Regulation of Magnesium Uptake and Release in the Heart and in Isolated Ventricular Myocytes

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Perfused rat hearts release or accumulate approximately 10% of total Mg²⁺ content when stimulated with norepinephrine (NE) or carbachol, respectively. Collagenase-dispersed rat ventricular myocytes increase or decrease total cell Mg²⁺ by 1 mM within 5 minutes when stimulated with these same transmitters. Measurements of Mg²⁺ transport using ²⁸Mg or atomic absorbance spectrophotometry indicate that the rate and the extent of both stimulated Mg²⁺ efflux and influx are independent of the concentration of extracellular Mg²⁺ (0 to 1.2 mM). Mg²⁺ release induced by NE is rapidly reversed by the addition of carbachol, and Mg²⁺ uptake induced by carbachol is reversed by NE. Decreasing extracellular Na⁺ or Ca²⁺ decreases or abolishes Mg²⁺ efflux from myocytes. Cd²⁺ or other Ca²⁺ channel blockers also inhibit Mg²⁺ influx in the presence of a physiological concentration of extracellular Ca²⁺. Replacement of extracellular Ca²⁺ with Sr²⁺ or with Mn²⁺ decreases or abolishes both stimulated efflux and influx of Mg²⁺. Redistribution of ⁸⁶Sr in myocytes and in the supernatant indicates that under those conditions Sr²⁺ is released or accumulated by NE or carbachol in a manner similar to that of Mg²⁺. Hence, at least in the case of Sr²⁺, the inhibition of Mg²⁺ fluxes can be explained by the transport of Sr²⁺ rather than Mg²⁺ through the transport(s) systems. By contrast, replacement of extracellular Ca²⁺ with Ba²⁺ inhibits stimulated Mg²⁺ uptake but not Mg²⁺ release. These results indicate that cardiac myocytes have a major pool of Mg²⁺ that can be rapidly mobilized upon hormonal stimulation. The net uptake and release of Mg²⁺ are quantitatively similar and appear to be independent of the extracellular Mg²⁺ concentrations but are affected, to various degrees, by the presence of other cellular or extracellular cations. (Circulation Research 1993;72:1139–1148)

KEY WORDS • Mg²⁺ • myocytes • β-adrenergic stimulation

Mg²⁺ is one of the most abundant cellular cations,¹⁻³ and increasing evidence indicates that it plays a "key" role in regulating ion channels,⁴⁻¹¹ enzymes,¹²⁻¹⁸ and a variety of cell functions.⁹,¹⁰ Yet, this increased knowledge of individual cellular processes that are regulated by Mg²⁺ has not been paralleled by a better understanding of the mechanism(s) involved in intracellular Mg²⁺ homeostasis.

In bacteria, the existence of specific transporter(s) for Mg²⁺ has recently been demonstrated by Maguire and coworkers.¹¹,¹² In eukaryotes, the existence of a powerful Mg²⁺ transport has been inferred by Bond et al,¹³ Jakob et al,¹⁴ and Somlyo et al,¹⁵,¹⁶ who reported major cellular Mg²⁺ redistribution under hormonal stimulation in liver cells,¹³,¹⁴ muscle cells,¹⁵ and bee photoreceptors,¹⁶ respectively. The mechanism of Mg²⁺ mobilization under hormonal stimulation has also been investigated by our group. We have recently reported¹⁷ that β-adrenergic stimulation of cardiac cells induces a major extrusion of Mg²⁺ from the cells. This efflux can be mimicked by stimulating isolated cardiac ventricular myocytes with different permeant cyclic AMP analogues (i.e., dibutyryl-cAMP, 8-Cl-cAMP, 8-Br-cAMP) or with forskolin, which directly stimulates adenylate cyclase activity.¹⁸ Moreover, the addition of nanomolar concentrations of cAMP to permeabilized hepatocytes or to isolated liver mitochondria¹⁹ induces the mobilization of large amounts of Mg²⁺ from the mitochondrial pool.

We also observed that cardiac or liver cells accumulate Mg²⁺ from the extracellular compartment when stimulated with carbachol or vasopressin.¹⁷,¹⁸ This influx pathway seems to be mediated by protein kinase C, since the stimulation by protein kinase C activators, such as DAG analogues (OAG and SAG) or TPA (or PDBU), results in a quantitatively similar Mg²⁺ uptake in either cell type.²¹ However, these data fall short of identifying the type of plasma membrane transport mechanism(s) (i.e., an exchanger or a channel) involved in the Mg²⁺ influx and efflux stimulated by cAMP or protein kinase C, respectively. Recently, Gunther and coworkers proposed that a Na⁺/Mg²⁺ exchanger, formerly identified in chicken erythrocytes,²²,²³ could account for Mg²⁺ extrusion in cardiac²⁴ and liver cells.²⁵ The stoichiometry of this exchanger (2 Na⁺ out:1 Mg²⁺ in) was clearly defined in erythrocytes²² but not in the other cell types.

