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Magnesium homeostasis in mammalian cells

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1. ABSTRACT

Mammalian cells tightly regulate cellular Mg^{2+} content despite undergoing a variety of hormonal and metabolic stimulatory conditions. Evidence from several laboratories indicates that stimulatory conditions that increase cellular cAMP level result in a major mobilization of Mg^{2+} from cells and tissues into the bloodstream. Conversely, hormones or agents that decrease cAMP level or activate protein kinase C signaling induce a major accumulation of Mg^{2+} into the tissues. These Mg^{2+} fluxes are quite large and fast suggesting the operation of powerful transport mechanisms. At front of the recent identification of several Mg^{2+} entry mechanisms, the Mg^{2+} extrusion pathway(s) still remain(s) poorly characterized. Similarly, it remains not completely elucidated the physiological significance of these Mg^{2+} fluxes in the various tissues in which they occur. In the present review, we will attempt to provide a comprehensive framework of the modalities by which cellular Mg^{2+} homeostasis and transport are regulated, as well as examples of cellular functions regulated by changes in cellular Mg^{2+} level.

2. INTRODUCTION

The presence of high concentrations of total and free magnesium (Mg^{2+}) within mammalian cells is essential to regulate a broad number of cellular functions and enzymes. Ion channels, metabolic cycles, signaling pathways and more than three hundred and fifty enzymes require and/or are regulated to a varying extent by concentrations of Mg^{2+} that are well within the physiological range observed in mammalian tissues and cells. The relevance of such a role sharply contrasts with the limited knowledge of the mechanisms, genes and proteins that regulate Mg^{2+} homeostasis at the cellular and sub-cellular level, as well as in the whole body. Conceptual and methodological reasons can be adduced to explaining such a limitation. The abundance of both total and free [Mg^{2+}] within the cell, the absence of changes of significant amplitude in free [Mg^{2+}], and the relatively slow turn-over of the cation across the cell plasma membrane under quiescent condition, all have supported for a long time the assumption that no major changes in cellular Mg^{2+} concentration occur or are required for Mg^{2+} to play its role

as a co-factor for enzymes or channels. In turn, this conceptual point of view has limited the development of techniques and methodologies suitable to accurately quantify changes in cellular Mg^{2+} concentration.

In recent years, a large body of experimental observations has significantly reverted this way of thinking. Compelling evidence now demonstrates the occurrence of major fluxes of Mg^{2+} across the cell membrane in either direction following a variety of hormonal and non-hormonal stimuli, which result in major changes in total and plasma Mg^{2+} levels. As a result of these fluxes, limited variations in free Mg^{2+} concentration but larger changes in total Mg^{2+} content within tissues and organelles have been observed. At the same time, the increased interest in understanding the physiological and pharmacological roles of Mg^{2+} have resulted in the development of techniques and methodological approaches to better detect and quantify variations in cellular Mg^{2+} homeostasis and distribution. Furthermore, the identification of Mg^{2+} transport mechanisms in bacteria and yeast has provided new tools to characterize the presence of similar proteins in mammalian cells. The present review will attempt to provide a comprehensive frame-work to understand how cellular Mg^{2+} content changes as a result of hormonal and non-hormonal stimuli, and how these changes can modify the activity rate of several cellular enzymes.

3. CELLULAR Mg^{2+} DISTRIBUTION

Direct and indirect measurement of total cellular Mg^{2+} content by various techniques consistently indicates that Mg^{2+} concentration ranges between 17 to 20 mM in the majority of mammalian cell types examined (see Table 1 in refs. 1 and 2). Determinations of total and free Mg^{2+} concentrations by electron probe X-rays microanalysis (3,4), ^{31}P-NMR, selective Mg^{2+}-electrode, ^{13}C-NMR citrate/isocitrate ratio or fluorescent indicators localize major amounts of Mg^{2+} within sub-cellular organelles and cytoplasm, and support the presence of a very limited chemical Mg^{2+} gradient across the cell membrane (5). Within the cell, Mg^{2+} is more or less homogeneously distributed among nucleus, mitochondria and endo(sarco)plasmic reticulum (3,4), with total concentrations ranging between 15-and 18 mM in each of these organelles. The presence of such an elevated concentration of total Mg^{2+} within these organelles suggests an involvement of phospholipids, proteins, nucleic acids, chromatin and nucleotides in binding Mg^{2+} so that a relatively small fraction of the cation is actually free in the lumen of these structures. Consistent with this hypothesis, concentrations of 0.8 and 1.2 mM free [Mg^{2+}] have been determined in the mitochondria matrix (6,7). In contrast, no determination of free Mg^{2+} concentration is currently available for the nucleus or the endo-sarco-plasmic reticulum. In the absence of direct determinations, the porous structure of the nuclear envelope suggests that the intra-nuclear free Mg^{2+} concentration is similar to that observed in the cytoplasm, as already reported for mitochondria. Attempts to quantitate luminal free [Mg^{2+}] within the endo-sarco-plasmic reticulum have been hampered by the elevated concentration of Ca^{2+} inside the organelle (3-4 mM, (8)), and the relative affinity of the fluorescent dyes Mag-Fura or Mag-Indo for Ca^{2+} *versus* Mg^{2+} (~50 μM *versus* 1.5 mM, respectively), to the point that various authors have actually utilized the incorporation of Mag-Fura within the endo-sarco-plasmic reticulum to measure variations in reticular Ca^{2+} under diverse stimulatory conditions (9). Lastly, very little has been uncovered in terms of proteins able to bind Mg^{2+} within the cell and cellular organelles. Aside from calmodulin (10), troponin C (11), parvalbumin (12), and possibly S100 protein (13), it is presently unknown whether other cytosolic or intra-organelle proteins can bind substantial amount of Mg^{2+} and participate in the cellular buffering of the cation. In this respect, an early report by Bogucka *et al.* (14) about the presence of two Mg^{2+}-binding proteins in the intermembraneous space of the mitochondrion has been neither confirmed nor expanded by additional studies.

^{31}P-NMR (15), fluorescent indicators (16), microelectrodes and null-point-titration techniques (17) indicate that an additional conspicuous pool of Mg^{2+} is located in the cytoplasm in the form of a complex with adenine phosphonucleotides and other phosphometabolites (18). Because of its abundance (~ 5 mM) and binding affinity (K_D ~78 μM), ATP constitutes the largest binding moiety for Mg^{2+} in the cytoplasm. The binding/buffering capacity of ATP, phosphonucleotides and phosphometabolites, and possibly proteins maintains cytosolic *free* [Mg^{2+}] between 0.5-1 mM, or less than 5% of total cellular Mg^{2+} content in almost all the cells and tissues examined (Table 1 in ref. 1).

Taking into account this cellular distribution and assuming a Mg^{2+} concentration in the plasma and extracellular fluid of 1.2-1.4 mM, one-third of which is binding extracellular proteins (e.g. albumin) or other biochemical moieties (19), it appears that most cells are near a *zero trans* condition as far it concerns the chemical concentration of free Mg^{2+} across the plasma membrane, or the bio-membrane of cellular organelles (e.g. mitochondria) and the cytoplasm. As the electrochemical equilibrium potential for intracellular free Mg^{2+} is approximately 50 mM in most mammalian cells under resting conditions (20), it is evident that mechanisms must operate in the cell membrane to maintain cytosolic *free* Mg^{2+} and total cellular Mg^{2+} content within the measured levels.

4. Mg^{2+} TRANSPORT ACROSS BIOLOGICAL MEMBRANES

Under resting conditions, the majority of mammalian cells do not significantly change total and free cellular Mg^{2+} over several h or days of incubation in the presence of a physiological extracellular Mg^{2+} concentration (21-24). Yet, different cells or tissues exhibit varying turn-over rates ranging from 1 hour in the case of adipocytes to several days in the case of lymphocytes, brain tissue and skeletal muscle. Radioisotopic ($^{28}Mg^{2+}$) equilibrium in isolated cells, tissues or in the whole animals has provided comparable results for the most part, although some exceptions have been observed. For example, isolated cardiac myocytes achieve $^{28}Mg^{2+}$ equilibrium

within 72-80 h when incubated at 37 ^{o}C, or over a longer period of time when the experimental temperature is reduced to 20 ^{o}C (21,25-27). The performance of similar experiments in the whole animal, instead, indicates that the ventricular myocardium exchanges total cellular Mg^{2+} content at a faster rate, usually within 3 h (21). The relative slow Mg^{2+} turn-over observed in several tissues or cells has contributed to the overall impression that mammalian cells do not change total Mg^{2+} content, or change it very slowly, despite ample variation in physiological and metabolic conditions.

This perception has been largely reconsidered following reports by numerous laboratories (29-41), indicating that lymphocytes (42), erythrocytes (43,44), cardiac myocytes (36,39) and liver cells (37,38,40) can extrude a considerable amount of cellular Mg^{2+} within 5-6 min from the administration of various hormonal or non-hormonal stimuli. Consequently, interest has grown in trying to characterize and identify the mechanisms by which Mg^{2+} is transported across the cell membrane.

Based upon their modality of operation, the Mg^{2+} transport mechanisms in biological membranes can be divided into Na^{+}-dependent and Na^{+}-independent pathways.

