Hormonal Stimulation of Mg$^{2+}$ Uptake in Hepatocytes
REGULATION BY PLASMA MEMBRANE AND INTRACELLULAR ORGANELLES

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Collagenase dispersed rat liver hepatocytes release Mg$^{2+}$ when stimulated with norepinephrine or accumulate Mg$^{2+}$ when stimulated with vasopressin, respectively. Mg$^{2+}$ fluxes in either direction account for a net loss or gain of approximately 10% of total cell magnesium and are rapidly reversible.

Both stimulated Mg$^{2+}$ efflux and Mg$^{2+}$ influx require physiological concentration of extracellular NaCl and Ca$^{2+}$. In the absence of extracellular Na$^+$, Mg$^{2+}$ efflux, but not influx, can be observed in the presence of extracellular Cl$^-$. Under these conditions, the efflux is inhibited by the Cl$^-$/HCO$_3^-$ exchanger inhibitor 4,4'-dinitrostilbene-2,2'-disulfonic acid.

In hepatocytes, Mg$^{2+}$ influx, but not efflux, is completely inhibited by thapsigargin, a specific inhibitor of the endoplasmic reticulum Ca$^{2+}$ ATPase. Several lines of evidence, such as measurements of cytosolic Ca$^{2+}$ or of cytosolic Ca$^{2+}$ buffering, indicate that the effect of thapsigargin in inhibiting Mg$^{2+}$ influx could not be explained by an increase in cytosolic Ca$^{2+}$. Instead, the inhibition of hepatocyte Mg$^{2+}$ influx was found to be the result of the depletion of the Ca$^{2+}$ stored within the endoplasmic reticulum.

Magnesium is one of the most abundant cations within mammalian tissues and cells (1-4). Recently, there has been a surge of interest in the role of Mg$^{2+}$ in preventing acute and chronic metabolic derangements in the human body. Highly publicized findings of the role of extracellular magnesium in preventing ischemic damage to tissue have been reported. These observations have been paralleled by findings that the limited development of cellular Mg$^{2+}$ homeostasis, both intracellular and extracellular Mg$^{2+}$ homeostasis are far less understood than that of other cations such as H$^+$, Ca$^{2+}$, Na$^+$, or K$^+$. The single main reason for such limited knowledge is the fact that available techniques for measuring free or total magnesium within cells or extracellular fluid are less developed than those available for measuring other cations.

The interest in the regulation of intracellular Mg$^{2+}$ has grown significantly in recent years. A large number of recent observations have shown that under physiological (5-8) and pathological (9-13) conditions, a major redistribution of Mg$^{2+}$ across cells and organelles occurs in liver (14-19), heart (5, 12, 18-20), and other tissues (6, 21, 22).

For instance, this and other laboratories have shown that a net Mg$^{2+}$ efflux can be induced in perfused organs or isolated cells upon noradrenergic stimulation and consequent increase in cell cAMP (18, 19). It was further demonstrated that some of this efflux can be accounted for by a release of Mg$^{2+}$ from mitochondria induced by cAMP interacting with the adenine nucleotide translocase (16). Another recent observation showed that either vasopressin or carbachol, via stimulation of protein kinase C, can induce a massive uptake of Mg$^{2+}$ into hepatocytes through a pathway that remains to be elucidated (15).

Even less well understood is the mechanism through which various cell types handle large net fluxes of Mg$^{2+}$ across their plasma membranes. Evidence has been provided in the literature that extracellular Na$^+$ is important to permit Mg$^{2+}$ efflux in mammalian (17, 20, 21) and non-mammalian cells (1, 6, 23, 24).

In this study, we investigated the role of extracellular Na$^+$, and of other cations, in regulating not only Mg$^{2+}$ efflux but also Mg$^{2+}$ influx in liver cells. We also investigated cellular mechanisms responsible for the recently observed hormonal regulation of cellular Mg$^{2+}$ influx. Evidence is provided that the endoplasmic reticulum, and its Ca$^{2+}$ storage properties within hepatocytes, is necessary for the observed stimulated Mg$^{2+}$ influx but not Mg$^{2+}$ efflux.