The present study shows that physiological perturbations of perfused rat hearts or isolated myocytes can
result in major fluxes of $\text{Mg}^{2+}$ to and from the cells. It then investigates if these vectorially opposite $\text{Mg}^{2+}$ transport pathways have a comparable requirement for intracellular and extracellular ions and result in the mobilization of $\text{Mg}^{2+}$ from and to similar cellular $\text{Mg}^{2+}$ pools.

**Materials and Methods**

**Perfused Hearts**

Hearts were quickly removed from pentothal-anesthetized male Sprague-Dawley rats (220–250 g). Hearts were perfused at a rate of 10 ml/min through a cannula inserted in the aorta, with a buffer containing (mM) NaCl 120, KCl 3, CaCl$_2$ 1.2, MgCl$_2$ 1.2, KH$_2$PO$_4$ 1.2, glucose 10, NaHCO$_3$ 12, and HEPES 10, pH 7.2 (at 37°C in the presence of 95% O$_2$–5% CO$_2$). After 10 minutes of perfusion, the buffer was replaced with a similar buffer containing either a lower $\text{Mg}^{2+}$ concentration (50 μM) or only contaminant $\text{Mg}^{2+}$ (2–5 μM). The use of low $\text{Mg}^{2+}$ in the buffer was necessary to obtain adequate sensitivity for measuring changes in $\text{Mg}^{2+}$ content of the perfusate by atomic absorbance spectrophotometry (AA). Hearts were then stimulated by the indicated concentrations of norepinephrine or carbachol, which were directly dissolved in the perfusate. The perfusate was continuously collected, pooled in test tubes each containing 20 seconds of perfusate, and the $\text{Mg}^{2+}$ content was then measured by AA (Varian AA-575).

**Isolation of Cardiac Ventricular Myocytes**

Cardiac ventricular myocytes were prepared by collagenase digestion, according to the procedure of De Young et al.$^{26}$ After isolation, cells were stabilized for 1 hour at room temperature in Joklik medium supplemented with 1.2 mM CaCl$_2$, 12 mM NaHCO$_3$, 10 mM glucose, and 95% O$_2$–5% CO$_2$, pH 7.2. CaCl$_2$ was introduced progressively to prevent cell contracture and cell damage due to Ca$^{2+}$ overloading. Cell viability was assessed by microscopy. Vital myocytes (77±3%, n=8) of this cell preparation maintained a rod-shaped configuration.

**$\text{Mg}^{2+}$ Transport in Cardiac Ventricular Myocytes**

One hour after isolation, myocytes were washed three times and resuspended, at the final concentration of 1×10$^6$ cells/ml, in a medium containing (mM) NaCl 120, KCl 3, KH$_2$PO$_4$ 1.2, MgCl$_2$ 1.2, CaCl$_2$ 1.2, NaHCO$_3$ 12, glucose 10, and HEPES 10, pH 7.2 (at 37°C in the presence of 95% O$_2$–5% CO$_2$). Henceforth, this solution will be referred to as the resuspension buffer. During these manipulations no changes in cell viability were observed. Myocytes were incubated, at a concentration of 300 µg protein/ml, in the same medium but without added $\text{Mg}^{2+}$. After 5 minutes of stabilization, various agents (norepinephrine, forskolin, carbobachol) were added to the incubation medium. At the times noted in the figures, aliquots of the experimental samples were withdrawn and rapidly sedimented in microfuge tubes. $\text{Mg}^{2+}$ content of the supernate was measured by AA. For the experiments reported in Table 1, aliquots of the incubation mixture were withdrawn at the indicated times and centrifuged in microfuge tubes through an oil layer (dibutyl-phthalate:diocetyl-phthalate [2:1]). The supernate was removed by vacuum suction, and the pellet was digested overnight in 1 ml 10% HNO$_3$. The cellular $\text{Mg}^{2+}$ content was measured in the acid extracts by A.A.

**Ion Dependence**

The role of extracellular Na$^+$ and Ca$^{2+}$ on $\text{Mg}^{2+}$ transport was investigated by incubating isolated ventricular myocytes in a medium in which sodium chloride content was isosmotically replaced with choline chloride. In other experiments, instead of Na$^+$, the CaCl$_2$ content of the medium was decreased to the concentration shown in the figures.

The role of extracellular Ca$^{2+}$ was also studied in myocytes incubated in reaction media in which Ca$^{2+}$ was replaced by Ba$^{2+}$, Mn$^{2+}$, or Sr$^{2+}$. Alternatively, myocytes were incubated in the presence of 120 μM Cd$^{2+}$ to block Ca$^{2+}$ channels and displace Ca$^{2+}$ from plasma membranes.$^{27}$ In each of these experimental conditions, only contaminant $\text{Mg}^{2+}$ was present (2–5 μM).

