg^{2+}]_i$ increase was independent of the extracellular Mg^{2+} concentration. In this report, the Mg^{2+} efflux mechanism in salivary cells was examined using Mg^{2+}-loaded rat sublingual mucous acini.

2. Materials and methods

2.1. Materials

Male 150–250 g Wistar strain rats (Charles River, Kingston Facility, NY) were used in all experiments. Earle's minimal essential medium was purchased from Biofluids (Rockville, MD). Hyaluronidase (type I-S), Hepes, quinidine, carbachol, db-cAMP and BSA (type V) were from Sigma (St. Louis, MO). Collagenase (type CLSPA) was from Worthington Biomedical (Malvern, PA). Mag-fura-2/AM, ionomycin, Fura-2/AM and BAPTA were from Molecular Probes (Eugene, OR). All other chemicals used were of the highest grade available.

2.2. Solutions

The digestion medium consisted of Earle's minimal medium, 1% BSA, 100 U/ml collagenase and 0.2 mg/ml hyaluronidase. PSS contained (in mM): 110 NaCl, 25 $NaHCO_3$, 20 Hepes, 10 glucose, 5.4 KCl, 1.2 $CaCl_2$, 0.8 $MgSO_4$, 0.4 KH_2PO_4 and 0.33 NaH_2PO_4, and pH was adjusted to 7.4 with NaOH after gassing with 95% O_2 and 5% CO_2 for more than 45 min. The Mg^{2+}-loading medium contained (in mM): 115 KCl, 25 $KHCO_3$, 20 Hepes, 10 glucose, 0.4 KH_2PO_4, 0.2 BAPTA (K salt), 4.2 $MgCl_2$ and 0.8 $MgSO_4$, and pH was adjusted to 7.4 with KOH after gassing with 95% O_2/5% CO_2 for more than 2 h. The free Ca^{2+} concentration in the Mg^{2+}-loading medium determined using fura-2 acid was 31.9 nM. The Mg^{2+}-free/Na^+-containing medium contained: 115 NaCl, 25 $NaHCO_3$, 20 Hepes, 10 glucose, 5.4 KCl, 0.4 KH_2PO_4, 0.33 NaH_2PO_4 and 0.2 BAPTA (K salt), and pH was adjusted to 7.4 with NaOH after O_2/CO_2 gassing. The free Ca^{2+} concentration in Mg^{2+} efflux medium determined using fura-2 acid was 14 nM. In ion substitution experiments, Na^+ and K^+ were substituted with the cations indicated in Table 1.

2.3. Isolation of rat sublingual mucous acini

Dispersed rat sublingual acini were isolated as described previously [17,18]. In short, the sublingual glands removed from 3 rats were placed in ice-cold digestion medium and dissected free of connective tissue and minced, then, incubated in 10 ml of the same solution at 37°C with continuous gassing (95% O_2 and 5% CO_2, humidified) and shaking (80 cycles/min). The mince was dispersed by gently pipetting 10× with a 10-ml plastic pipette at 15-min intervals. After 45 min of digestion, the preparation was centrifuged at 50 × *g* for 30 s and resuspended in 10

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Abbreviations: BAPTA, bis(*O*-aminophenoxy)ethane-*N,N,N',N'*-tetraacetic acid; BSA, bovine serum albumin; $[Ca^{2+}]_i$, cytosolic free Ca^{2+} concentration; db-cAMP, N^6,2'-*O*-dibutyladenosine 3',5'-cyclic monophosphate; Hepes, 4-(2-hydroxyethyl)-1-piperazine ethanesulphonic acid; $[Mg^{2+}]_i$, cytosolic free Mg^{2+} concentration; NMDG, *N*-methyl-D-glucamine; PSS, physiological salt solution.

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ml of fresh medium and incubated for another 45 min. After digesting for a total of 1.5 h, the dispersed acini were washed 3× with PSS containing 0.01% BSA at 23°C. The resulting preparation was isolated into two parts; small and large aggregates by centrifugation at 400 × g for 1 min. The small aggregates, consisting mainly of 3–15 acini/clump, were used.

2.4. Mg^{2+} loading

Isolated sublingual acini were loaded with Mg^{2+} and depleted of Na^+ as described by Günther et al. [19]. Briefly, the acini were incubated 30 min at 37°C in the Mg^{2+}-loading medium containing 0.01% BSA and 10 μM ionomycin. After 30 min incubation, the acini were rinsed and resuspended in the same medium without ionomycin for 10 min. This step was repeated 3×, then the acini were suspended in the same medium and kept at room temperature until use.