**EXPERIMENTAL PROCEDURES**

Isolated Hepatocytes—Collagenase dispersed hepatocytes were prepared from male Sprague-Dawley rats (200–250 g), according to the procedure of Seglen (25). After isolation, cells were resuspended in a buffer containing (in mM): 120 NaCl, 3 KCl, 1 KH$_2$PO$_4$, 1.2 MgCl$_2$, 1 CaCl$_2$, 10 glucose, 12 NaHCO$_3$, 10 HEPES (pH 7.2 at 37 °C) in the presence of O$_2$CO$_2$ (95:5 v/v). Cell viability (88 ± 2%, n = 8), assessed by the trypan blue exclusion test, did not change appreciably during the first 5 h after isolation.

For measuring Mg$^{2+}$ movements, hepatocytes (300–350 μg/ml protein) were incubated in the medium described above, in the absence of added Mg$^{2+}$ (Mg$^{2+}$-free buffer). At selected times, aliquots of the incubation mixture were withdrawn and quickly sedimented in microcentrifuge tubes. The supernatant was removed and the Mg$^{2+}$ content determined by atomic absorbance spectrophotometry using a Varian AA-575 instrument. The Mg$^{2+}$ present as contaminant in the Mg$^{2+}$-free buffer ranged between 2 and 5 μM as determined by atomic absorbance spectroscopy. In the experiments performed to evaluate the Na$^+$ dependence of Mg$^{2+}$ movements, the Na$^+$ concentration was decreased to 60 or to 0 mM. In both cases Na$^+$ was isotonically replaced with choline or N-methyl-d-glucamine. No changes in hepatocyte viability were observed during the first 15 min of incubation with either of the Na$^+$ replacement buffers (87 ± 4%, n = 6).

In another set of experiments, hepatocytes were incubated in a...
Na+-free buffer having the same composition as the Mg2+-free buffer described above, but with NaCl isosmotically replaced with choline chloride. At the times indicated in the figures, 120 mM NaCl (final concentration) was added to the incubation system by diluting the reaction mixture with an identical volume of isosmotic Mg2+-free buffer containing 440 mM NaCl, or different concentrations of sodium isothionate, previously equilibrated at pH 7.2 and 37 °C. An identical procedure was used to investigate the role of extracellular Cl− on Mg2+ movements. For these experiments, hepatocytes were incubated in 200 mM sucrose, 20 mM HEPES, Tris (pH 7.4 at 37 °C). At the times indicated in the figures, the final concentrations of Cl− were obtained by diluting the reaction mixture with an identical volume of buffer containing twice the desired Cl− concentration, previously equilibrated at the same pH and temperature.

Loading of the Hepatocytes with BAPTA—After isolation, hepatocytes were resuspended, at the final concentration of 106 cells/ml, in the medium, reported under Isolated Hepatocytes, containing 1.2 mM MgCl2 and loaded for 30 min with 5 μM BAPTA-AM at room temperature. After loading, hepatocytes were washed 3 times with the same medium and incubated in the Mg2+-free buffer for measuring Mg2+ movements. Mg2+ determinations were carried out as previously reported.

Loading of the Hepatocytes with Fura-2—After isolation, hepatocytes were resuspended, at the final concentration of 106 cells/ml, in the resuspension medium containing 1.2 mM MgCl2 and loaded for 20 min with 2 μM Fura-2-AM at room temperature. After loading, hepatocytes were washed 3 times with the same medium and incubated in the Mg2+-free buffer, in order to detect cytosolic Ca2+ movements. Calibration was performed at the end of the experiments as reported elsewhere (26).

Other Techniques—In all of the procedures, protein amount was determined with the Bradford technique (27). Alternatively, hepatocytes were counted in a hemacytometer. In all of the preparations tested (n = 5), the protein concentration was found to be 2.7 ± 0.3 mg of protein/million cells.

Chemicals—Collagenase (CLS-2) was from Worthington (Freehold, NJ). Thapsigargin was from LC Services Corp. (Woburn, MA). BAPTA-AM and Fura-2-AM were from Molecular Probes (Eugene, OR). All other chemicals and reagents were from Sigma.

RESULTS

Magnesium Transport in Hepatocytes—Fig. 1A shows that the addition of 10 μM NE to a suspension of collagenase dispersed hepatocytes induces the release of 6 nmol of Mg2+/million cells during an 8-min period, consistent with previously reported data (15, 16, 18). The addition of TPA, 4 min after treatment with NE, results in a reversal of the release and in a large, time-dependent Mg2+ accumulation by the hepatocytes.