$^{28}\text{Mg}$ and $^{85}\text{Sr}$ Loading of Ventricular Myocytes

Myocytes, resuspended in resuspension buffer without added MgCl$_2$, were incubated for 4 hours with 1 μCi/ml $^{28}\text{Mg}$. After equilibration of the cells with the radiolabeled cation, cells were washed three times in the resuspension buffer containing 1.2 mM MgCl$_2$ to remove excess radioisotope and resuspended in the same buffer, at a final concentration of 1×10$^6$ cells/ml. For the measurements of $\text{Mg}^{2+}$ movements, cells were incubated in the same incubation buffer, with 1.2 mM MgCl$_2$ labeled with 1 μCi/ml $^{28}\text{Mg}$.

In other experiments, the Mg$^{2+}$ in the medium was replaced by 1.2 mM SrCl$_2$ or with 1.2 mM SrCl$_2$ labeled with 5 μCi/ml $^{85}\text{Sr}$. In all of these experiments, aliquots of the incubation mixture were withdrawn at selected times and sedimented through an oil layer in 1-ml centrifuge tubes.$^{28}$ The supernatant and oil layer were removed by vacuum suction, and the radioactivity in the pellet was detected by liquid scintillation counting in a Beckman LS 7000 counter (for $^{28}\text{Mg}$) or a Packard Cobra Auto-gamma counter (for $^{85}\text{Sr}$).

**Permeabilized Myocytes**

After isolation, myocytes were resuspended, at a final concentration of 1×10$^6$ cells/ml, in a medium containing (mM) KCl 100, NaCl 3, KH$_2$PO$_4$ 1.2, MgCl$_2$ 0.8, glucose 10, NaHCO$_3$ 12, HEPES 10, pH 7.2 (at 37°C in the presence of 95% O$_2$–5% CO$_2$), CaCl$_2$ 1, and EGTA 1. The free Ca$^{2+}$ concentration of this medium was estimated according to Fabiato’s program$^{29}$ and found to range between 120 and 150 nM. Cells were permeabilized by treatment with digitonin (80 µg/ml) for several minutes at room temperature. The efficiency of the permeabilization, with respect to the concentration of digitonin and the time of treatment, to yield full permeabilization, was assessed by the trypan blue exclusion test and confirmed by the ethidium bromide test.$^{30}$ After permeabilization, cells were washed three times in the same buffer to remove excess digitonin and resuspended in this buffer, at a final concentration of 1×10$^6$ cells/ml. For the measurement of $\text{Mg}^{2+}$ movements, cells were incubated, at a protein concentration of 300 µg/ml, in the same buffer without added MgCl$_2$, but in the presence of 3 mM ATP (or 1 mM ATP plus a regenerating system containing 30 mM phosphocreatine and 10 µM creatine-phosphokinase). Under these conditions,
Mg\(^{2+}\) contaminant was approximately 5 \(\mu\)M (measured by AA), and the free Ca\(^{2+}\) concentration was 80–100 nM (estimated as before). Mg\(^{2+}\) determinations were carried out as previously reported (see “Mg\(^{2+}\) Transport in Cardiac Ventricular Myocytes”).

Other Techniques

In all of the procedures, protein was assayed by using the Bradford technique.\(^{31}\) Cells were also counted in the hemacytometer, and a value of 3.8–4 mg protein/10\(^6\) cells was found.

Chemicals

Collagenase (CLS-II) was from Worthington Biochemicals Corp., Freehold, N.J. \(^{85}\)SrCl\(_2\) (5 mCi/ml) was from New England Nuclear-DuPont, Wilmington, Del. \(^{28}\)MgCl\(_2\) (1 mCi/ml) was from the Isotope Distribution Laboratory of Brookhaven National Laboratories. All other chemicals and reagents were from Sigma Chemical Co., St. Louis, Mo.

Results

We have previously shown\(^{37}\) that isolated rat hearts, perfused in a Langendorff system, release large amounts of cellular Mg\(^{2+}\) when stimulated by \(\beta\)-adrenergic agonists. However, the measurements of the Mg\(^{2+}\) content in the perfusate by AA required a nominal zero Mg\(^{2+}\) concentration in the perfusion system, a condition that could have prompted or magnified Mg\(^{2+}\) efflux along its concentration gradient.

In the present study, Mg\(^{2+}\) efflux was induced in hearts perfused in the presence of higher submillimolar concentrations of extracellular Mg\(^{2+}\). Figure 1 shows the results obtained in three hearts in the presence of 50 \(\mu\)M Mg\(^{2+}\) in the perfusate. In a control heart, over the period of observation, no Mg\(^{2+}\) leakage from the cardiac cells was observed, and for each 20 seconds, the content of Mg\(^{2+}\) in the perfusate closely resembles that of the medium used for the perfusion (50 \(\mu\)M). The total amount of cellular Mg\(^{2+}\) extruded during the 7 minutes following the addition of 10 \(\mu\)M norepinephrine is approximately 800 nmol (calculated as the difference between \(\bullet\) and \(\square\) values), which is very close to that obtained in the presence of contaminant Mg\(^{2+}\) only (≈860 nmol). In contrast, when a heart was stimulated with 100 \(\mu\)M carbachol, an accumulation of approximately 1 \(\mu\)mol Mg\(^{2+}\) was observed.