2.5. Determination of $[Mg^{2+}]_i$

Mg^{2+}-loaded sublingual acini were loaded with mag-fura-2 by incubation with 2 μM mag-fura-2/AM for 30 min at room temperature. The mag-fura-2-loaded acini were rinsed twice with Mg^{2+}-loading medium containing 0.01% BSA. $[Mg^{2+}]_i$ was determined as previously reported at 23°C [17,20]. Briefly, 0.5 ml of acinar suspension was removed, centrifuged at 400 × g for 15 s and the acinar pellet resuspended in the same medium without BSA to promote attachment to a coverslip mounted in a perfusion chamber. A pinhole turret was used to locate five to eight cells on the stage of a Nikon inverted microscope with a Nikon fluor X40 1.3NA oil immersion lense. The mag-fura-2 fluorescence was monitored using an AR-CM fluorometer (SPEX Industries, Edison, NJ). Dye was alternately excited with light of 340 and 380 nm wavelengths while the fluorescence emitted at 500 nm was sampled. The fluorescence ratios (340/380) were converted to $[Mg^{2+}]_i$ by calibration as previously described [17,20].

2.6. Determination of $[Ca^{2+}]_i$

$[Ca^{2+}]_i$ was determined by using the Ca^{2+}-sensitive fluorescent indicator, fura-2, as previously described [18,21,22]. Briefly, Mg^{2+}-loaded sublingual acini were loaded with fura-2 by incubation with 2 μM fura-2/AM for 30 min at 23°C. The fura-2-loaded acini were rinsed twice with Mg^{2+}-loading medium containing 0.01% BSA and resuspended in the same medium. Determination of $[Ca^{2+}]_i$ was essentially the same procedure as used in determination of $[Mg^{2+}]_i$. Calibration was performed according to the method described previously [18,21,22]. $[Ca^{2+}]_i$ was calculated as described by Grynkiewicz et al. [23] using 224 nM as the K_d of fura-2 for Ca^{2+}.

2.7. Statistics

All values are presented as mean ± S.E. of separate experiments using different preparations. The significance between values was examined by the unpaired Student's *t* test.

3. Results

The resting $[Mg^{2+}]_i$ in isolated rat sublingual acini was 0.35 ± 0.01 mM (n = 24) as previously reported [17,20]. After loading the acini with 5 mM Mg^{2+} and depleting of Na^+ for 30 min, the $[Mg^{2+}]_i$ was increased to 0.66 ± 0.01 mM (n = 75). Superfusion of the unloaded acini with a Mg^{2+}-free/Na^+-containing medium induced only a very slow and slight decrease in $[Mg^{2+}]_i$ (29.8 ± 9.1 μM/30 min, n = 6). In contrast, the $[Mg^{2+}]_i$ in Mg^{2+} (5 mM)-loaded acini decreased dramatically when superfused with the Mg^{2+}-free/Na^+-containing medium (343.7 ± 30.2 μM/30 min, n = 8; $P < 0.001$ compared with unloaded acini; Fig. 1). The initial rate of the $[Mg^{2+}]_i$ decrease in Mg^{2+}-free/Na^+-containing medium was 81.4 ± 7.1 μM/min (n = 8). The possibility that the increased membrane permeability to Mg^{2+} was due to residual ionomycin was tested by using the same loading procedure but physiological external Mg^{2+} (0.8 mM). After treatment with 0.8 mM Mg^{2+} for 30 min $[Mg^{2+}]_i$ was 0.33 ± 0.01 mM (n = 6), virtually identical with the physiological resting level (0.35 mM). Superfusion of these control