Fig. 1B shows that the addition of TPA induces a Mg2+ uptake corresponding to 6 nmol of Mg2+/million cells within 8 min. If during the stimulated uptake, NE was added, a release of 8 nmol of Mg2+/million cells was observed. Changes qualitatively similar to those reported in the experiments shown in Fig. 1 (A and B) were also observed when forskolin or vasopressin were added instead of NE or TPA, respectively (not shown). These data indicate that either a large Mg2+ influx or efflux could be observed in hepatocytes upon the addition of two classes of agonists and that this stimulation was rapidly reversible.

Recently, Gunther and co-workers (17) provided evidence that a Na+/Mg2+ exchanger regulates Mg2+ efflux from liver cells. This pathway, blocked by amiloride (17), appears to be also located in the plasma membrane of cardiac cells (20), chicken erythrocytes (23), and thymocytes (21). The next set of experiments was designed to determine if this exchanger is involved not only in the cAMP-mediated Mg2+ efflux but also in the TPA (or vasopressin)-induced Mg2+ influx.

Fig. 2 shows that both the NE-induced Mg2+ efflux and the TPA-stimulated Mg2+ uptake require the presence of a "physiological" concentration of NaCl (120 mM) in the extracellular medium. In fact, both the efflux and the influx of Mg2+, which were maximal in the presence of 120 mM extracellular NaCl, were consistently decreased, or totally abolished, when cells were incubated in the presence of 60 or 0 mM NaCl, respectively. Under these experimental conditions, Na+ was isosmotically replaced with choline and no changes in cell viability were observed during the first 10 min of Na+ replacement.

The same results were also observed when the cells were stimulated with concentrations of NE or TPA higher than those reported in Fig. 2 or by other stimulatory agents (i.e. forskolin or vasopressin; data not shown), suggesting that the decrease, or the absence, of stimulation is not attributable to a reduced effectiveness of these agents.

Additional evidence that Mg2+ movements require extracellular physiological NaCl concentrations is provided by the experiments shown in Fig. 3. Hepatocytes incubated in the absence of external NaCl and stimulated by NE or TPA neither released (Fig. 3A) nor accumulated Mg2+ (Fig. 3B). However, the addition of 120 mM NaCl after 4 min of incubation fully restored both Mg2+ efflux (Fig. 3A) and Mg2+ uptake (Fig. 3B).
Transport in Hepatocytes

**Fig. 2.** Norepinephrine-induced magnesium efflux and TPA-induced magnesium uptake in hepatocytes incubated in the presence of 120 (A, ◦), 60 (■, □), or 0 mM NaCl (▲, ○) in the extracellular medium. Intact hepatocytes were incubated in the presence of different concentrations of extracellular NaCl. NaCl was partially or totally replaced by choline chloride or N-methyl-D-glucamine. Where indicated in the figure, 10 μM NE or 20 nM TPA was added. The data represent means ± S.E. of 5 different preparations.

**Fig. 3.** The reintroduction of NaCl in the incubation medium fully restored magnesium efflux (A) and uptake (B). Collagenase-dispersed hepatocytes were incubated in a medium containing 120 mM choline chloride instead of NaCl. A few min after the cell addition, 10 μM NE (A) or 20 nM TPA (B) were added in the incubation medium. Four min later (second arrow) 120 mM NaCl was added. Data are means ± S.E. of 4 different experiments.

**Fig. 4.** Effect of the Na⁺ (A) or Cl⁻ (B) in determining Mg²⁺ efflux or uptake in intact hepatocytes stimulated by norepinephrine or vasopressin. Intact hepatocytes were incubated in the Mg²⁺-free medium described under “Experimental Procedures,” and stimulated with 10 μM NE or 20 nM vasopressin (first arrow). Four min later 35 mM sodium isothionate (A) or 35 mM choline chloride (B) were added. Data are means ± S.E. of 3 different experiments.

Influx (Fig. 3B). In the absence of NE or TPA stimulation, the addition of 120 mM NaCl per se did not induce any change in Mg²⁺ movements (not shown).