The possible concern is that the Mg\(^{2+}\) efflux or influx is not specific to agonist stimulation, but could be the result of an increase in heart rate or force of contraction. The experiments of Figure 1 were carried out with unpaced, spontaneously beating rat hearts. No increase in Mg\(^{2+}\) efflux was observed by pacing the hearts at 100–150 times per minute, after cutting the atrioventricular conduction system. On the other hand, unpaced hearts and hearts paced at 100–150 times per minute show virtually identical response to the addition of norepinephrine (data not shown). The force of contraction was also changed by perfusing hearts with concentrations of Ca\(^{2+}\) of 1 and 3 mM or by adding 30 mM KCl or 2 mM caffeine to the perfusion medium. Under those conditions no stimulation of Mg\(^{2+}\) efflux was observed (data not shown).

Figure 2 shows the results of an experiment where dispersed ventricular myocytes, which were loaded 4 hours with \(^{28}\)Mg to reach isotopic equilibration, were incubated in the presence of a physiological concentration of extracellular Mg\(^{2+}\) (1.2 mM) labeled with 1
μCi/ml 28Mg. The isotope distribution indicates that myocytes, within 5 minutes of the addition of norepinephrine or forskolin, lose approximately 10% of cellular 28Mg. On the basis of 28Mg distribution and assuming a cell volume of 10 pl (calculated from Table 1 of Reference 32), the total cellular Mg2+ concentration was estimated to be approximately 10 mM in control conditions and to decrease to 8.5–9 mM after the addition of norepinephrine or forskolin. By contrast, when myocytes were stimulated by carbachol, they accumulated Mg2+ so that the nominal total concentration was 11.5–12 mM. In both cases (efflux and influx), the amount of Mg2+ moving across the plasma membrane is similar and corresponds to 10–15% of the total cell Mg2+.

An attempt was also made to quantify Mg2+ efflux and influx in the presence of different concentrations of extracellular Mg2+. Table 1 illustrates Mg2+ movements in collagenase-dispersed ventricular myocytes incubated in the presence of 0, 0.3, 0.5, 0.8, or 1.2 mM MgCl2 in the external medium and stimulated by 10 μM norepinephrine or 100 μM carbachol. Under these experimental conditions, myocytes released or accumulated approximately the same amount of Mg2+, irrespectively of the external Mg2+ concentration. Values were estimated by measuring total cellular Mg2+ by AA, after sedimentation of the cells through an oil layer, and assuming a cell volume of 10 pl. Concentrations of Mg2+ higher than 1.2 mM (3 and 5 mM) were also tested and yielded quantitatively similar results as those reported in Table 1 (data not shown).

Mg2+ efflux or influx can be rapidly reversed. Figure 3 shows that the addition of 10 μM norepinephrine stimulated a major and time-dependent Mg2+ efflux (=25 nmol/106 cells after 7–8 minutes of stimulation). When 100 μM carbachol was added 4 minutes after norepinephrine, this efflux was appreciably decreased (12 nmol/106 cells). Conversely, the addition of norepinephrine (or forskolin, not shown) prompted a large Mg2+ efflux during a phase of carbachol-stimulated influx.

Both uptake and release of cell Mg2+ are temperature-dependent processes. Figure 4 shows myocytes incubated in a thermostated vessel at 37°C, 20°C, and 4°C and then stimulated with norepinephrine. By decreasing the temperature of incubation to 20°C or 4°C, a significant decrease or a complete inhibition of the stimulated efflux rate was observed. The restoration of the temperature to 37°C restored the Mg2+ efflux. A similar temperature dependence was also observed for the cell uptake induced by carbachol (not shown).

Recent experiments by Gunther et al. on plasma membrane of chicken erythrocytes suggested that Mg2+...
release is the result of the operation of a 2:1 Na\(^+\):Mg\(^{2+}\) exchange.

To investigate whether a similar mechanism for Mg\(^{2+}\) release was present in cardiac cells, myocytes were incubated in a medium where sodium chloride was partially or totally replaced isosmotically by choline chloride. Figure 5 shows that when myocytes were incubated in a medium containing 60 mM Na\(^+\), the norepinephrine-induced Mg\(^{2+}\) efflux was consistently decreased with respect to the movement occurring in presence of 120 mM Na\(^+\). A further decrease in extracellular Na\(^+\) content to 20 mM resulted in a more marked decrease of Mg\(^{2+}\) extrusion. When Na\(^+\) was completely removed from the incubation medium, replaced by choline chloride, Mg\(^{2+}\) release was totally abolished. Similar results were also obtained when Na\(^+\) was replaced by N-methyl-d-glucamine. This decrease in Mg\(^{2+}\) efflux was not the consequence of irreversible damage in cardiac cells. In fact, even if the normal rod-shaped configuration of myocytes was affected, probably as a consequence of the modified ionic distribution across the plasma membrane and Ca\(^{2+}\) overload, the readdition of a physiological concentration of Na\(^+\) (120 mM) in the external medium during the first few minutes restored both the cell configuration and the Mg\(^{2+}\) efflux (data not shown).