4.1. Na^{+}-dependent Mg^{2+} extrusion

Magnesium-loaded erythrocytes tend to re-establish their normal Mg^{2+} content by exchanging excess cellular Mg^{2+} for extracellular Na^{+} (43,44). The Na^{+}-dependence of this mechanism is supported by *1)* the inability of lithium, potassium or other monovalent cations to effectively substitute for Na^{+} in supporting Mg^{2+} extrusion (44), and *2)* the ability of amiloride, a diuretic commonly used to inhibit Na^{+} transport, to block Mg^{2+} extrusion (43). The initial observation by Günther and Vormann (44) has been further corroborated by various other groups (45-50). Feray and Garay (45) have reported that other Na^{+} transport inhibitors such as imipramine and quinidine are also effective at inhibiting Mg^{2+} extrusion, and that Mg^{2+} efflux is closely regulated by the cell energetic status (46). From a physiological stand-point, changes in cellular Mg^{2+} content appear to contribute to regulation of Na^{+},K^{+}-ATPase activity (51), phospho-inositide turnover (52), Na^{+}/H^{+} exchange (53), K^{+}/Cl^{-} co-transport (54), and hemoglobin metabolism, both directly (55) and indirectly via modulation of 3-phosphoglycerate (56). Also, the linear correlation existing between total and free Mg^{2+} (57) allows for a direct estimate of the concentrations of free Mg^{2+} in erythrocytes under resting and stimulatory conditions and the effect that changes in such a level may have for various cellular enzymes, in particular those connected with glycolysis (58). Yet, it is still controversial whether this Mg^{2+} extrusion mechanism is electroneutral or electrogenic in its operation. The electroneutral stoichiometry ratio of $2Na^{+}_{in}$:1 Mg^{2+}_{out} originally reported by Günther and Vormann (44) has not been confirmed by other groups (49,50). This discrepancy has been justified with the different source of the erythrocytes (*e.g.*, human or rat *versus* turkey), the experimental conditions utilized (*e.g.*, variation in extracellular Na^{+} concentration rather than cellular Mg^{2+} loading), or the modality by which Mg^{2+} transport has been elicited (*e.g.*, loading *versus* hormonal stimulation).

This Na^{+}-dependent Mg^{2+} extrusion mechanism appears to be ubiquitously present and operative in mammalian cells. Cardiac myocytes (36,39,41), hepatocytes (37,38,40), lymphocytes (42), ascites cells (59,60), sublingual mucosal acini (61,62), trophoblasts (63), and erythrocytes (43,44,64), just to name a few cellular models, all appear to possess a Mg^{2+} transport mechanism tentatively defined as a Na^{+}/Mg^{2+} exchanger for its Na^{+}-dependence (see ref. 65 for a list). Yet, inconsistent or negative reports involving the same or similar cell types can also be found in the literature. For example, two reports from the group of Murphy and Lieberman (66,67) do not support the operation of this transport mechanism in cardiac cells under basal conditions, whereas its operation has been observed by Handy *et al.* (68), Tashiro and Konishi (69), and others. Discrepancy also exists about the activation of this transporter in cardiac cells following hormonal stimulation. The Mg^{2+} extrusion observed by Günther and Vormann (36), Romani *et al.* (39,70) and Weiss and coworkers (71) has not been confirmed by Altschuld *et al.* (72). Similarly, a Na^{+}-dependent Mg^{2+} transport pathway appears to operate in freshly isolated spleen lymphocytes (42) but not in S49 lymphoma cells (32). The reasons for these discrepancies may involve the experimental conditions, modality of isolation, and phenotypic modifications in immortalized *versus* freshly isolated cells. Substantial differences between cell types also exist in terms of sensitivity of the transporter to inhibitory agents. Although not specific, amiloride and imipramine are the most effective inhibitors of the Na^{+}/Mg^{2+} exchanger in the majority of mammalian cells, with an ED_{50} of 100-150 μM (69) and 10 μM (68), respectively. Yet, amiloride derivatives are effective in certain cell types (59,73) but not in others (74). Because these derivatives exhibit some selectivity at inhibiting the Na^{+}/H^{+} antiport (75,76), it is possible that a Na^{+}/H^{+}exchanger is coupled to some extent to the operation of the Na^{+}/Mg^{2+} exchanger or modulates it *via* changes in cellular pH or Na^{+} content, at least in specific cell types.

Lastly, a particular form of Na^{+}-dependent Mg^{2+} extrusion mechanism appears to be the Na^{+}/Ca^{2+} exchanger. Magnesium-loaded cardiac myocytes over-expressing isoforms 1 or 3 of the Na^{+}/Ca^{2+} exchanger would extrude excess Mg^{2+} *via* this transporter in exchange for extracellular Na^{2+} (77). Yet, the lengthy period of time (more than 45 min) required to mobilize any significant amount of Mg^{2+} makes debatable whether the Na^{+}/Ca^{2+} exchanger actually plays a significant role in cells that do not over-express the transporter and/or contain a physiological amount of cellular Mg^{2+}. Recent data, however, suggest that the Na^{+}/Ca^{2+} exchanger may play a significant role in modulating cellular Mg^{2+} at least under Ca^{2+}-free conditions. Uetani *et al.* (78) have reported that the administration of amiloride to pig carotid artery smooth muscle cells in the presence of a physiological Ca^{2+} concentration in the extracellular medium results in a significant decrease in cytosolic free Mg^{2+} concentration.

Switching to Ca^{2+}-free incubation conditions, however, rapidly reverts this initial decrease into an increase in Mg^{2+} concentration (plus 13% after 100 min). Similar changes in free Mg^{2+} concentration can be observed in the presence of KB-R7943, a selective antagonist of the operation in reverse of the Na^+/Ca^{2+} exchanger (78), and suggest that the Na^+/Ca^{2+} exchanger can indeed directly modulate the activity of the Na^+/Mg^{2+} exchanger in a Ca^{2+}-dependent manner, at least under conditions in which extracellular Ca^{2+} is reduced and the passive Mg^{2+} influx is facilitated. However, it still remains questionable to which extent such a mechanism operates under physiological conditions and whether hormonal stimuli can modulate it.

4.2. Na^+-independent Mg^{2+} extrusion

One possible explanation for the variable stoichiometry of the Na^+/Mg^{2+} exchanger discussed previously (44,49,50) is that - as for other cations - the cell possesses more than one pathway to extrude Mg^{2+}. This hypothesis is supported by the observation that erythrocytes (79-81), and other mammalian cell types as well (70,82,83), can still extrude significant amounts of Mg^{2+} in the absence of extracellular Na^+ (70,79-82) or in the presence of Na^+-transport inhibitors (81,84). Divalent cations such as Mn^{2+} (70, 80,81), Ca^{2+} (70,82,85) or Sr^{2+} (70,74), or anions such as HCO_3^- or Cl^- (85,86) have been reported to be counter transported for or co-transported with Mg^{2+} respectively. Because of the heterogeneity of ions utilized for Mg^{2+} transport and its operation under conditions in which extracellular Na^+ is below the physiological concentration, this pathway has been generically termed as *Na^+-independent*. Depending on the ionic moiety favoring Mg^{2+} extrusion, a varying stoichiometry ratio has been measured. Under conditions in which a divalent extracellular cation is counter-transported for cellular Mg^{2+} a stoichiometry ratio of 1:1 has been commonly reported (81,83,87). With monovalent anions, an electroneutral co-transport of 2 negatively charged ions for 1 Mg^{2+} has been observed (84-87). Mass spectroscopy determinations utilizing non-radioactive Mg^{2+} isotopes (*e.g.*, ^{25}Mg,) or radioactive determination of ^{28}Mg also supports the operation of a Mg^{2+}:Mg^{2+} exchanger in erythrocytes (85) and cardiac cells (33), respectively. The physiological presence and significance of such a mechanism is not clear. In fact, a $1Mg^{2+}{}_{in}$:$1Mg^{2+}{}_{out}$ exchange ratio of this transporter makes difficult to explain how such a mechanism can account for the increase in cytosolic free Mg^{2+} observed by various authors (68,69,88). Further, although it is possible for the Na^+-dependent Mg^{2+} exchanger to accommodate Mg^{2+} instead of Na^+ at its extra-cellular site (89), it remains unresolved which electrochemical gradient this transport would utilize to move Mg^{2+} across the cell membrane under virtually *zero trans* conditions. A possibility could be that the 1:1 exchange between extracellular and intracellular Mg^{2+} results from the sequential operation of two distinct transport mechanisms coupled by the electrochemical gradient of another, intermediate cation. In this respect, Günther (89) has proposed that the Na^+ (Mg^{2+})/Mg^{2+} exchanger can accommodate another divalent cation at the extracellular site, which could thereby activate the transporter. Kinetic evaluation of the transporter activity (84) has resulted in K_m values comparable to those previously determined for the Na^+/Mg^{2+} exchanger (32,74,90), and consistent with the activation of a Na^+-independent Mg^{2+} extrusion pathway only in the presence of low extracellular Na^+ concentration (74,81,83). However, because the cell is never exposed to low extracellular Na^+ concentration, the physiological relevance of this transporter remains elusive. Further, the observation that ATP depletion appears to inhibit the Na^+-independent pathway irrespective of the cation or anion involved in Mg^{2+} transport suggests that this pathway requires ATP hydrolysis for activation (phosphorylation ?) (46). The involvement of anions in Mg^{2+} transport in mammalian cells is corroborated by recent data from Ebel and Günther (91), who have reported a role of intracellular Cl^- in stimulating the activity rate of the Na^+/Mg^{2+} antiport in rat erythrocytes *via* a cooperative effect. Kinetic evaluation indicates that 2 Cl^- can be actively transported per cycle of the transporter, thus contributing to its overall electrogenicity. This stimulatory effect is not restricted to Cl^- but it is shared by all the halogen anions ($Cl^->Br^->I^->F^-$) (91). In addition, these authors have provided evidence that rat erythrocytes can extrude Mg^{2+} *via* the choline exchanger (92). Erythrocytes appear to possess two distinct types of Na^+-independent Mg^{2+} efflux, one mechanism operating in sucrose medium whereas the second operates in high Cl^- medium, in particular choline chloride medium. Under the latter conditions, Mg^{2+} efflux occurs independent of the presence or the absence of various monovalent cations in the extracellular medium but is inhibited by quinine, cinchonine, and other cinchona alkaloids, all agents that normally inhibit the choline exchanger (92). As the extent of inhibition is the same for Mg^{2+} efflux and choline efflux, the authors have proposed that cells incubated in choline chloride medium can extrude Mg^{2+} via a non-selective transport, namely the choline exchanger (92).