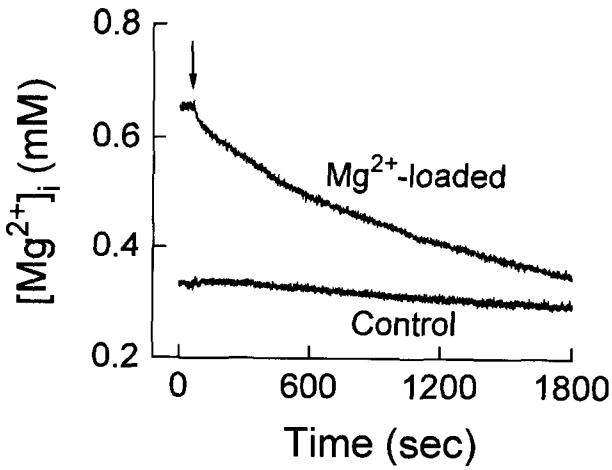

Fig. 1. $[Mg^{2+}]_i$ changes in Mg^{2+}-loaded sublingual acini. Rat sublingual acini were loaded with either 5 mM Mg^{2+} (Mg^{2+}-loaded) or 0.8 mM Mg^{2+} (Control) for 30 min and washed 4 ×. Acini were then loaded with 2 μM mag-fura-2 for 30 min. The Mg^{2+}- and mag-fura-2-loaded acini were superfused with the Mg^{2+}-loading media without ionomycin and the $[Mg^{2+}]_i$ was determined. At the time indicated by the arrow, the acini were superfused with Mg^{2+}-free/Na^+-containing medium. Each trace is representative of separate experiments using different cell preparations (control, n = 6; Mg^{2+}-loaded, n = 8).

acini with Mg^{2+}-free medium induced a decrease in $[Mg^{2+}]_i$ (32.8 ± 6.4 μM/30 min, n = 6; Fig. 1) comparable to that seen in acini not exposed to ionomycin (see above). These results suggest that the $[Mg^{2+}]_i$ decrease after Mg^{2+}-loading was not derived from an ionomycin-mediated membrane permeability.

It has been reported that Ca^{2+} interferes with $[Mg^{2+}]_i$ determination when using mag-fura-2 [24–26]. In order to avoid this interference, the specific Ca^{2+} chelator BAPTA (0.2 mM) was used to eliminate changes in $[Ca^{2+}]_i$. As shown in Fig. 2, after Mg^{2+}-loading $[Ca^{2+}]_i$ was not significantly altered by either muscarinic stimulation (10 μM carbachol) or Mg^{2+}-free medium. Under the experimental conditions used here, the observed $[Mg^{2+}]_i$ decrease was clearly not derived from the interference of Ca^{2+} on the mag-fura-2 fluorescence.

To examine whether the $[Mg^{2+}]_i$ decrease is mediated by an electrogenic Mg^{2+} efflux pathway, the plasma membrane was depolarized by addition of 70 mM K^+. The rate of the $[Mg^{2+}]_i$ decrease (77.2 ± 19.5 μM/min; n = 5; Fig. 3) in depolarized acini was similar to the rate in physiological $[K^+]$ and the same extracellular $[Na^+]$ (87.4 ± 11.2 μM/min, n = 7; Fig. 3). These results suggest that the $[Mg^{2+}]_i$ decrease is mediated by an electroneutral process.

The influence of extracellular Mg^{2+} on the $[Mg^{2+}]_i$ decrease was examined. The Mg^{2+}-loaded acini were superfused with a medium containing 5 mM Mg^{2+} and 140 mM Na^+. High external Mg^{2+} completely prevented the $[Mg^{2+}]_i$ decrease (Fig. 4). A physiological concentration (0.8 mM) of Mg^{2+} only slightly blunted the $[Mg^{2+}]_i$ decrease (74.6 ± 9.1 μM/min, n = 5; Fig. 4) compared with the Mg^{2+}-free condition (see Fig. 1).

The $[Mg^{2+}]_i$ decrease from the Mg^{2+}-loaded acini was Na^+-dependent. Sucrose and $NMDG^+$ medium inhibited 79% and 82% of the $[Mg^{2+}]_i$ decrease, respectively (Table 1, Fig. 5), indicating that the Na^+-dependent $[Mg^{2+}]_i$ decrease is mediated by a Na^+/Mg^{2+} antiport mechanism. K^+ could partly substitute for Na^+, however, the initial rates of the $[Mg^{2+}]_i$ decreases in