In hepatocytes a large fraction of the cell Mg\(^{2+}\) release following the addition of norepinephrine is accounted for by a release of mitochondrial Mg\(^{2+}\) through the adenine nucleotide translocase induced by cAMP.\(^{19}\) Figure 6a shows the results of an experiment where a large fraction of Mg\(^{2+}\) was released by the addition of cAMP to ventricular myocytes previously permeabilized with digitonin. The presence of 100 µM atractyloside in the incubation medium completely prevented the effect of cAMP on Mg\(^{2+}\) release. Figure 6b shows that the redistribution of mitochondrial Mg\(^{2+}\), induced by cAMP, was not affected by changes in Na\(^+\) concentrations.

Figure 7 shows the results of an experiment where myocytes were incubated in a medium containing a physiological concentration of Na\(^+\) (120 mM) but different concentrations of added Ca\(^{2+}\) (0, 0.2, 0.5, or 1.2 mM) to investigate the dependence of Mg\(^{2+}\) efflux on extracellular Ca\(^{2+}\). No EGTA was used to decrease the extracellular concentration of Ca\(^{2+}\) because of its chelating properties on extracellular Mg\(^{2+}\). By decreasing the external Ca\(^{2+}\) from 1.2 mM to nominal 0 Ca\(^{2+}\), the norepinephrine-induced Mg\(^{2+}\) efflux decreased in a manner similar to that observed by decreasing the external Na\(^+\) concentration (see Figure 5 for comparison). The decrease in extracellular Ca\(^{2+}\) also decreased...
the influx of Mg\(^{2+}\) stimulated by carbachol (data not shown).

To exclude the possibility that changes in external Na\(^{+}\) or Ca\(^{2+}\) concentration may affect the stimulation of adrenergic receptors and the following Mg\(^{2+}\) efflux, these experiments were repeated by stimulating the cells with forskolin. Also, in this case results qualitatively similar to those reported in Figures 5 and 7 were observed (data not shown).

Figure 8 shows experiments where 120 μM Cd\(^{2+}\) was used to block the Ca\(^{2+}\) channels in the plasma membrane of ventricular myocytes. In the presence of a physiological concentration of extracellular Ca\(^{2+}\) (1.2 mM), the addition of Cd\(^{2+}\) strongly decreased the norepinephrine-induced Mg\(^{2+}\) efflux and completely abolished the carbachol-stimulated Mg\(^{2+}\) influx (see Figure 8a and Figure 10a for comparison). By decreasing the Ca\(^{2+}\) concentration to 0.5 mM, the inhibitory effect of Cd\(^{2+}\) was even more evident (Figure 8b).

The importance of Ca\(^{2+}\) in controlling Mg\(^{2+}\) movement is strengthened by the data reported in Figure 9, showing that the presence of the Ca\(^{2+}\) blockers verapamil (50 μM) or nifedipine (1 μM) in the incubation mixture almost completely prevented the norepinephrine-induced Mg\(^{2+}\) efflux from collagenase-dispersed myocytes (from -25 nmol Mg\(^{2+}\)/10⁶ cells in the absence of the blockers to 5 nmol Mg\(^{2+}\)/10⁶ cells when the blockers were present).

In the experiments shown in Figures 10 and 11, Ca\(^{2+}\) in the incubation buffer was replaced by Ba\(^{2+}\), Mn\(^{2+}\), or Sr\(^{2+}\). When Ba\(^{2+}\) was used to replace Ca\(^{2+}\) (Figure 10b), the rate of Mg\(^{2+}\) efflux stimulated by norepinephrine was only slightly decreased with respect to that observed in the presence of Ca\(^{2+}\) (Figure 10a). By contrast, the carbachol-induced Mg\(^{2+}\) influx was completely abolished (Figures 10a and 10b). When Mn\(^{2+}\) was used to replace Ca\(^{2+}\), both Mg\(^{2+}\) movements were completely blocked (Figure 10c).

The replacement of Ca\(^{2+}\) by Sr\(^{2+}\) significantly affects both Mg\(^{2+}\) movements. Figure 11a shows that, in the presence of 1.2 mM Sr\(^{2+}\), no Mg\(^{2+}\) movements were detectable after the addition of 10 μM norepinephrine or 100 μM carbachol. This phenomenon was further studied by detecting Sr\(^{2+}\) movements by using the redistribution of ⁸⁵Sr. Cells were loaded for 4 hours with 5 μCi/ml ⁸⁵Sr (see “Materials and Methods”). Sr\(^{2+}\) movements following norepinephrine or carbachol addition were detected by measuring the radioactivity in the pellet and/or in the supernatant. Under these experimental conditions, shown in Figure 11b, Sr\(^{2+}\) appears to behave like Mg\(^{2+}\) in terms of uptake and release. A major efflux of cellular ⁸⁵Sr was induced by 10 μM norepinephrine, whereas a sizable influx of the radioisotope followed the stimulation of myocytes with 100 μM carbachol.