Fig. 4 shows the results of two experiments where the effect of changes in extracellular Na⁺ or Cl⁻ on Mg²⁺ efflux or Mg²⁺ uptake was investigated. Hepatocytes were incubated in the Mg²⁺-free medium described under “Experimental Procedures,” where NaCl content was isosmotically replaced with sucrose, and were stimulated with 10 μM NE or with 20 nM vasopressin. Fig. 4 (A and B) shows that in this medium the addition of either agonist had no effect on Mg²⁺ efflux or influx in hepatocytes during the first 6 min. Fig. 4A shows that the addition of sodium isothionate caused an efflux of Mg²⁺ in cells previously stimulated with NE and an uptake in cells previously stimulated with TPA. In contrast, Fig. 4B shows that when Cl⁻ was added to stimulated cells in the form of choline chloride Mg²⁺ efflux, but not Mg²⁺ uptake, was restored.

These experiments indicate that the presence of extracellular Na⁺ is necessary for both stimulated Mg²⁺ efflux and uptake. On the other hand, extracellular Cl⁻ alone is sufficient for Mg²⁺ efflux, but not for Mg²⁺ uptake.

The effective concentrations of Cl⁻ necessary to induce Mg²⁺ efflux after addition of NE were further investigated by incubating the hepatocytes in the Mg²⁺-free buffer reported under “Experimental Procedures.” Cells were stimulated with various concentrations of choline chloride, under conditions...
where osmolarity changes of the system were minimized (see "Experimental Procedures"). Fig. 5A shows that the addition of Cl− induced a consistent Mg2+ efflux, which was already maximal in the presence of 10 mM Cl−. When the same experiment was repeated in the presence of 50 μM DNDS, an inhibitor of the plasma membrane Cl−/HCO3− exchanger (28, 29), Mg2+ efflux was almost completely abolished in the presence of 25 mM Cl− or significantly decreased in the presence of 75 mM Cl− (Fig. 5B).

Fig. 6 shows that, in addition to extracellular Na+ and Cl−, extracellular Ca2+ was also required for both stimulated efflux and influx of Mg2+ in hepatocytes. The decrease in extracellular Ca2+ concentration from 1.2 to 0.3 mM and to 0 mM decreased or abolished the vasopressin-mediated Mg2+ uptake and the NE-stimulated Mg2+ release.

In principle, extracellular Ca2+ could exchange for intracellular Ca2+ and such an exchange could account for the release of Mg2+ induced by NE. On the other hand, lack of extracellular Ca2+ should facilitate, rather than inhibit, the Mg2+ uptake induced by vasopressin.

It is possible that changes in extracellular Ca2+ result in changes of intracellular Ca2+ or total cell Ca2+ bound within the cytosol or internalized within organelles.

Fig. 7 shows the results of an experiment where cytosolic free Ca2+ in hepatocytes was highly buffered by the previous loading with the Ca2+ buffer BAPTA. In the presence of a physiological extracellular Ca2+ concentration, the buffering of cytosolic free Ca2+ abolishes both the NE-dependent Mg2+...
release (Fig. 7A) and the vasopressin-dependent Mg$^{2+}$ uptake (Fig. 7B).

The data of Fig. 7A, where BAPTA inhibits stimulated Mg$^{2+}$ efflux, cannot be unequivocally explained in term of cytosolic Ca$^{2+}$ buffering, since BAPTA might also buffer cytosolic Mg$^{2+}$. This explanation, however, is not consistent with the data in Fig. 7B, which shows that even the stimulated Mg$^{2+}$ uptake is inhibited by BAPTA. Under these conditions, cytosolic Mg$^{2+}$ buffering should have enhanced, or maintained unaffected, Mg$^{2+}$ uptake.

Fig. 8 shows the results of an experiment where intact hepatocytes were incubated in the presence of 5 nM thapsigargin, a specific blocker of the ER Ca$^{2+}$-ATPase (30, 31). Thapsigargin, which is lipophilic, can cross the plasma membrane of liver cells, reach its intracellular target, and effectivelly deplete the ER Ca$^{2+}$ store(s) (30, 31). When thapsigargin was added to a suspension of intact hepatocytes, no detectable loss or gain in Mg$^{2+}$, with respect to control samples, was observed. However, under these conditions a small, but long lasting, increase in cytosolic free Ca$^{2+}$ was observed in cells loaded with the calcium-selective fluorescent dye Fura-2 (see below), consistent with a depletion of the ER Ca$^{2+}$ pool(s) following the inhibition of the ER Ca$^{2+}$-ATPase.