An additional parameter to be considered when investigating Mg^{2+} transport in mammalian cells is the role plaid by the membrane potential or by cations other than Na^+ in facilitating Mg^{2+} movement. Marek and Martens have reported that in rumen epithelial cells, which exhibit apical and basolateral K^+ conductances, transcellular Mg^{2+} transport has an electrogenic component. In this particular experimental model, changes in apical membrane potential constitute the link between an increase in K^+ concentration in the rumen and a decrease in Mg^{2+} absorption (93). Although the transcellular Mg^{2+} absorption is for the most part electrically silent (~65%), experimental evidence supports the operation of a Na^+/Mg^{2+} exchanger in the cell membrane of ruminant epithelial cells, in that cytosolic *free* Mg^{2+} in these cells increases in a manner directly proportional to the extracellular Mg^{2+} concentration after stimulation by butyrate or HCO_3^- (94). The increase in cytosolic *free* Mg^{2+} is pH- and K^+- independent but is accompanied by a decrease in cytosolic free Na^+. Further, it is inhibited by imipramine and quinidine (94). Whether a co-transport of Mg^{2+} and HCO_3^- occurs in these cells as already suggested by Günther *et al.* in erythrocytes (85,86) is at the present undefined.

4.3. Mg^{2+} transport in purified plasma membranes

Recently, the number and modality of operation of Mg^{2+} transporters have been investigated in purified plasma membrane vesicles. This system presents several practical advantages, including the absence of signaling pathways and intracellular buffering components or organelles, and the ability to providing a well defined ionic extra- and intra-vesicular milieu composition to determine the modality of operation of the various Mg^{2+} transporters.

By using purified liver plasma membrane vesicles (74,83), Cefaratti and colleagues have reported the operation of a bi-directional Na^+-dependent Mg^{2+} transport mechanism in the basolateral domain of the hepatocyte (83). In contrast, the apical portion of the liver cell possesses two apparently distinct, unidirectional Mg^{2+} transport mechanisms, which extrude intravesicular Mg^{2+} for extravesicular Na^+ and Ca^{2+}, respectively (83). The basolateral and apical Mg^{2+} transport mechanisms possesses distinct kinetic and pharmacological properties. The basolateral Mg^{2+} transporter has a K_m for Na^+ lower than 20 mM, in good agreement with kinetic data obtained in isolated hepatocytes (82), and other cell types (69,90). This transporter is selectively activated by Na^+ (74,83,95) and inhibited by imipramine, but not amiloride and amiloride derivates (83). The Na^+-dependent Mg^{2+} transporter in the apical domain of the hepatocyte presents a K_m for Na^+ comparable to the basolateral transporter, selectively uses Na^+ *versus* other monovalent cations, but is inhibited by both imipramine and amiloride (83). The apical Ca^{2+}-dependent mechanism is also inhibited by amiloride or imipramine, and is activated by micromolar Ca^{2+} concentration ($K_m \leq 50$ μM). This transporter, however, does not possess Ca^{2+} specificity, as Mg^{2+} extrusion can be elicited by the extravesicular addition of micromolar concentrations of other divalent cations ($Ca^{2+} >> Co^{2+} = Mn^{2+} > Sr^{2+} >> Ba^{2+} > Cu^{2+} >> Cd^{2+}$) (74). The Ca^{2+}-dependent Mg^{2+} transporter appears to be electroneutral ($1Ca^{2+}_{in}:1Mg^{2+}_{out}$), while both the basolateral and apical Na^+-dependent Mg^{2+} transporters are electrogenic, transporting $1Na^+_{in}:1Mg^{2+}_{out}$ under the majority of experimental conditions tested (95). The transporters continue to operate in the presence of *zero trans* transmembrane conditions for Mg^{2+} (*i.e.*, 20 mM Mg^{2+} inside and outside the vesicle), an indication that Mg^{2+} extrusion utilizes the electrochemical gradient of the counter-transported cation (74,83). The operation of functionally similar Na^+- and Ca^{2+}-dependent Mg^{2+} extrusion mechanisms has also been observed in cardiac sarcolemma vesicles (96). Neither liver nor cardiac plasma membrane vesicles require intravesicular ATP for the operation of the various Mg^{2+} transporters (74,96). Pre-treatment of the vesicles with phosphatases at the time of purification completely abolishes the ability of the transporters to move Mg^{2+} across the membrane (97). This observation is consistent with the idea that a (cAMP-dependent) phosphorylation process is required to activate the Mg^{2+} transporter mechanisms in intact cells (89,98).

The operation of specific Mg^{2+} accumulation mechanisms has also been observed in plasma membrane vesicles from brush border cells of rabbit ileum (99) and from the duodenum and jejunum of rat (100,101). By using membrane vesicles from rabbit ileum and cell permeant and non-permeant Mag-Fura (99), Juttner and Ebel have observed the operation of a saturable Mg^{2+} uptake mechanism when the intracellular Na^+ concentration is higher than the extracellular one, the process being inoperative when the gradient is reversed (*i.e.*, $[Na^+]_i < [Na^+]_o$), the vesicles are in *zero trans* condition for Na^+, or external Na^+ is removed. At variance with the transporter observed in liver plasma membrane, the pathway in ileum vesicles is not reversible and appears to be electroneutral. Yet, it possess a K_m for Na^+ of 16 mM, a value similar to the K_m calculated in liver plasma membranes (74,83), in smooth muscle cells from guinea pig tenia caecum (90), and in chicken erythrocytes (89). Another similarity with the transporter operating in basolateral liver plasma membranes is the lack of inhibition by amiloride analogs. The transporter is modulated by intravesicular anions, especially Cl^- and SCN^-, and markedly stimulated by antagonists of anion transport (*e.g.*, H_2-DIDS).

The main difference between plasma membrane vesicles from duodenum and jejunum (100,101) is that a single Mg^{2+} uptake mechanism operates in the duodenum with a K_m of 0.8 mM, whereas two transporters operate in the jejunum with K_m values of 0.15 and 2.4 mM, respectively. In both these experimental models, Mg^{2+} but not Ca^{2+} accumulation is reduced in the presence of alkaline phosphatase inhibitors (100), suggesting that Ca^{2+} and Mg^{2+} are transported *via* distinct pathways. This hypothesis is further supported by the observation that Mg^{2+} accumulation is not inhibited by Ca^{2+} channel antagonists while it is blocked by amiloride. Consistent with the report by Juttner and Ebel (99), Mg^{2+} accumulation is stimulated by an intravesicular electronegative potential (101) or by an alkaline pH_o. The effect of external pH, however, is lost when $[Mg^{2+}]_o > 1$ mM (100). Under the latter condition, Mg^{2+} accumulation is enhanced by the presence of Na^+ or K^+ in the extravesicular space but is inhibited by the presence of divalent cations ($Co^{2+} > Mn^{2+} > Ca^{2+} > Ni^{2+} > Ba^{2+} > Sr^{2+}$) (101).

4.4. Newly identified Mg^{2+} transport mechanisms

As indicated above, the presence and operation of Mg^{2+} transport mechanism(s) in eukaryotic cells is essentially supported by pharmacological and biophysical evidence, or manipulation of extracellular medium composition. Up to now, in fact, no functional Na^+-dependent or independent Mg^{2+} transporter has been cloned or purified in mammalian cells, in stark contrast with the identification of distinct magnesium transporters in bacteria (102), *Archaea*, and yeasts (102-104).

The operation of a selective Mg^{2+} channel in *Paramecium* has been observed and characterized by Preston and collaborators (105-107). The initial observation of a Mg^{2+} current in this monocellular organism (105) has been further corroborated by the report that the Mg^{2+} transported through this mechanism is specifically utilized to control the operation of the flagellum and direct the *Paramecium* in a specific direction

(106). More recently, Preston and collaborators have provided evidence that the structure responsible for the large Mg^{2+} current in *Paramecium* has some similarity with Na^{+}/Ca^{2+} exchangers rather than with ion channels (107).