**Discussion**

Previous evidence from this laboratory\(^{17,19,20}\) indicates that the increase in cell cAMP following β-adrenergic stimulation or treatment with forskolin or permeant cAMP analogues induces a sizable release of cellular Mg\(^{2+}\) from perfused hearts or livers and isolated ventricular myocytes or hepatocytes. A large fraction of this Mg\(^{2+}\) release is the result of the mobilization of a mitochondrial Mg\(^{2+}\) pool, which occurs because cAMP increases Mg\(^{2+}\) efflux through the adenine nucleotide translocase (AdNT)\(^{19}\).

Similarly, hormones or agents activating protein kinase C stimulate cell Mg\(^{2+}\) uptake in isolated cells,\(^2^1\) and this work shows that vasopressin can stimulate a major Mg\(^{2+}\) influx in perfused heart. The present study was aimed at better characterizing the requirement of extracellular Mg\(^{2+}\), Ca\(^{2+}\), Na\(^{+}\), and different cations on Mg\(^{2+}\) release and uptake by myocytes.

**Dependence on Extracellular Mg\(^{2+}\)**

As we have previously shown, perfused hearts and isolated myocytes lose approximately 10% of their total Mg\(^{2+}\) content within 5 minutes when cytosolic cAMP

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**Figure 6.** Graphs showing Mg\(^{2+}\) efflux in digitonin-permeabilized myocytes incubated in the absence or in the presence of atraclyside (top panel) and in the absence or in the presence of a “cytosol-like” concentration of Na\(^{+}\) (bottom panel). Digitonin-permeabilized myocytes were incubated in a medium containing (nM) KCl 100, NaCl 3, KH₂PO₄ 1.2, glucose 10, NaHCO₃ 12, HEPES 10, pH 7.2 (at 37°C in the presence of 95% O₂-5% CO₂), CaCl₂ 1, EGTA 1, ATP 1, phosphocreatine 30, and creatine-phosphokinase 10 μM, without added Mg\(^{2+}\). Where indicated, 50 nM cAMP was added to the cell suspension in the absence of Na\(^{+}\) or presence of 10 μM Mg\(^{2+}\) (Figures 6a, 6b). Data were mean±SEM of three different experiments.

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**Figure 8.** Graphs showing Mg\(^{2+}\) efflux in digitonin-permeabilized myocytes incubated in the absence or in the presence of atraclyside (top panel) and in the absence or in the presence of a “cytosol-like” concentration of Na\(^{+}\) (bottom panel). Digitonin-permeabilized myocytes were incubated in a medium containing (nM) KCl 100, NaCl 3, KH₂PO₄ 1.2, glucose 10, NaHCO₃ 12, HEPES 10, pH 7.2 (at 37°C in the presence of 95% O₂-5% CO₂), CaCl₂ 1, EGTA 1, ATP 1, phosphocreatine 30, and creatine-phosphokinase 10 μM, without added Mg\(^{2+}\). Where indicated, 50 nM cAMP was added to the cell suspension in the absence of Na\(^{+}\) or presence of 10 μM Mg\(^{2+}\) (Figures 8a, 8b). Data were mean±SEM of three different experiments.
Because increases.17 Mg2+ concentration.

measurement procedure, those experiments were carried out in the presence of an artificially low extracellular Mg2+ concentration. This condition could have facilitated the efflux of Mg2+ from the cytosol, where it is about 0.7-1 mM,2,3 to the extracellular space down its concentration gradient. The experiments reported here show that this is not the case because 1) perfused hearts and isolated myocytes do not lose cellular Mg2+ in the absence of carbachol or norepinephrine (Figures 1 and 3); 2) under identical low extracellular Mg2+, cell Mg2+ uptake is detectable upon stimulation by carbachol (Figures 1 and 3); 3) the process is temperature dependent (Figure 4), and no Mg2+ loss is observable at low temperature; 4) increasing the extracellular Mg2+ toward or above physiological concentrations results in no change of rate and amount of Mg2+ release (and uptake). The data reported here clearly shows that a similar amount of Mg2+ is accumulated or released when the external Mg2+ concentration is in the micromolar (2-5 μM [Figure 3] or 50 μM [Figure 1]), submillimolar (0.3, 0.5, and 0.8 mM [Table 1]), or millimolar range (1.2 mM [Figure 2 and Table 1]).

The redistribution of 25Mg (Figure 2) confirms that, within a few minutes of stimulation, the total intracellular concentration of Mg2+ is either increased or reduced by 1 mM. This amount is equal to or greater than

**Figure 7.** Graph showing Mg2+ efflux in ventricular myocytes incubated in the presence of decreasing concentrations of extracellular Ca2+. Cardiac myocytes were incubated in the medium described in the legend of Figure 2, partially or totally depleted of extracellular Ca2+. Where indicated in the figure, 10 μM norepinephrine (NE) was added. Data are mean±SEM of four different experiments.