Therefore, intact hepatocytes were treated with 5 nM thapsigargin for varying periods of time (20 s, 1 min, 3 min, or 5 min) before being stimulated by 20 nM vasopressin. As shown in Fig. 9 (bottom panel), hepatocytes were still able to accumulate Mg$^{2+}$ from the extracellular medium when vasopressin was added 20 s or 1 min after the addition of thapsigargin. In contrast, in hepatocytes treated for 3 min or for 5 min with

![Fig. 8](image)

Fig. 8. Thapsigargin does not modify the norepinephrine-induced magnesium efflux (A) but completely prevents the TPA-stimulated magnesium uptake in intact hepatocytes (B). Intact hepatocytes were incubated in the Mg$^{2+}$-free medium (see "Experimental Procedures") in the absence or in the presence of 5 nM thapsigargin. A few min after their addition, cell were stimulated by adding 10 μM NE or 50 μM forskolin (Fig. 8A). Mg$^{2+}$ effluxes, similar to those reported in Fig. 1A and Fig. 2, were observed. In contrast, no Mg$^{2+}$ influx was observed when 20 nM TPA (Fig. 8B) or 20 nM vasopressin (not shown) were used under similar experimental conditions. Higher doses of these agents were equally ineffective.

The increase in cytosolic Ca$^{2+}$ following the inhibition of the ER Ca$^{2+}$-ATPase by thapsigargin is a process that requires several minutes for a maximal effect to be observed (31). Therefore, intact hepatocytes were treated with 5 nM thapsigargin for varying periods of time (20 s, 1 min, 3 min, or 5 min) before being stimulated by 20 nM vasopressin. As shown in Fig. 9 (bottom panel), hepatocytes were still able to accumulate Mg$^{2+}$ from the extracellular medium when vasopressin was added 20 s or 1 min after the addition of thapsigargin. In contrast, in hepatocytes treated for 3 min or for 5 min with
thapsigargin, the vasopressin-induced Mg\(^{2+}\) influx was completely abolished. Parallel experiments, where intracellular free Ca\(^{2+}\) was measured with the Ca\(^{2+}\)-fluorescent dye Fluo-3 (Fig. 9, top panel), indicated that after 20 s or 1 min of thapsigargin treatment, cytosolic free Ca\(^{2+}\) increased and some ER Ca\(^{2+}\) could still be mobilized by vasopressin. Hepatocytes treated for 2 min with thapsigargin, instead, presented a level of cytosolic free Ca\(^{2+}\) consistently higher than the untreated cells and no Ca\(^{2+}\) was further mobilizable by vasopressin from the endoplasmic reticulum Ca\(^{2+}\) pools.

The inhibition of Mg\(^{2+}\) uptake by thapsigargin could be due to the increase in cytosolic Ca\(^{2+}\) following the inhibition of the ER Ca\(^{2+}\) pump or to the depletion of Ca\(^{2+}\) from the ER itself. The data of Fig. 9 provide evidence supporting the latter alternative.

**DISCUSSION**

**Overall Mg\(^{2+}\) Fluxes in Hepatocytes**—Previous reports from our laboratory have provided evidence that isolated cardiac (18, 19) and liver cells (15, 16, 18) can regulate Mg\(^{2+}\) movements across the plasma membrane in response to hormonal stimulation. More specifically, the increase in cytosolic cAMP level, resulting from the stimulation of β-adrenergic receptors or from the use of forskolin or permeant cAMP analogs (i.e. dibutyryl cAMP, 8-chloro-cAMP, 8-bromo-cAMP), prompts a marked Mg\(^{2+}\) efflux from both cell types. A cAMP-dependent Mg\(^{2+}\) efflux has been reported to occur in thymocytes as well (21), supporting the idea that the CAMP-mediated Mg\(^{2+}\) efflux has been reported to occur in thymocytes as well.

The amount of net Mg\(^{2+}\) translocated in either direction within seconds or vice versa, by alternatively activating signaling pathways.

Two striking conclusions can be drawn from these data. First, both Mg\(^{2+}\) influx or efflux are rapidly reversible. Second, the amount of net Mg\(^{2+}\) translocated in either direction within a few minutes is very large and corresponds to a net decrease or increase in total cell Mg\(^{2+}\) by 1 mM (−10%) (16, 19). Based upon reported values of hepatocyte surface area (36, 37), either flux will correspond to a net flux of 0.2 nmol of Mg\(^{2+}\)/min/cm\(^2\), which is one of the highest values reported for exchanges occurring in cell for net ion translocation.