Conceptually, the operation of a Mg^{2+} channel in mammalian cells is not inconsistent with the *zero trans* conditions present across the cell membrane, as discussed in *Section 3*. In recent years, mounting experimental evidence indicates the operation of Mg^{2+} channels in mammalian cells. Quamme and co-workers were the first to hypothesize the presence of a channel responsible for the accumulation of Mg^{2+} in cardiac and kidney cells based on the inhibition of Mg^{2+} influx by nifedipine. Support for their hypothesis has been recently provided by Fleig and her group (108), who identified a Mg-ATP regulated divalent cation channel that can specifically transport Mg^{2+} under well defined conditions and is strictly required for cell viability (108). This channel, identified as LTRPC7 (108), TRP-PLIK (109), Chak (110) or Chak1 (111) by the four laboratories that independently cloned it, is a member of the TRP channel family (112). TRP channels are classically associated with Ca^{2+} entry across the plasma membrane of various cell types following specific hormonal and non-hormonal stimuli that induce Ca^{2+} release from IP_3-sensitive reticular Ca^{2+} pools and a subsequent entry of Ca^{2+} across the cell membrane (thus the term SOC or Store Operated Channels, (112)). According to a recent unified nomenclature for TRP channels, LTRPC7 is now termed TRPM7. Interestingly, TRPM7 possesses an alpha-kinase domain at its C-terminus (113), which specifically phosphorylates threonine residues within an alpha helix structure of the substrate(s) (114). The kinase domain does not control TRPM7 gating, although it appears to play a not well-defined role in modulating TRPM7 channel operation (113). Presently, annexin I (115), myosin IIA heavy chain (116), and calpain (117) have been identified as substrates of TRPM7 kinase domain. The dual ability of this protein to act as a channel and a kinase at the same time suggests its involvement in regulating both Mg^{2+} homeostasis (109,113) and cellular function, in particular cell adhesion (116,117). At the present time, however, only bradykinin (116) and angiotensin II (118) have been reported to activate TRPM7. Hence, it is still undetermined whether hormones that elicit cellular Mg^{2+} accumulation (see *Section 5.4*) operate *via* TRPM7 activation. While experimental evidence indicates that increasing concentrations of intracellular Mg^{2+} or Mg-ATP selectively inhibit the channel component of TRPM7, it is less clear which second cellular messengers modulate the channel activity, if at all. The initial observation that PIP2 can inhibit TRPM7 channel reported by Clapham and his group (119) has not been confirmed by Fleig and colleagues, who instead reported a regulatory role by cAMP (120). The latter observation would suggest the presence of a cAMP binding consensus in the kinase domain of TRPM7. Another member of the TRP family, the TRPM6 channel, also appears to be involved in mediating Mg^{2+} influx in intestinal and renal cells (121). The best evidence for such a role is provided by the observation that specific mutation in TRPM6 sequence results in familial hypomagnesemia with secondary hypocalcemia (122). Also TRPM6, originally cloned as Chak2 (123), is a chan-zyme as it possesses an alpha-kinase domain at its C-terminus (114,123). As no substrate for the TRPM6 kinase domain has been presently identified, it is unclear whether TRPM6 phosphorylates the same substrates targeted by TRPM7. The tissue distribution of the two channels, however, is different. While TRPM6 is exclusively expressed in kidney and colon cells (121) TRPM7 appears to be ubiquitously expressed in all the tissues and cells tested so far. The attempt to over-express TRPM6 in cells not expressing the channel under normal conditions (*e.g.*, HEK 293) has resulted in conflicting results in that the presence of a TRPM6-mediated current reported by Nilius *et at.* (121) has not been confirmed by Chubanov *et al.*, (124). The group of Ryazanov has actually observed that TRPM7 needed to be co-expressed to rescue TRPM6 to the plasma membrane, to form a heterodimeric channel for Mg^{2+} entry into the cell (125), suggesting that TRPM6 remains internalized in cells that do not natively express the channel. This result together with the observation that TRPM6 kinase domain can phosphorylate TRPM7 but not *vice versa*, and that the two kinases are functionally non-redundant despite a highly conserved amino acid sequence (125) strongly indicates that the two chan-zymes play different roles and functions in the cells in which they are constitutively expressed.

Another protein that has a direct impact on renal Mg^{2+} homeostasis and resorption is Paracellin-1 (126). This protein, a component of the tight junctions of the thick ascending limb of Henle, exhibits a high Mg^{2+} conductance and a high impermeability to water (126). Its role in regulating Mg^{2+} resorption is confirmed by the observation that autosomal recessive mutations in the gene (PCLN-1) encoding for Paracellin-1 result in renal hypomagnesemia with severe Mg^{2+} wasting, hypercalciuria and nephrocalcinosis. The protein is 305 amino acids in length with 4 transmembrane domains, and appears to be specifically present in the kidney (126), where it is restricted to distal tubular segments including the thick ascending limb of Henle's loop, the distal tubule, and the collecting duct, all sections of the kidney in which Mg^{2+} resorption is maximal.

More recently the presence of specific Mg^{2+} transport mechanisms with close homology to bacterial transporters has been evidenced in the plasma membrane or in the mitochondria of mammalian cells. Aasheim and collaborators have identified a solute carrier, termed SLC41A1, which present a close homology to the bacterial MgtE family (127). This transporter consists of 10 trans-membrane domains for a total of 513 amino acids with an estimated molecular weight of 56 kDa, and is ubiquitously represented in human tissues, with the highest expression in heart and testis. Homologs of the transporter have been found in species as diverse as *H. sapiens, M. musculus, D. melanogaster, A. gambiae,* and *C. elegans*, thus suggesting the presence of a well distributed gene family (127). Three isoforms of this transporter (SLC41A1, A2 and A3) have now been identified. At the functional level, the expression of SLC41A2 in *Xenopus laevis* oocyte voltage-dependent and saturable Mg^{2+} uptake with a Michaelis constant of

~0.35 mM, which does not appear to depend on the extracellular Na^+ concentration (128). Neither SLC41A1 nor SLC41A2 are selective for Mg^{2+} as they can transport other divalent cations although with some differences. SLC41A1 can transport Sr^{2+}, Zn^{2+}, Cu^{2+}, Fe^{2+}, Co^{2+}, Ba^{2+}, and Cd^{2+}, but not Ca^{2+}, Mn^{2+}, or Ni^{2+}(128), whereas SLC41A2 can transport Ba^{2+}, Ni^{2+}, Co^{2+}, Fe^{2+}, or Mn^{2+}, but not Cu^{2+}, Zn^{2+}, or Ca^{2+} (129) Large concentrations of Ca^{2+} actually inhibit Mg^{2+} transport through SLC41A2 (129) but not SLC41A1 (128). An additional difference between these two isoforms is their genetic expression in response to a low external Mg^{2+} concentration. While SLC41A1 expression increases in the presence of a low extracellular Mg^{2+} concentration (128), SLC41A2 expression remains unchanged (129). Overall, these data suggest that both SLC41A1 and SLC41A2 are general divalent metal cation transporters that can transport Mg^{2+} under specific conditions.

Using oligonucleotide microarray analysis, Goytan and Quamme have recently identified two additional genes that encode for Mg^{2+} transport mechanisms. One of these genes encodes for a novel Mg^{2+} transport protein that has no amino acid sequence identity to other known transporters (130). This protein, termed MagT1 (for Magnesium Transporter 1) has a molecular weight of 38 KDa and presents a sequence of 335 amino acids arranged to form 5 transmembrane spanning domains. At its N-terminus 4 cAMP phosphorylation sites and 4 protein kinase C phosphorylation sites have been identified (130). The functional expression of MagT1 in oocytes elicited large Mg^{2+}-evoked currents with little permeability to other divalent cations. MagT1 is widely distributed among tissues, particularly in epithelia, in which its expression appears to be regulated by external Mg^{2+}. Hence, MagT1 can be considered a dedicated mammalian Mg^{2+} transporter.

The second gene that is differentially expressed under low Mg^{2+} conditions encoded instead for the Ancient Conserved Domain Protein (ACDP2) (131). At variance of MagT1, this protein has no cAMP phosphorylation sites but presents 4 possible phosphorylation sites for protein kinase C. Real-time RT-PCR of mRNA isolated from distal epithelial cells cultured in low-magnesium media and from kidney cortex of mice maintained on low-magnesium diets confirmed that the ACDP2 transcript is up-regulated as compared to cells maintained in normal external Mg^{2+} or tissue from mice maintained on normal Mg^{2+} diet. In particular, ACDP2 expression increases in the distal convoluted tubule cells, kidney, heart, and brain of animals maintained with a Mg^{2+}-deficient diet. When expressed in oocytes, ACDP2 mediates saturable Mg^{2+} uptake with a Michaelis constant of 0.5-0.6mM. This uptake is voltage-dependent, and not associated to Na^+ or Cl^- ion movement. ACDP2 also transports other divalent cations in addition to Mg^{2+} (*i.e.*, Co^{2+}, Mn^{2+}, Sr^{2+}, Ba^{2+}, Cu^{2+}, and Fe^{2+} but not Ca^{2+}, Cd^{2+}, Zn^{2+}, and Ni^{2+}). In terms of inhibition, only Zn^{2+} appears to be an effective blocking agent. In terms of tissue distribution, ACDP2 transcript is abundantly present in kidney, brain, and heart but has a low expression in liver, small intestine, and colon. These results suggest that ACDP2 may operate as a transporter for Mg^{2+} and other divalent cations especially in epithelial cells.

As for the identification of Mg^{2+} transporters in cellular organelles, a series of interesting data from Schweyen's group have shed a new light in understanding the molecular bases of Mg^{2+} transport in mitochondria from yeast, mammals and plants. This group, in fact, has identified two proteins located in the yeast mitochondrial inner membrane, Mrs2p and Lpe10p, that have closed homologs in mammals and plants, and which appear distantly related by sequence to the bacterial CorA Mg^{2+} transporter (132-135). The yeast genome also encodes three additional proteins, Alr1p, Alr2p and Mnr2p, which are distantly related to CorA, Mrs2P and Lpe10p. Alr1 is specific located in the yeast plasma membrane (135), and not only controls Mg^{2+} homeostasis but is subject to Mg^{2+}-dependent control of its synthesis and degradation (135). In contrast, Mrs2p appears to be an essential component of the major electrophoretic Mg^{2+} influx system in mitochondria (132). Structurally, Mrs2p possesses 2 transmembrane domains and is able to sustain a rapid and high capacity Mg^{2+} influx in isolated yeast mitochondria. This influx is sustained by the mitochondrial membrane potential and is inhibited by cobalt(III)hexaammine (132), as already reported by Maguire and colleagues for bacterial CoA (102-104). While overexpression of this transporter increases Mg^{2+} influx rate several fold, the deletion of the related MRS2 gene completely abolishes Mg^{2+} accumulation. The bacterial CorA (133) or the human mitochondrial MRS2 gene can functionally complement the yeast mutant (134) and restore Mg^{2+} influx across the yeast inner mitochondrial membrane. Cross-linking experiments suggest that Msr2p forms homo-oligomers within the mitochondrial membrane with a molecular weight of ~110kDa (dimer) or ~270 kDa (most likely a tetramer), depending on the cross-linking agent utilized (132). It is presently unresolved whether Mrs2p is also subject to Mg^{2+}-dependent control of its synthesis and degradation as observed for Alr1.