**Figure 8.** Graphs showing Mg2+ efflux and influx in ventricular myocytes incubated in the presence of Cd2+. Ventricular myocytes were incubated in a medium identical to that reported in the legend of Figure 2 but containing 120 μM Cd2+ in the presence of 1.2 mM (top panel) or 0.3 mM (bottom panel) extracellular Ca2+. Where indicated in the figures, 10 μM norepinephrine (●) or 100 μM carbachol (▲) was added. Data are mean±SEM of four different experiments.

**Figure 9.** Graph showing Mg2+ efflux induced by 10 μM norepinephrine (NE) in collagenase-dispersed ventricular myocytes incubated in the absence or in the presence of verapamil or nifedipine. Cardiac myocytes were incubated in the medium described in the legend of Figure 2, containing 1.2 mM Ca2+, in the absence (○) or presence (■) of 25 μM verapamil or 1 μM nifedipine (▲) as Ca2+ channel blockers. Where indicated in the figure, 10 μM NE was added. Data are mean±SEM of two different preparations.
the total pool of free cytosolic Mg\(^{2+}\), strong evidence that a large fraction of the mobilizable Mg\(^{2+}\) is bound in the cytosol or within cell compartments. While the data shown in Figure 6 is consistent with a major mobilization of mitochondrial Mg\(^{2+}\) upon increase of cAMP, it is not clear whether mitochondria are the destination pool for Mg\(^{2+}\) uptake.

Taken together these data indicate that a large net flux of Mg\(^{2+}\) can be induced in either direction across the sarcolemma of myocytes upon hormonal stimulation and that both these fluxes are independent of the extracellular Mg\(^{2+}\) concentrations. This observation is intriguing and may not be consistent with a mechanism of "pump and leak" of Mg\(^{2+}\) across the sarcolemma but with a control of sarcolemmal exchanger(s) and/or pumps whose operations are finely regulated by the concentrations of intracellular Mg\(^{2+}\) and intracellular signals.

**Dependence on Extracellular Na\(^{+}\)**

The previous observations indicate that Mg\(^{2+}\) exchanges or is cotransported with other ions, and it may use the electrochemical gradient of other ions to cross the plasma membrane. Flatman et al. have suggested that Na\(^{+}\) may be the counterion exchanged with Mg\(^{2+}\). Evidence for a Na\(^{+}\):Mg\(^{2+}\) exchange has been obtained in giant squid axon and in chicken erythrocytes. Measurements of the exchange ratio of this mechanism have been attempted in both the experimental models and found to be 1 Na\(^{+}\):1 Mg\(^{2+}\) in squid axon and 2 Na\(^{+}\):1 Mg\(^{2+}\) in erythrocytes. The existence of a similar mechanism, inhabitable by amiloride, has been suggested in liver cells, thymocytes, and also in cardiac cells. However, the data of Murphy et al. do not confirm the presence of this antiporter in cardiac cells.

Under our experimental conditions, the norepinephrine-induced Mg\(^{2+}\) efflux strictly depends on the Na\(^{+}\) concentration in the extracellular medium (Figure 5). In fact, by decreasing the Na\(^{+}\) concentration, a concomitant reduction in Mg\(^{2+}\) efflux is observed (Figure 5). The same phenomenon is observed when forskolin, instead of norepinephrine, is used to mobilize Mg\(^{2+}\). This suggests that the failure in Mg\(^{2+}\) mobilization is not attributable to a defect in cAMP rise after the β-adrenergic stimulation but is directly related to the reduction in Na\(^{+}\) concentration. This hypothesis is supported by the observation that digitonin-permeabilized myocytes (Figure 6b) and isolated rat heart mitochondria (unpublished data) release Mg\(^{2+}\) when stimulated by 50 nM cAMP either in the presence or in the absence of Na\(^{+}\) in the incubation medium.

Although these data support the existence of a Na\(^{+}\):Mg\(^{2+}\) exchanger in the plasma membrane of cardiac myocytes, the attempt to measure a fixed stoichiometry of exchange was unfruitful. The estimation of total cellular Na\(^{+}\) and Mg\(^{2+}\) contents before and after norepinephrine stimulation indicated an increase in total cellular Na\(^{+}\), which correlated well with a decrease in total Mg\(^{2+}\) content. However, the Na\(^{+}\):Mg\(^{2+}\) ratio exchange failed in our measurements to provide a consistent value. This may suggest that other ion(s) may be involved in the exchange process. The addition of norepinephrine induces beating of isolated ventricular myocytes in various degrees and, although this phenomenon does not induce Mg\(^{2+}\) efflux per se (authors'
unpublished observations), it may affect the transmembrane movement of other ions, such as Na⁺ and Ca²⁺, and change their intracellular distribution.