**Dependence of Cell Mg\(^{2+}\) Uptake and Release on Extracellular Ions**—An increasing number of reports suggest that a Na\(^{+}/Mg\(^{2+}\) exchanger, located in the plasma membrane of mammalian cells (17, 20, 21), chicken erythrocytes (6, 23), and giant squid axon (24), is the main mechanism responsible for Mg\(^{2+}\) efflux under stimulatory conditions. Herefore, no evidence has been provided about the dependence of Mg\(^{2+}\) influx on extracellular Na\(^{+}\) and whether or not this sodium-dependent pathway could be involved in Mg\(^{2+}\) uptake as well. The importance of a physiological concentration of Na\(^{+}\) in the extracellular medium for Mg\(^{2+}\) efflux is confirmed by the data reported in Figs. 2 and 3. On the other hand, these same data indicate that stimulated Mg\(^{2+}\) influx is equally dependent on extracellular Na\(^{+}\).

Hence, the proposed obligatory exchange of extracellular Na\(^{+}\) for intracellular Mg\(^{2+}\) during Mg\(^{2+}\) efflux may not be operative in hepatocytes for at least two reasons. First, extracellular Na\(^{+}\) is required for both uptake and release of Mg\(^{2+}\). Second, the exchange ratio between Na\(^{+}\) and Mg\(^{2+}\) measured in these hepatocytes is extremely variable, ranging between 1 and 4 Na\(^{+}\) for 1 Mg\(^{2+}\) (not shown).

Both types of evidence suggest that, although Na\(^{+}\) could exchange with Mg\(^{2+}\) under certain conditions, other cations or anions could be involved in exchanging for Mg\(^{2+}\).

For instance, extracellular Cl\(^{-}\) can support a stimulated Mg\(^{2+}\) efflux, but not influx (Fig. 4B). This observation, which clearly demonstrates a dissociation between the two processes, requires additional investigation, since changes in extracellular Cl\(^{-}\) can result in changes in intracellular Cl\(^{-}\) concentration, pH, and membrane potential.

The extracellular Ca\(^{2+}\) requirement for both stimulated Mg\(^{2+}\) uptake and release can also be explained by different mechanisms. For instance, under certain conditions, extracellular Ca\(^{2+}\) could exchange for intracellular Mg\(^{2+}\), and this putative pathway could account for an exchange of Ca\(^{2+}\) for Mg\(^{2+}\), during the Mg\(^{2+}\) efflux mediated by NE. However, lack of extracellular Ca\(^{2+}\) resulted in a similar inhibition of cellular Mg\(^{2+}\) uptake by liver cells. If a Ca\(^{2+}\)/Mg\(^{2+}\) exchange were involved in both Mg\(^{2+}\) release and uptake, a decrease in extracellular Ca\(^{2+}\) should affect only Mg\(^{2+}\) release and enhance or be without effect on Mg\(^{2+}\) uptake.

Changes in extracellular Ca\(^{2+}\) also resulted in changes in intracellular Ca\(^{2+}\) and in a decrease of the Ca\(^{2+}\) bound within cytosolic buffers and/or stored within intracellular organelles. The possibility exists that cytosolic free Ca\(^{2+}\) may be important in mediating Mg\(^{2+}\) uptake from the extracellular medium. Another, not mutually exclusive, hypothesis is that intracellular organelles play a role on intracellular Mg\(^{2+}\) homeostasis. Accordingly, most of the observed Mg\(^{2+}\) uptake and release by hepatocytes could be the observable consequence of a previous Mg\(^{2+}\) uptake by or Mg\(^{2+}\) release from these organelles. Two lines of evidence support this hypothesis. First, the amount of net Mg\(^{2+}\) uptake or release observed 3–5 min after stimulation is equivalent to 10% of the total cell Mg\(^{2+}\) content and is greater than the values of free cytosolic Mg\(^{2+}\) reported in the literature (1, 2, 4). Second, in the case of cellular Mg\(^{2+}\) release due to NE, most of the Mg\(^{2+}\) translocated outside the cell is Mg\(^{2+}\) mobilized from the mitochondria (16).