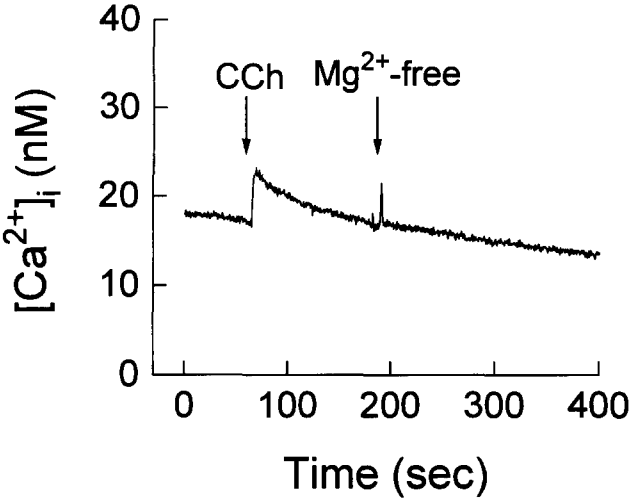

Fig. 2. Effects of muscarinic stimulation and Mg^{2+}-free medium on $[Ca^{2+}]_i$ in Mg^{2+}-loaded acini. Fura-2- and Mg^{2+}-loaded sublingual acini were superfused with Mg^{2+}-loading medium and the $[Ca^{2+}]_i$ monitored. At the time indicated by the arrows, 10 μM carbachol (CCh) was added or the Mg^{2+}-free medium was applied. The trace is representative of 5 separate experiments using different cell preparations.

the presence of other monovalent cations, Rb^+ and Cs^+, were not significantly different from the Na^+-free conditions (Table 1).

To further confirm the mechanism mediating the $[Mg^{2+}]_i$ decrease in sublingual acinar cells, P-type ATPases were inhibited with vanadate. The $[Mg^{2+}]_i$ decrease was not affected by vanadate (83.6 ± 6.3 μM/min, n = 5; Fig. 6) suggesting that the $[Mg^{2+}]_i$ decrease is not mediated by a P-type ATPase. In contrast, the Na^+/Mg^{2+} antiport inhibitor quinidine (50 μM) significantly blocked the $[Mg^{2+}]_i$ decrease (11.4 ± 2.7 μM/min, n = 5; $P < 0.005$; Fig. 6) indicating that the Na^+-dependent $[Mg^{2+}]_i$ decrease is mediated by a Na^+/Mg^{2+} antiport system.

In myocytes and hepatocytes, an increase of cellular cAMP results in Mg^{2+} efflux [27–29]. In the present study, Mg^{2+}-loaded acini were stimulated by cAMP or carbachol in the Na^+-free ($NMDG^+$) medium for 2 min, then superfused with the Mg^{2+}-free/Na^+-containing solution. The initial rate of the $[Mg^{2+}]_i$ decrease (64.0±13.7 μM/min, n = 7; Fig. 7) in the acini stimulated with the membrane-permeable cAMP analog db-cAMP (0.2 mM) was comparable to the rate of the unstimulated control (56.0 ± 7.2 μM/min, n = 8; Fig. 7). Conversely, the $[Mg^{2+}]_i$ decrease was significantly inhibited by the muscarinic agonist carbachol (28.5 ± 4.9 μM/min, n = 8; $P < 0.01$; Fig. 7).

Table 1
Ion selectivity for Mg^{2+} efflux

Ion	n	Initial rate (μM/min)
Na^+	6	86.7 ± 17.9
(Sucrose)	7	18.0 ± 4.3*
$NMDG^+$	6	15.3 ± 2.3*
K^+	5	40.0 ± 3.9*#
Rb^+	4	22.0 ± 4.6*
Cs^+	5	22.4 ± 7.9*

Mg^{2+}- and mag-fura-2-loaded acini were superfused with Mg^{2+}-loading medium for 2 min, then superfused with a Mg^{2+}-free medium containing 20 mM Hepes, pH 7.4, 10 mM glucose, 0.2 mM BAPTA and either 140 mM NaCl, NMDGCl, KCl, RbCl or CsCl. Sucrose medium contained 280 mM sucrose, 20 mM Hepes, pH 7.4, 10 mM glucose and 0.2 mM BAPTA.
*: Significantly different from Na^+, $P < 0.001$; #: significantly different from $NMDG^+$, $P < 0.001$.