These data clearly indicate a requirement for extracellular Na⁺ in permitting stimulated Mg²⁺ efflux from myocytes, but fall short of indicating that there is an obligatory exchange of extracellular Na⁺ for intracellular Mg²⁺. Changes in extracellular Na⁺ result in changes of membrane potential, decrease in intracellular Na⁺ and increase in Ca²⁺ and H⁺ concentrations, all conditions that may directly or indirectly affect Mg²⁺ transport across the plasma membrane.

**Dependence on Extracellular Ca²⁺**

Recently, Quamme et al reported that chicken embryonic hearts and MDCK cells possess a Mg²⁺ transport pathway that is inhibitable by the Ca²⁺ channel inhibitor verapamil. A correlation between cytosolic Mg²⁺ and Ca²⁺ and other cations is also indirectly implied by the work of Agus et al and White and Hartzell.

As for the case of removal of extracellular Na⁺, a decrease in extracellular Ca²⁺ decreases or abolishes the stimulated efflux of Mg²⁺ (Figure 7). Although these results may suggest an exchange of cytosolic Mg²⁺ for extracellular Ca²⁺, several lines of evidence discount this possibility. First, lack of extracellular Na⁺ also inhibits Mg²⁺ efflux and under those conditions myocytes should have a decreased intracellular/extracellular Ca²⁺ gradient due to the Ca²⁺ loading in the absence of extracellular Na⁺. Second, the data of Figure 8 show that the presence of 120 μM Cd²⁺, an inhibitor of Ca²⁺ channels, inhibits both Mg²⁺ efflux and influx, irrespective of the presence of extracellular Ca²⁺ (Figure 8a).

Additional evidence that could support the hypothesis that extracellular Ca²⁺ is involved in Mg²⁺ movements is provided by the inhibitory effect of two selective Ca²⁺ channel blocking agents, verapamil and nifedipine, on the Mg²⁺ efflux in isolated cardiac myocytes (Figure 9).

Taken together, the observed inhibition of Mg²⁺ efflux that occurs upon removal of extracellular Ca²⁺ or following the inhibition of plasma membrane Ca²⁺ influx are more consistent with a role of cytosolic Ca²⁺ rather than extracellular Ca²⁺. Again, this hypothesis is not consistent with the inhibition of Mg²⁺ efflux observed in the absence of extracellular Na⁺ where an increase, rather than a decrease of cytosolic Ca²⁺, occurs.

The pivotal role of both extracellular Na⁺ and Ca²⁺ on the Mg²⁺ efflux suggests a role of the Na⁺-Ca²⁺ exchanger, which is particularly active in the plasma membrane of cardiac cells. Further study will be necessary to assess whether the Na⁺-Ca²⁺ exchanger is directly or indirectly involved in sarclemmal Mg²⁺ fluxes.

**Dependence on Extracellular Divalent Cations**

Replacement of extracellular Ca²⁺ with various divalent cations also has, to various degrees, inhibitory effects on Mg²⁺ efflux and/or influx. Replacement of Ca²⁺ with Mn²⁺ (Figure 10c) or Sr²⁺ (Figure 11a) completely inhibits both Mg²⁺ efflux and influx. Interestingly, replacement of Ca²⁺ with Ba²⁺ inhibits Mg²⁺ influx but not Mg²⁺ efflux (Figure 10b).

Because of the redistribution of those divalent cations within the cell and the different handling by intracellular organelles, it is clearly outside the scope of this work to conclusively present a mechanistic view for those effects. For instance, Sr²⁺ and Mn²⁺, but not Ba²⁺, are accumulated by mitochondria at a rate and an extent similar to that of Ca²⁺. Like contrast, neither Mn²⁺ nor Ba²⁺ are accumulated by isolated sarcoplasmic reticulum vesicles but to a different extent, both ions can replace Mg²⁺ in activating Ca²⁺-ATPase. More complex and less well known are the effects of those cations on Na⁺-Ca²⁺ exchange, plasma membrane Ca²⁺-ATPase, and Ca²⁺ channels.

The evidence of Figure 11a indicate that the inhibition by Sr²⁺ on Mg²⁺ efflux can be entirely accounted for by the fact that Sr²⁺ could substitute for Mg²⁺. In fact, under conditions of isotopic equilibrium, labeled Sr²⁺ is released or accumulated by myocytes upon stimulation with norepinephrine or carbachol, respectively (Figure 11b). This finding, if more widely documented, is potentially important because it will enable the use of Sr²⁺
rather than $^{25}$Mg, which is seldom available, expensive, and short-lived, to routinely measure Mg$^{2+}$ fluxes. Mn$^{2+}$ could similarly inhibit Mg$^{2+}$ uptake and release by replacing Mg$^{2+}$ and/or by other mechanism(s). The substitution with Ba$^{2+}$ is more intriguing because it inhibits only Mg$^{2+}$ uptake and not release. This disassociation of inhibition by Ba$^{2+}$ may become a useful tool for future studies aimed at assessing whether Mg$^{2+}$ influx and efflux occur through the same or different transporters.

References

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