In concluding this section, some recent information on two additional Mg^{2+} transport mechanisms has to be reported. The first transporter is a Mg^{2+}/H^+ exchanger presently identified only in plants (136). This transporter, originally identified in *A. thaliana* and termed AtMHX, appears to be present in all plants. It presents 11 putative transmembrane domains, is exclusively localized in the vacuolar membrane of the plant and electrogenically exchanges protons with Mg^{2+} or Zn^{2+}. Interestingly, the ectopic overexpression of the transporter in tobacco plants sensitizes the plant to grow in the presence of elevated concentrations of Mg^{2+} (or Zn^{2+}) (136). At the moment, no corresponding gene or transporter has been identified in mammalian cells, although evidence for a direct or indirect exchange of Mg^{2+} for H^+ under certain conditions has been provided by Günther (79,87). The second transporter, for which we do not yet have functional cloning or protein sequence is a ~70 KDa protein band tentatively corresponding to the Na^+/Mg^{2+} exchanger discussed in *Section 4.1*. The group of Schweigel, Martens and colleagues has worked extensively (93,94,137,138, and

refs. therein) at characterizing how Mg^{2+} is transported in the rumen, and their results support the operation of a Na^{+}/Mg^{2+} exchanger with kinetic parameters and characteristics similar to those described by Günther and other groups in a variety of mammalian cell types (see *Section 4.1*). By incubating sheep rumen epithelial cells in the presence of monoclonal antibodies raised against the porcine red blood cell Na^{+}/Mg^{2+} exchanger, these authors observed that the transport of Mg^{2+} *via* this exchanger was significantly hampered by the antibodies (137). This is the first time that information about the molecular size of the elusive Na^{+}/Mg^{2+} exchanger is obtained, and the availability of these antibodies can clearly speed up the identification and recognition of this transporter in mammalian tissues.

Although the information relative to the nature and modality of operation of these mechanisms is still fragmentary and incomplete, a new picture is emerging whereby distinct Mg^{2+} transporters have been identified in various compartments within the cell, in which they operate to maintain Mg^{2+} homeostasis under a variety of metabolic conditions.

5. HORMONAL REGULATION OF Mg^{2+} TRANSPORT

In the last fifteen years a large body of evidence has been provided by various laboratories, which indicates that several hormones can induce the extrusion of a large amount of Mg^{2+} out of mammalian cells and tissues into the extracellular space and ultimately the blood stream, or the accumulation of a sizable amount of Mg^{2+} from these spaces into the cell. While a clear understanding of the physiological relevance of these fluxes in and out of the cells has not yet been obtained, it is undeniable that the amount of Mg^{2+} transported in either direction across the cell membrane results in major changes in total and free Mg^{2+} content within the cell or selective cellular organelles. The present section is aimed at presenting the available evidence for hormonal-regulated Mg^{2+} transport and providing, where possible, the physiological implications of this process.

5.1. Mg^{2+} extrusion following β-adrenergic receptor stimulation: role of cAMP

The first observation of an effect of hormones on Mg^{2+} transport dates back to 1974, when Elliott and Rizack (139) reported an accumulation of Mg^{2+} in adipocytes stimulated by epinephrine and adrenocorticotrophic hormone, although the specificity and modality of transport was not elucidated. In the early eighties, Maguire and colleagues provided the first extensive characterization of the modulatory effect of beta-adrenergic agonists and PGE_1 on Mg^{2+} fluxes in S49 lymphoma cells, primary lymphocytes and other cell types (29-32). By using $^{28}Mg^{2+}$ to distinguish influx *versus* efflux, Maguire and Erdos (35) reported that beta-adrenergic agonists inhibited Mg^{2+} influx in S49 lymphoma cells while agents modulating protein kinase C activity stimulated it. None of these agents, however, appeared to affect Mg^{2+} efflux. By using S49 lymphoma cells clones lacking protein kinase A or adenylyl cyclase, these authors also provided evidence that cAMP and protein kinase A were not involved in transducing the inhibitory effect of beta-adrenergic agonists on Mg^{2+} influx (34,35). Interestingly, Mg^{2+} influx in S49 lymphoma cells does not depend on extracellular Na^{+} or membrane potential (R.D. Grubbs and M.E. Maguire, unpublished observations), significantly differing from what observed by Wolff *et al.* in freshly isolated primary lymphocytes (59). In addition, total Mg^{2+} turnover in S49 lymphoma cells is very slow (more than 40 h) compared to Ca^{2+} turnover (accomplished in less than 3 h) (28).

Since these initial observations, reports from various laboratories have provided a clear indication that various hormones control Mg^{2+} homeostasis in numerous cell types and tissues. At variance of what reported in S49 cells, cardiac myocytes (36,39,41,70), hepatocytes (37,38,40,140,141), erythrocytes (142), lymphocytes (59), Erhlich ascites cells (143), sublingual mucous acini (61,62), thymocytes (98) and HL-60 promyelocytic leukemia cells (144) are just a few examples of tissues or cell types that respond to the administration of catecholamine (epinephrine or norepinephrine) or β-adrenergic agonist (*i.e.*, isoproterenol) by extruding a significant amount of cellular Mg^{2+} within a few minutes from the application of the stimulus. This extrusion becomes evident within 1-2 min from the addition of the agonist to the perfusate or the incubation system, and reaches the maximum within 5-6 min, irrespective of the dose of agonist administered. As Mg^{2+} extrusion then returns towards basal levels independent of the persistence of the agonist in the perfusate (36,39,40,141), evidence is there that Mg^{2+} is mobilized from a specific but still ill-defined cellular pool(s). The sequential addition of submaximal doses of agonist to the perfusate within a few minutes from each other results in a Mg^{2+} extrusions that progressively decrease in amplitude (39), thus suggesting a progressive depletion of the cellular Mg^{2+} store(s) by the sequence of stimulation applied to the tissue, or a desensitization of the receptor and associated extrusion signaling mechanism. Magnesium extrusion can be inhibited by the administration of beta-adrenergic antagonists (39,40), by Rp-cAMPs (42), an isomer of cAMP able to cross the cell plasma membrane and prevent protein kinase A activation by endogenous cAMP (145), or by hormones such as carbachol (146), vasopressin (147) or insulin (148,149) that decrease cAMP production within the cell. In contrast, Mg^{2+} extrusion can be stimulated by cell permeant cAMP analogs (*e.g.*, di-butyryl-cAMP, 8-Cl-cAMP or 8-Br-cAMP) (39,40) or forskolin (39,40) to an extent comparable to that elicited by beta-adrenergic agonists. The cAMP-mediated Mg^{2+} extrusion appears to be a more general phenomenon, irrespective of the pathway leading to cAMP increase. Cittadini and collaborators, for example, have reported a cAMP-mediated Mg^{2+} extrusion in spleen lymphocytes (42) and Erhlich ascites cells (143,150) following administration of prostaglandin PGE_1 or PGE_2 and arachidonic acid, respectively, whereas Fagan and Romani have reported a cAMP-mediated Mg^{2+} extrusion in liver cells stimulated by glucagon (151). Taken together, these observations strongly suggest that cAMP plays a major role in determining Mg^{2+} extrusion, most likely *via*

direct phosphorylation of the transport mechanism (98) or, alternatively, a regulatory protein. Consistent with the information reported in *Sections 4.1 and 4.2*, under all the stimulatory conditions indicated above, Mg^{2+} extrusion across the plasma membrane requires the presence of a physiological concentration of Na^+ and Ca^{2+} (70,82) in the extracellular milieu to occur. In fact, the removal of extracellular Na^+ or Ca^{2+} (70,82), or the presence of amiloride (36), imipramine (45) or quinidine (46) as Na^+-transport inhibitors, or verapamil or nifedipine (87) as Ca^{2+}-channel inhibitors are all conditions that significantly reduce or abolish the hormone-activated Mg^{2+} extrusion in mammalian cells.

5.2. Mg^{2+} extrusion following α_1-adrenergic receptor stimulation: role of Ca^{2+}

More recently, evidence has been provided about the occurrence of Mg^{2+} extrusion following activation of $alpha_1$-adrenergic receptor in addition or alternative to stimulation of beta-adrenergic receptor.

Jakob *et al.* (38) were the first to report a Mg^{2+} extrusion in phenylephrine-stimulated liver cells. Support for the operation of both $alpha_1$- and a beta-adrenergic receptor-mediated Mg^{2+} extrusion in liver cells has been provided by Keenan *et al.* (141). In these cells, the pre-treatment with insulin completely prevents the Mg^{2+} extrusion normally elicited by β-adrenergic agonist or cell permeant cAMP analogs administration and reduces by approximately 10-15% the extrusion induced by norepinephrine or epinephrine while leaving unaffected the Mg^{2+} extrusion stimulated by phenylephrine.

The modality by which $alpha_1$-adrenergic stimulation induces Mg^{2+} extrusion has been further investigated by Fagan and Romani (151,152). The amount of Mg^{2+} mobilized by catecholamine has been estimated to be equivalent to the sum of the amounts of Mg^{2+} mobilized by the separate stimulation of $alpha_1$- and beta-adrenergic receptor by phenylephrine and isoproterenol, respectively (151). Further, while beta-adrenergic receptor stimulation strictly requires the presence of a physiological concentration of extra-cellular Na^+ to elicit Mg^{2+} extrusion, the stimulation of $alpha_1$-adrenergic receptor requires the presence of both Na^+ and Ca^{2+} in the extracellular compartment (151). Following phenylephrine stimulation, the extrusion of Mg^{2+} *via* a Ca^{2+}-dependent mechanism accounts for ~15-20% of the total, the remaining 80-85% depending on what appears to be a Ca^{2+}-activated Na^+-dependent mechanism (151). From these data, it can be envisaged that Na^+ is required as the cation counter-transported for Mg^{2+} at the plasma membrane level while Ca^{2+} plays a key *signaling* role within the cell to mediate $alpha_1$-adrenergic receptor induced Mg^{2+} extrusion. Consistent with this hypothesis, the prevention of reticular Ca^{2+} release, cytosolic Ca^{2+} concentration increase or capacitative Ca^{2+} entry across the cell membrane almost completely inhibits hepatic Mg^{2+} extrusion (152). Conversely, the administration of thapsigargin, which increases intracellular Ca^{2+} concentration by inhibiting reticular Ca^{2+}-ATPases, mimics phenylephrine-induced Mg^{2+} extrusion even in the absence of extracellular Ca^{2+} (82,152). This would suggest that a certain level of cytosolic Ca^{2+} concentration has to be attained to activate the transporter, possibly *via* calmodulin (153) and/or displacement of Mg^{2+} from specific cellular binding sites (153).