Under vasopressin stimulation, a transient rise in cytosolic Ca\(^{2+}\) occurs in liver cells (7, 38–40) (Fig. 9) following the mobilization of ER Ca\(^{2+}\) pool by inositol 1,4,5-trisphosphate (7, 41, 42). Additionally, protein kinase C (33, 42) appears to induce Ca\(^{2+}\) mobilization, probably via the same or by an alternative route. Less clear is the link between these stimulatory agents and Mg\(^{2+}\). Bond et al. (7) reported a major redistribution of cellular Mg\(^{2+}\) in rat liver "in vivo" stimulated by vasopressin and glucagon, whereas Baumann et al. (8) and Somlyo et al. (13) suggested that Mg\(^{2+}\) can be counter-transported with Na\(^{+}\) during Ca\(^{2+}\) release from the ER or the sarcoplasmic reticulum, both in physiological and pathological conditions. An alternative or additional mechanism involved in Mg\(^{2+}\) influx and intracellular redistribution could be the exchanger localized in the inner mitochondrial membrane, responsible for the transport of Mg\(^{2+}\)/ATP complex into the mitochondria, in exchange for the PO\(_4\)\(^{-}\) present in the matrix (43–45). This pathway appears to be activated by the rise in cytosolic Ca\(^{2+}\) that follows the vasopressin stimulation of liver cells (44, 45).

Taken together, the data presented here do not unequivocally support the hypothesis that changes in cytosolic free
Ca$^2+$ concentration alone can modulate Mg$^2+$ uptake (or release) from hepatocytes. However, the results do indicate a role for the Ca$^2+$ cycling by ER in the stimulated Mg$^2+$ uptake, but not the Mg$^2+$ release, in hepatocytes. There is only one set of experimental data to indicate that the rise in cytosolic Ca$^2+$ could explain Mg$^2+$ uptake, either by exchanging with Mg$^2+$ across the plasma membrane or by an alternative mechanism. This is the finding that agents which promote cellular Mg$^2+$ uptake, such as vasopressin or TPA, increase cytosolic Ca$^2+$, at least transiently (32, 38-42).

However, there is much evidence against this hypothesis. (i) NE or other adrenergic agonists, which also transiently increase cytosolic Ca$^2+$ in hepatocytes (46), cause Mg$^2+$ release rather than Mg$^2+$ uptake. (ii) Thapsigargin resulted in a large increase in cytosolic Ca$^2+$ (Fig. 9, top panel) but still abolished Mg$^2+$ uptake (Fig. 9, bottom panel).

To the extent that the effect of thapsigargin is selectively confined to the Ca$^2+$-ATPase of the ER, and no evidence to the contrary exists in the literature, the data presented in this report indicate that functional ER Ca$^2+$ cycling is necessary for Mg$^2+$ uptake into hepatocytes. The lag period observed between the inhibition of the Ca$^2+$-ATPase after thapsigargin addition (Fig. 9 and Ref. 31) and the inhibition of the stimulated Mg$^2+$ uptake suggest that the size of the ER Ca$^2+$ pool, rather than the Ca$^2+$-ATPase itself, is responsible for cellular Mg$^2+$ uptake.

Hence, all these data are consistent with the ER being the destination site for the observed Mg$^2+$ uptake under conditions where Ca$^2+$ uptake by the ER is present and Ca$^2+$ is stored within the ER.

Previous results from this laboratory (16) and others (17) clearly indicate that the cAMP-stimulated Mg$^2+$ release observed in hepatocytes depends, to a great extent, on the mobilization of Mg$^2+$ from mitochondria, followed by the extrusion of cytosolic Mg$^2+$. We have shown that this release is not affected by thapsigargin. In contrast, ER functioning in terms of Ca$^2+$ cycling is necessary for the observed Mg$^2+$ uptake stimulated by vasopressin, carbachol, or TPA.

The process of stimulated Mg$^2+$ uptake described in this work is novel although not mechanistically defined. However, the identification of two different cellular organelles as the origin and destination sites for hepatocytes Mg$^2+$ release and uptake, and the disassociation of conditions affecting the two processes, provide a basic foundation and experimental tools for a better elucidation of the integrated Mg$^2+$ redistribution and homeostasis in hepatocytes.

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