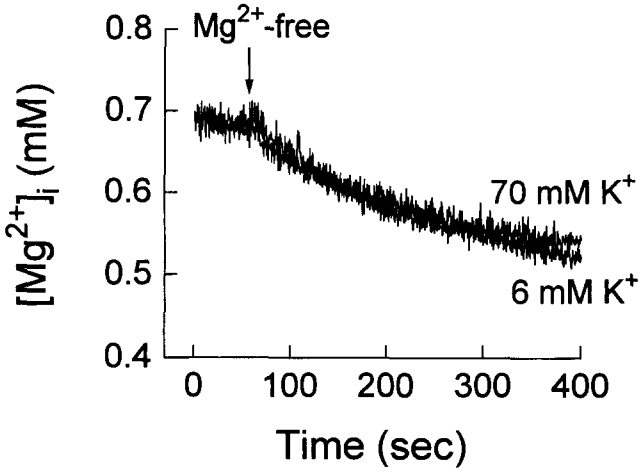

Fig. 3. Effect of membrane potential on the $[Mg^{2+}]_i$ decrease. Mag-fura-2- and Mg^{2+}-loaded acini were superfused with the Mg^{2+}-loading medium. At the time indicated by the arrow, the acini were superfused with either a Mg^{2+}-free medium (6 mM K^+) which contained (in mM): 128.4 sucrose, 70 NaCl, 20 Hepes, 10 glucose, 5.8 KCl, 0.2 BAPTA/K, pH 7.4, or with a high K^+/Mg^{2+}-free medium (70 mM K^+) in which sucrose was substituted by 64.2 mM KCl (70 mM Na^+ and 70 mM K^+). Each trace is representative of separate experiments using different cell preparations (70 mM K^+, n = 5; 6 mM K^+, n = 7).

4. Discussion

The intracellular free Mg^{2+} level in sublingual acinar cells is ~0.35 mM [17,20, this study]. To maintain such a low $[Mg^{2+}]_i$ requires active Mg^{2+} extrusion mechanism(s). The present study showed that removal of extracellular Mg^{2+} induced a relatively small decrease in $[Mg^{2+}]_i$ in unloaded cells. In contrast, the same treatment resulted in a large $[Mg^{2+}]_i$ decrease in Mg^{2+}-loaded cells (Fig. 1) suggesting that Mg^{2+} efflux is acti-

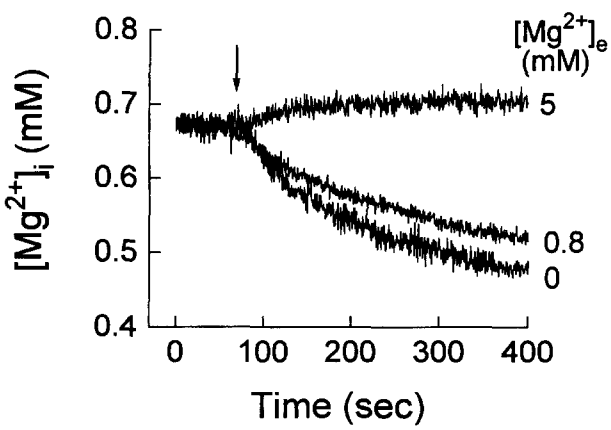

Fig. 4. Effect of extracellular Mg^{2+} on the $[Mg^{2+}]_i$ decrease. Mag-fura-2-and Mg^{2+}-loaded acini were superfused with Mg^{2+}-loading medium and the $[Mg^{2+}]_i$ was monitored. At the time indicated by the arrow, acini were superfused with the Mg^{2+} efflux solutions containing 0, 0.8 or 5 mM Mg^{2+}. Each trace is representative of separate experiments using different cell preparations (0 mM, n = 6; 0.8 mM, n = 5; 5 mM, n = 6).