Based upon these results, it is evident that both $alpha_1$- and beta-adrenergic receptor activations can induce Mg^{2+} extrusion from a tissue following stimulation by catecholamine or by specific receptor agonists. This redundancy in signaling and Mg^{2+} extrusion mechanisms may acquire particular relevance under conditions in which one of the signaling/extrusion pathways is inhibited (*e.g.*, insulin secretion, (142)). The limited information currently available does not permit any conclusion as to whether $alpha_1$- and beta-adrenergic receptor signaling induce a differential mobilization of Mg^{2+} from the apical *versus* the basolateral domain of the liver cell or whether the different Mg^{2+} extrusion pathways evidenced by Cefaratti *et al.* in discrete portions of the hepatocyte cell membrane (83) are differentially regulated.

An insight about a physiological role of Mg^{2+} extrusion in liver cells is provided by the report of Jakob *et al.* (38), and by the recent observation by Fagan and Romani (152) and Torres *et al.* (154). Jakob *et al.* (38) were the first to observe that phenylephrine administration induces both Mg^{2+} extrusion and glucose output from the hepatocyte. Fagan and Romani (152) confirmed this observation and further reported that in the absence of extracellular Na^+ (*i.e.*, a condition that prevents Mg^{2+} mobilization) both adrenergic-mediated Mg^{2+} extrusion and glucose output from liver cells are inhibited. In addition, these authors indicated that inhibition of glucose transport by phloretin markedly decreases the amplitude of glucagon- or catecholamine-mediated Mg^{2+} extrusion from the hepatocyte (152). In agreement with these reports, Torres *et al.* (154) have provided further evidence in support of the physiological association of Mg^{2+} extrusion to glucose output. These authors, in fact, observed an absence of Mg^{2+} mobilization following *in vitro* administration of β- or α_1-adrenergic agonist to liver cells from overnight starved animals. When analyzed for Mg^{2+} content, hepatocytes from starved rats presented already a 10-15% decrease in total Mg^{2+} level as compared to liver cells from fed animals (154). This decrease is quantitatively comparable to that observed in liver cells from fed animals following *in vitro* stimulation by catecholamine (37,40,151,152). This observation is consistent with the idea that during starvation the increase in the serum level of pro-glycemic hormones (*i.e.*, catecholamine, glucagon, etc.) mobilizes Mg^{2+} from liver cells while stimulating hepatic glycogenolysis and glucose output to maintain euglycemia.

5.3. Mg^{2+} extrusion following non-hormonal stimulation

While hormonal stimulation may represent the most dynamic modality by which a cell can rapidly mobilize an amount of Mg^{2+} equivalent to 10-15% of its total content, Mg^{2+} can also be mobilized from a cell following the treatment with cyanide (155), mitochondria uncouplers (155,156), fructose (157, 158), ethanol (159), or

hypoxia (160). All these agents decrease cellular ATP content by affecting either the ability of mitochondria to generate energy or by altering the pyridine nucleotide level within the cytosol (ethanol). Because ATP represents the most abundant Mg^{2+} chelating moiety within the cell (18), any significant decrease in its concentration will result in an increase in cytosolic *free* Mg^{2+}. Thus, it is not surprising that an increase in cellular Mg^{2+} extrusion might result. In the absence of significant changes in cellular cAMP level (139) that could explain the activation (by phosphorylation?) of the Mg^{2+} extrusion mechanism, it appears that an increase in cytosolic free Mg^{2+} *per se* is sufficient to activate this pathway. The increase in cytosolic free Mg^{2+} observed in hepatocytes undergoing anoxia (161), or treated with cyanide (155) or fructose (157) is considerably lower than the increase expected to occur merely based upon the decrease in cellular ATP content. While not excluding a possible redistribution of Mg^{2+} within the intracellular compartments, this observation would be consistent with the extrusion of a portion of *free* Mg^{2+} across the cell plasma membrane (155). A small increase in cytosolic free Mg^{2+} concentration (usually 70-100 μM) as compared to a much larger mobilization of total Mg^{2+} from the cell (~1 mM or more, (88)) is also observed following adrenergic stimulation. Under adrenergic stimulatory conditions, however, the removal of extracellular Na^+ results in a larger increase in cytosolic *free* Mg^{2+}, indication that extrusion rather than redistribution is the main mechanism limiting the increase in free Mg^{2+} within the cell

Finally, the role cellular ATP plays in regulating Mg^{2+} extrusion must be mentioned. In squid axon (162,163), a physiological level of ATP is required to support the Na^+-dependent Mg^{2+} efflux, the process being inhibited under conditions in which ATP level decreases (162,163). In mammalian cells, however, the scenario is far more complex. As mentioned previously, the Na^+-independent pathway is specifically inhibited by ATP depletion, at least in erythrocytes (84) or hepatocytes (46). Because no evidence supports the notion that cellular Mg^{2+} is extruded *via* an outwardly oriented Mg^{2+}-ATPase, it would appear more likely that ATP is required to maintain the transporter (or a regulatory protein) in a phosphorylated, active state (46,84). Under conditions in which cellular ATP level is decreased (*e.g.*, chemical hypoxia (155,161), diabetes (164), or acute ethanol treatment, (159)), the extrusion of Mg^{2+} *via* a Na^+-dependent mechanism appears to be enhanced (155,161) in a manner that is proportional to the decrease in ATP level. This observation is consistent with the role of ATP as the main ligand for Mg^{2+} in the cytoplasm, whereby a decrease in ATP content results in an increase in free Mg^{2+} concentration and subsequent Mg^{2+} extrusion across the plasma membrane (155,161).

5.4. Mg^{2+} accumulation following hormonal and non-hormonal stimuli

As briefly indicated in *Section 5.2*, hormones like insulin or carbachol can prevent adrenergic receptor-induced Mg^{2+} extrusion, at least under certain conditions. In the majority of the studies reviewed here Mg^{2+} extrusion has been measured by atomic absorbance spectrophotometry, an approach that measures net mass change in Mg^{2+} content in the extracellular compartment under conditions in which external Mg^{2+} concentration is maintained sufficiently low to enhance signal versus noise ratio. A limit of this approach is that it cannot distinguish whether the observed changes depend on an enhanced Mg^{2+} extrusion or an inhibition of Mg^{2+} accumulation with or without changes in efflux rate. Similarly, it is unclear whether the increase in total cellular Mg^{2+} content observed under certain stimulatory conditions results from an enhanced Mg^{2+} uptake or an inhibition of Mg^{2+} extrusion with or without modifications in accumulation rate.

While at the whole body level the renal apparatus represents the major site where hormones operate to regulate Mg^{2+} reabsorption and, consequently, normomagnesemia (165-169), an increasing number of reports indicate that various hormones can change total and free Mg^{2+} content inside various cell types within minutes of application of the stimulus. Insulin, carbachol and vasopressin are among the hormones most commonly used to induce an increase in Mg^{2+} content within cells. One of the possible mechanisms by which these agents can induce Mg^{2+} accumulation is by decreasing cellular cAMP level (146-148), thereby reinforcing the notion that this second messenger plays a key role in modulating cellular Mg^{2+} homeostasis.

Data from our laboratory (141,170) indicate that in addition to preventing the beta-adrenergic receptor-induced cAMP-mediated Mg^{2+} extrusion in cardiac (170) and liver cells (141), insulin also induces a Mg^{2+} accumulation in 3T3 fibroblasts (171), cardiac myocytes (170), and pancreatic beta cells (172). Vasopressin (140,173-175) and angiotensin-II (175) also induce Mg^{2+} accumulation within cells. The effect of vasopressin has been documented in hepatocytes (82,140), in which it induced an accumulation of Mg^{2+} *via* a Na^+-dependent mechanism. A similar effect has been observed in renal epithelial cells (173) and in smooth muscle cells from mesenteric artery (174,175). Overall, vasopressin stimulation results in a net increase in total Mg^{2+}, measured by atomic absorbance spectrophotometry (82,140), and in cytosolic free Mg^{2+}, measured with the fluorescent dye Mag-Fura (173-175). The increase in cytosolic free Mg^{2+} induced by vasopressin is transient (174), the initial increase being followed by a decrease within 2 minutes from application of the hormone. Because the determination of total cellular Mg^{2+} by atomic absorbance spectrophotometry indicates, at least in other cell types, an increase in Mg^{2+} content that lasts for 5-6 minutes (82,140,176), it is conceivable that the decline in cytosolic free Mg^{2+} observed by Touyz and Schiffrin (174) represents a redistribution of Mg^{2+} from the cytosol into an intracellular compartment(s). In addition to bombesin (171), an increase in cytosolic free Mg^{2+} concentration has been observed in pancreatic islets (172), cardiac ventricular myocytes (88) and sublingual mucosa acini (61) following stimulation by carbachol

5.5. Involvement of protein kinase C in Mg^{2+} accumulation

The inhibitory effect of insulin (141,170), vasopressin (146,148) or carbachol (177) on adenylyl

cyclase/cAMP pathway mentioned above does not exclude a stimulatory effect of these agents on other signaling pathways within the cell, primarily protein kinase C, which represents the *alter ego* of protein kinase A for many functions within the cell. Supporting evidence for a role of protein kinase C in mediating Mg^{2+} accumulation is provided by a series of reports from various laboratories (178-180), including ours (176), which indicate that Mg^{2+} accumulation can occur to a similar extent in cells stimulated by diacylglycerol analogs or phorbol myristate acetate analogs, all agents that can cross the cell membrane to directly activate protein kinase C (181). In contrast, the inhibition of protein kinase C by calphostin (174) or staurosporine (158) or its down-regulation by exposure to a supra-maximal dose of phorbol myristate acetate (158), are all conditions that result in the abolishment of Mg^{2+} accumulation within the cell. Support to a role of PKC in Mg^{2+} accumulation is also provided by observation from Altura's laboratory (182) and our laboratory (183) indicating that alterations in PKC activity or distribution between cytoplasm and membrane fraction are responsible for the defective accumulation of Mg^{2+} in arterial smooth muscle cells of alcohol treated animals (182), or in liver cells isolated from rats fed with alcohol for three weeks (183,184) or animals rendered diabetic by streptozotocin injection (164).