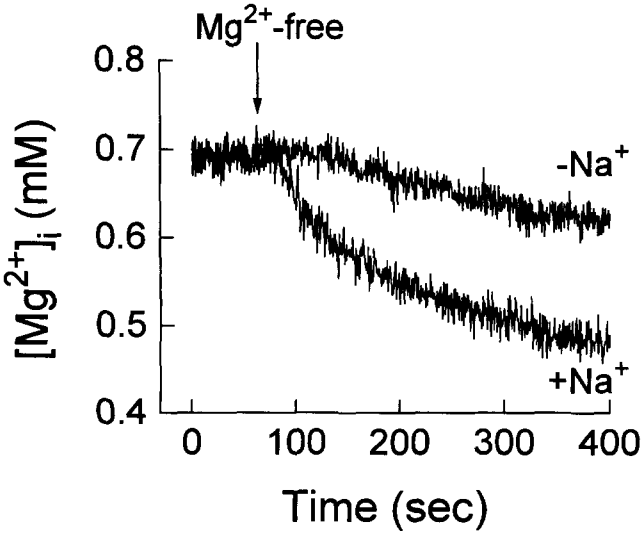

Fig. 5. Na^+ dependence of the $[Mg^{2+}]_i$ decrease. Mag-fura-2- and Mg^{2+}-loaded acini were superfused with Mg^{2+}-loading medium. At the time indicated by the arrow, the acini were superfused with a Mg^{2+}- and Na^+-free medium (-Na^+) which contained (in mM): 140 NMDGCl, 20 Hepes, 10 glucose, 0.2 BAPTA acid, pH 7.4, or a Mg^{2+}-free/Na^+-containing medium (+Na^+). Each trace is representative of separate experiments using different cell preparations (-Na^+, n = 7; +Na^+, n = 6).

vated by an elevated $[Mg^{2+}]_i$. This result is consistent with the Na^+-dependent Mg^{2+} efflux system previously described by Günther et al. [19] where the Mg^{2+} efflux mediated by Na^+/Mg^{2+} antiport only occurs at elevated $[Mg^{2+}]_i$. When the physiological $[Mg^{2+}]_i$ is reached, the Mg^{2+} efflux is greatly diminished [19]. This dual regulation of Mg^{2+} efflux by the cytosolic $[Mg^{2+}]_i$ prevents both Mg^{2+} loading and over-reduction in cytosolic Mg^{2+}.

The present study used a Ca^{2+} chelator BAPTA to prevent

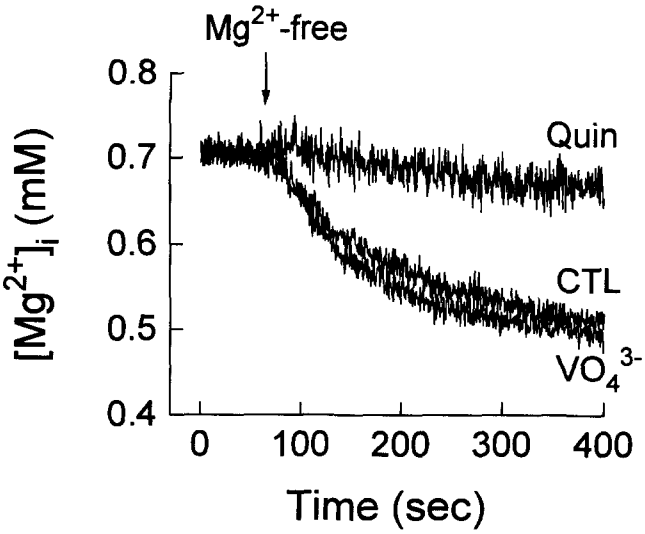

Fig. 6. Effects of quinidine and vanadate on the $[Mg^{2+}]_i$ decrease. Mag-fura-2- and Mg^{2+}-loaded acini were superfused with Mg^{2+}-loading medium (CTL), or the same medium containing 50 μM quinidine (Quin) or 0.2 mM vanadate (VO_4^{3-}). At the time indicated by the arrow, the acini were superfused with Mg^{2+} efflux medium containing quinidine or vanadate. Each trace is representative of separate experiments using different cell preparations (CTL, n = 6; Quin, n = 5; VO_4^{3-}, n = 5).