Because Ca^{2+} signaling is part of the integral response of vasopressin or angiotensin-II receptor activation, it is not immediately clear the role, if any, this cation plays in modulating Mg^{2+} uptake. The loading of hepatocytes with BAPTA-AM, an intracellular Ca^{2+} chelating agent, not only inhibits Mg^{2+} extrusion (152) but also prevents the Mg^{2+} accumulation induced by vasopressin or phorbol myristate acetate (82). The artificial increase in cytosolic Ca^{2+} elicited by thapsigargin treatment also prevents Mg^{2+} accumulation (82) and actually induces a Mg^{2+} extrusion from the liver cell when applied for a sufficiently long time (3-5 min) (82-152). The different time-scale and amplitude of the changes in cellular Ca^{2+} and Mg^{2+} content (185,186) make it difficult to properly correlate these experimental variations. Cytosolic free Ca^{2+} transiently increases several orders of magnitude while cytosolic free Mg^{2+}, which is already in the millimolar or submillimolar range, increases by ~10-15% (187), at the most, although in absolute terms this amount is far larger than the overall change in cytosolic Ca^{2+} mass.

An apparent inconsistency in a role Ca^{2+} and protein kinase C in regulating Mg^{2+} accumulation is provided by the reports that the administration of phenylephrine, which also activates protein kinase C signaling in addition to the inositol 1,4,5 trisphosphate/Ca^{2+} pathway, does not elicit Mg^{2+} accumulation but rather a Mg^{2+} extrusion from liver cells (152). This point rises the question as to what modulates the differential response of the cell to the administration of phenylephrine or vasopressin. One possibility could be that different isoform(s) of protein kinase C are activated under one condition and not the other. The hepatocyte possesses 3 classical protein kinase C isoforms and at least 2 novel protein kinase C isoforms (188). Thus, it is reasonable to envisage that one isoform (or class of isoforms) is involved in mediating Mg^{2+} accumulation whereas another isoform (or class of isoforms) is involved in modulating Mg^{2+} extrusion. Alternatively, it can be postulated that an additional signaling pathway(s) is/are involved in determining the differing response of the hepatocyte under apparently similar stimulatory conditions.

The characterization of PKC signaling is far from being completed. The mechanism ultimately responsible for Mg^{2+} accumulation into the cell has not been characterized nor is clear which PKC isoform(s) is involved in the process and whether this isoform exerts its effect directly or indirectly. In agreement with previous report from Altura's group (189) and Touyz's laboratory (190), we have recently provided evidence that pharmacological inhibition of ERK1/2 and p38 in liver cells abolishes PKC mediated Mg^{2+} accumulation (191). In this respect, it has to be noted that the inhibition of Mg^{2+} accumulation in vascular smooth muscle cells via MAPKs-regulated mechanism significantly affect the ability of the cells to properly progress in the cell cycle (190). This effect may occur via changes in nuclear functions directly regulated by Mg^{2+}, as proposed by Rubin (192) and/or via changes in nuclear functions of ERK2, which depends on Mg^{2+} level to properly dimerize, translocate and activate specific nuclear targets (193). The role of ERK1/2 in Mg^{2+} regulation, however, is far from being elucidated as this kinase appears to be involved to some extent in mediating also Mg^{2+} extrusion (191,194).

5.6. Presence of Mg^{2+} sensing mechanism?

As mentioned previously, the experimental evidence that mammalian cells accumulate or extrude Mg^{2+} under a variety of experimental conditions suggests the presence of a *sensor* for the cytosolic Mg^{2+} concentration, whereby the cell would operate accordingly either extruding the excess cation or accumulating it to restore the 'set-point'. Compelling evidence for the presence of such a *sensor* mechanism is provided by the reports by Quamme and his group. This group has reported that prolonged exposure to 0 mM $[Mg^{2+}]_o$ decreases cytosolic *free* Mg^{2+} concentration by approximately 50% in cardiac ventricular myocytes (195), MDKC cells (196) or MDCT cells (197-200). The new cytosolic Mg^{2+} level is maintained as long as the cells are incubated in the presence of 0 mM $[Mg^{2+}]_o$, but as soon as $[Mg^{2+}]_o$ is increased cytosolic free Mg^{2+} concentration returns to the normal level (195,196) in a frame of time that is directly proportional to the extracellular Mg^{2+} concentration utilized (196,197). The process occurs within a few minutes, and is prevented by the presence of the L-type Ca^{2+}-channel inhibitors verapamil or nifedipine, or La^{3+} in the extracellular milieu (195-197). Under these experimental conditions, no significant changes in cytosolic free Ca^{2+} concentration are observed, thus suggesting a direct effect of these inhibitory agents on Mg^{2+} entry mechanism (195-200). This observation led the authors to propose the operation of a Mg^{2+} channel in these cells. This possibility is significantly strengthened by the observation from Fleig *et al.* (108) that a specific Mg^{2+} current can be detected in TRPM7 and

possibly TRPM6 (122) channels under well defined conditions (see Section 3.4).

Mg^{2+} accumulation is also induced by ion redistribution. In fact, renal epithelial cells accumulate Mg^{2+} as a result of phosphate (198) or potassium (199) redistribution across the cell membrane. The dependence of Mg^{2+} accumulation on K^+ redistribution across the plasma membrane suggests that Mg^{2+} transport is the result of changes in membrane potential, possibly for charge compensation (201-203). In this respect, it is interesting to note that pathological conditions in which tissue Mg^{2+} content is markedly reduced (*e.g.*, diabetes) (164) are also characterized by an inability to properly transport potassium (204,205). Whether the K^+ effect occurs through changes in membrane potential or indirectly via Na^+/K^+ countertransport by the Na^+/K^+-ATPase coupled to the reverse operation of the Na^+/Mg^{2+} exchanger (74,83) is matter for future investigation.

6. CHANGES IN SERUM Mg^{2+} LEVEL

Humans and many mammals present a circulating Mg^{2+} level of ~1.2-1.4 mEq/L (19,165,206). Clinical and experimental evidence indicates that serum Mg^{2+} level decreases in humans and animals (205 -210) are associated with several chronic diseases. Yet, there is a remarkable lack of information as to whether serum Mg^{2+} undergoes circadian fluctuations following the physiological release of hormones or following non-hormonal stimuli (*e.g.*, fasting or exercise). The infusion of catecholamine in conscious humans (209,210) or ovine (211) for a period of time varying between 30 min to 5 h has resulted in a varying level of hypomagnesemia and in a marked excretion of Mg^{2+} in the urine. Contrasting results have also been obtained in anesthetized rats infused with isoproterenol (212) or epinephrine in the presence of α_1-adrenergic receptor blockade (211). While previous work has indicated negligible changes in serum Mg^{2+} level (211), more recent results indicate that isoproterenol infusion elicits in a marked dose- and time-dependent increase in circulating Mg^{2+} content (212, 213). The increase is serum Mg^{2+} is maximal within 20 min after the agent administration (212, 213), and remains unchanged up to 2 h even following the removal of the agonist (212). This time course indicates that the increase in serum Mg^{2+} is independent of the hemodynamic changes (*i.e.*, increase in heart rate and decrease in mean arterial pressure) elicited by the β-adrenergic agonist (213). In fact, the infusion of sodium nitroprusside does not affect serum Mg^{2+} level despite the fact it mimics the decrease in mean arterial pressure induced by isoproterenol (213). The persistence of an elevated serum Mg^{2+} level for up to 2 h also implies that operation of secondary and not fully elucidated mechanisms activated by the stimulation of β-adrenergic receptor. Consistent with the larger distribution of β_2 *versus* β_1 adrenergic receptors in the whole body (214, 215), the increase in serum Mg^{2+} occurs *via* the specific activation of β_2-adrenergic receptor, as the stimulation by the selective β_2-adrenergic agonist salbutamol and the inhibition by the specific β_2-antagonist ICI-118551 demonstrate (213). Consequently, the increase of Mg^{2+} into the bloodstream observed under these experimental conditions is most likely to be the result of a stimulatory effect of the adrenergic agent on more than one tissue (213). The involvement of bone has been proposed by Günther and co-workers (212) based upon their observation that infusion of carbonic anhydrase inhibitor in an anesthetized rat can prevent Mg^{2+} mobilization into the blood following isoproterenol administration. Changes in renal excretion do not appear to contribute significantly to the increase in serum Mg^{2+} level, at least during the first two h following adrenergic agonist infusion. Based upon the pre-infusion level of serum Mg^{2+}, the glomerular filtration rate (1.62 mL/min (19,216)) and the fractional excretion (17% (216)), Keenan *et al.* (213) have estimated that only one-third of the increase in serum Mg^{2+} level observed at 20 min would occur in the case of a total block of the renal fractional excretion. More difficult to assess is the extent to which a reduced glomerular filtration rate, together with a β-adrenergic stimulated cAMP-mediated increase in Mg^{2+} reabsorption at the level of the thick ascending portion of the loop of Henle (217) would contribute to the persisting elevated level of circulating Mg^{2+} up to 2 h after the end of agonist infusion (212). It is interesting to note, however, that hormones that stimulate Mg^{2+} extrusion from different organs or tissues, thereby increasing plasma Mg^{2+}, also increase Mg^{2+} reabsorption at the renal level, de facto preventing a net loss of the cation.