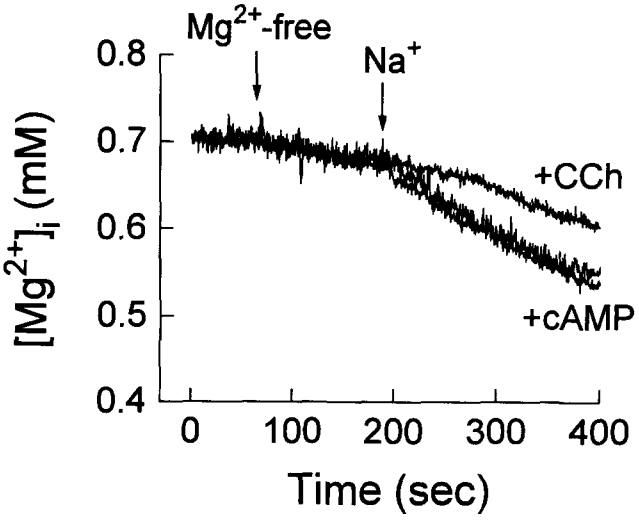

Fig. 7. Effects of db-cAMP and carbachol on the $[Mg^{2+}]_i$ decrease. Mag-fura-2- and Mg^{2+}-loaded acini were superfused with Mg^{2+}-loading medium and the $[Mg^{2+}]_i$ monitored. At the time indicated by the first arrow, acini were superfused with Mg^{2+}- and Na^+-free ($NMDG^+$) medium for 2 min. In the cAMP- and CCh-stimulation experiments, the Mg^{2+}- and Na^+-free medium contained either 0.2 mM db-cAMP (+cAMP) or 10 μM carbachol (+CCh). At the time indicated by the second arrow, acini were superfused with Mg^{2+}-free/Na^+-containing medium. In the cAMP- or CCh-stimulation experiments, 0.2 mM db-cAMP or 10 μM CCh was added. Each trace is representative of separate experiments (control, n = 8; +cAMP, n = 7; +CCh, n = 8).

cytosolic Ca^{2+} increase. The purpose of this treatment was to prevent the toxic effects of continuous $[Ca^{2+}]_i$ elevation and to avoid the interference of Ca^{2+} on $[Mg^{2+}]_i$ measurements [24–26]. Ca^{2+} interference of the $[Mg^{2+}]_i$ determination with mag-fura-2 has been reported in several cell systems [24,25] and can become a major concern [25,26]. Hurley et al. [25] reported that in exocrine pancreatic and submandibular acini, Ca^{2+} significantly interferes with the mag-fura-2 fluorescence. Indeed, we tested the contribution of Ca^{2+} interference on mag-fura-2 fluorescence and found that when the Mg^{2+} concentration is physiological, 330 nM of free Ca^{2+} induces an ~7% increase in $[Mg^{2+}]_i$ and 770 nM free Ca^{2+} results in an ~18% increase in $[Mg^{2+}]_i$ [unpublished observations]. However, in the present study, chelating Ca^{2+} with BAPTA during Mg^{2+}-loading prevented the muscarinic stimulation-induced $[Ca^{2+}]_i$ increase, indicating that the observed Mg^{2+} changes are not due to the interference of Ca^{2+} on mag-fura-2 fluorescence.

In MDCK cells, Mg^{2+} influx is mediated by a unique Mg^{2+} entry pathway which manifests similar characteristics to voltage-gated Ca^{2+} channels [30]. The membrane depolarization (Fig. 3) and a voltage-gated Ca^{2+} channel blocker diltiazem (data not shown) did not affect Mg^{2+} efflux from Mg^{2+}-loaded acini indicating that the $[Mg^{2+}]_i$ decrease process is not electrogenic and not mediated by these Ca^{2+} channels. Vanadate did not suppress the $[Mg^{2+}]_i$ decrease suggests that pump-ATPases are not involved in the $[Mg^{2+}]_i$ decrease. In red blood cells, Mg^{2+} efflux is mediated by two systems, a Na^+-independent pathway and a Na^+/Mg^{2+} antiport system. The Na^+-independent Mg^{2+} pathway appears to predominate in Mg^{2+} efflux [13,16]. In contrast, Na^+-free medium and Na^+/Mg^{2+} antiport inhibitor quinidine blocked ~80% of the $[Mg^{2+}]_i$ decrease in sublingual

acinar cells indicating that Na^+-dependent Mg^{2+} efflux is the main mechanism mediating the $[Mg^{2+}]_i$ decrease.

Mg^{2+} extrusion is activated by cAMP in ascites [31,32] and liver [27] cells. Increasing the cytosolic cAMP level with forskolin [27,31] and norepinephrine [27], or by application of membrane-permeable cAMP analogues [27,31], enhances Mg^{2+} efflux. In salivary cells, muscarinic stimulation, but not membrane-permeable cAMP, reduced the $[Mg^{2+}]_i$ decrease (Fig. 7). The inhibition of the $[Mg^{2+}]_i$ decrease by carbachol was not mediated by Ca^{2+} since changes in $[Ca^{2+}]_i$ were prevented with BAPTA. The lack of effect by cAMP may result from differences in the regulatory properties of the Na^+/Mg^{2+} antiporter in sublingual acinar cells compared with those found in ascites and liver cells.

In summary, Mg^{2+} extrusion in rat sublingual acinar cells is mediated by a Na^+/Mg^{2+} antiport system and is inhibited by muscarinic stimulation. Further investigations are needed to elucidate the underlying mechanism regulating Mg^{2+} extrusion during muscarinic stimulation.

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