The discrepancy between absence of changes in serum Mg^{2+} level obtained in the earliest studies as compared to the significant increase in serum Mg^{2+} levels reported in the most recent studies does not have a clear explanation. Several factors may contribute to this incongruity, including the relative proportion of β-adrenergic receptor subtypes in different experimental models, the ability of catecholamines and isoproterenol to stimulate with differing hierarchy α- and β-adrenergic receptors or distinct β-adrenergic receptor subtypes, and modality, rate and duration of drug infusion.

Despite significant advance in uncovering how tissue and whole body Mg^{2+} homeostasis is regulated, the physiological significance of the increase in serum Mg^{2+} level following catecholamine infusion still remains unclear. The observation that serum Mg^{2+} level increases under certain conditions implies that specific organs or tissues have the ability to *sense* these changes and act accordingly. No specific Mg^{2+} sensing mechanism has been identified. However, the Ca^{2+} sensing receptor (218) not only regulates calcemia, but also senses changes in circulating Mg^{2+} level in a range of concentrations higher than those of Ca^{2+} (219) and consistent with the reported increase in serum Mg^{2+} level (212,213). In addition, the observation by Bapty *et al.* (220) that in mouse distal convoluted tubule cells (MDCT) the Ca^{2+}-sensing mechanism operates with comparable sensitivity for both extracellular Ca^{2+} and Mg^{2+} suggests interesting hypotheses in terms of whole body physiology. The activation of this sensor mechanism would inhibit glucagon- or vasopressin-mediated entry of Mg^{2+} into the cell (221) and favor the elimination of the cation with the urine. Consequently, this

would explain the clinical and experimental evidence that hypermagnesemia and hypercalcemia inhibit hormone-stimulated cAMP-mediated reabsorption of Mg^{2+} and Ca^{2+} along the different segments of the nephron (222). It would also represent a distal regulatory mechanism to restore circulating Mg^{2+} to a physiological level following the increase observed in anesthetized animals infused with adrenergic agonists (212,213).

In physiological terms, a striking difference between an increase in serum Mg^{2+} and Ca^{2+} content is that increases in calcemia are associated with muscle weakness and arrhythmia whereas similar variations in serum Mg^{2+} content appear to be well tolerated *in vivo*. Rats infused with boluses of Mg^{2+} that increase serum Mg^{2+} level by 50% do not manifest significant systemic hemodynamic changes but induce a marked increase in coronary artery flow (223). Baboons infused with pharmacological doses of Mg^{2+} sufficient to prevent epinephrine-induced cardiac arrhythmias show a significant attenuation of the epinephrine-induced increase in mean arterial pressure and systemic vascular resistance (224). Studies *in vitro* suggest that an elevated level of $[Mg^{2+}]_o$ can regulate catecholamine release from peripheral and adrenal sources (225) and modulate cardiac contractility (41). Taken together, these observations indicate that Mg^{2+} can play a key role as an endogenous modulator of catecholamine release and activity. They also suggest that an increase in circulating Mg^{2+} following adrenergic stimulation can contribute to improved blood flow and O_2 delivery to the heart and possibly other tissues as well at a time when an increase in energy production is expected

7. Mg^{2+} AS AN INTRACELLULAR MESSENGER

The conclusions presented in the previous sections support the well recognized role for Mg^{2+} as an indispensable component for enzymes, phosphometabolites, and channel activity (1,226). Several glycolytic enzymes, including hexokinase, phosphofructokinase, phosphoglycerate mutase, phospho-glycerate kinase, enolase and pyruvate kinase, show activation at low and inhibition at high Mg^{2+} concentrations (58,227). An additional example of a key signaling pathway regulated by Mg^{2+} is the adenylyl cyclase, as work by Maguire and coworkers indicates (reviewed in 228). The Mg^{2+} concentrations at which these processes occur (0.5-1 mM) are well within the fluctuations in free Mg^{2+} content measured in the hepatocyte (10,227). With the exception of the glycolytic enzymes, studies attempting to evidence a signaling role of Mg^{2+} for cytosolic enzymes have been disappointing, especially because of the underlying assumption that Mg^{2+} would operate as Ca^{2+} in modulating enzyme activity. At variance from Ca^{2+}, Mg^{2+} concentration in the cytoplasm and extracellular fluids is in the millimolar or submillimolar range. Consequently, an increase or decrease in cytosolic Mg^{2+} level equivalent to the changes occurring for Ca^{2+} will go largely undetected by the common fluorescent or ^{31}P-NMR techniques. Heretofore, a role of Mg^{2+} as transient regulator of cytosolic enzymes would appear to be unlikely. Lastly, even under conditions in which major fluxes of Mg^{2+} cross the cell plasma membrane in either direction following hormonal and non-hormonal stimuli, resulting in massive translocations that increase or decrease total cellular Mg^{2+} by approximately 1-2mM (or 5-10% of total cell content), minor or no changes in cytosolic *free* Mg^{2+} content have been detected (88). The absence of significant changes in cytosolic *free* Mg^{2+} despite major changes in total cellular Mg^{2+} release or uptake can be explained by assuming that the source or destination of transported Mg^{2+} is a cellular compartment or organelle, or a major binding site, or that Mg^{2+} is highly buffered. Hence, regulation of cellular functions by Mg^{2+} should be expected to occur not in the cytosol but within organelles (or binding sites), and in the plasma, where Mg^{2+} concentration can rapidly increase or decrease more than 20% (212,213).

In the following pages we will focus mostly on what is known about regulatory effects of extracellular or intracellular Mg^{2+} on the operation of cation channels in the plasma membrane, as well as on mitochondrial metabolic parameters such as respiration and volume following changes in Mg^{2+} concentration within the organelle.

7.1. Mg^{2+} as a regulator of Ca^{2+}- and K^+-channels

White and Hartzell were first to indicate a regulatory effect of intracellular free Mg^{2+} on calcium channels (229). These authors reported that increasing intracellular free Mg^{2+} concentration from 0.3 to 3.0 mM in cardiac myocytes by internal perfusion has a small effect on the basal calcium current (I_{Ca}) of L-type Ca^{2+}-channels. In contrast, Mg^{2+} decreased by more than 50% the amplitude of I_{Ca} elevated by cAMP-dependent phosphorylation. The effect of Mg^{2+} was not due to changes in cAMP concentration or in the velocity of phosphorylation but appears to be a direct effect on the phosphorylated channel or on the channel dephosphorylation rate (229). Similar results were also reported by Agus and Morad in guinea pig cardiac myocytes (reviewed in 230). The block induced by Mg^{2+}on the Ca^{2+} current appears to be due to a direct effect on the steady-state inactivation of the channel, as the block persists in the presence or absence of cAMP and is not reversed by elevation of extracellular Ca^{2+} concentration or addition of catecholamine (230). Magnesium effects on Ca^{2+}-channels are not restricted to cardiac cells or to an action on the intracellular site. Bara and Bara have shown that $MgCl_2$ (and to a lesser extent $MgSO_4$) by acting at an extracellular site on L-type Ca^{2+}-channel regulates the influx of Ca^{2+} influx through voltage-gated Ca^{2+} channels in vascular smooth muscle cells and endothelial cells in human allantochorial placenta (231), thus modulating the tonus of the vessels. Evidence for a similar block by extracellular Mg^{2+} on T-type Ca^{2+}-channels has recently been provided by Serrano *et al.* (232). The modulatory effect of Mg^{2+} appears to take place at the EF-hand motif of the carboxy terminus of $Ca_v1.2$ channels as recently evidence by Catterall and his group (233).

Extracellular Mg^{2+} has also a modulatory role on the activity of store-operated Ca^{2+} channels. Studies in intact, pressurized rat mesenteric arteries with myogenic tone indicate that 10 mM extracellular Mg^{2+} can mimic

nifedipine in preventing or reversing the vasoconstriction elicited by phenylephrine administration, but not the contractions induced by K^+ depolarization (234). Thus, Mg^{2+} appears to be able to abolish the vasoconstriction attributed to Ca^{2+} entry through store-operated channels, which are activated following phenylephrine administration, and contribute to maintain both myogenic tone and α_1-adrenergic receptor-induced tonic vasoconstriction. While it remains to be assessed to which extent this Mg^{2+} effect occurs at more physiological extracellular concentrations, these data may help to elucidate some of the modifications that occur under hypertensive conditions, in which a decrease in plasma Mg^{2+} has often been reported.

Lastly, an effect of intracellular Mg^{2+} on the operation of the store-operated calcium release-activated Ca^{2+} (CRAC) channels has also been reported. CRAC channels are highly Ca^{2+}-selective under physiological ionic conditions whereas removal of extracellular divalent cations makes them freely permeable to monovalent cations, in particular Na^+. Experimental evidence had indicated that intracellular Mg^{2+} can modulate their activity and selectivity. While an effect of intracellular Mg^{2+} on CRAC channels cannot be completely excluded, a recent report by Prakriya and Lewis (235) would indicate that the channels modulated by intracellular Mg^{2+} are not CRAC channels, but a different class of channels that open when Mg^{2+} is washed out of the cytosol. These channels have therefore been termed Mg^{2+}-inhibited cation (MIC) channels, as they present distinctive functional parameters in terms of inhibition, regulation, ion permeation and selectivity (235). Overall, this new information has revealed intriguing similarities as well as differences in permeation mechanisms of voltage-gated and store-operated Ca^{2+} channels.

Potassium channels are also targets for Mg^{2+}. Matsuda (236) has reported that the presence of Mg^{2+} on the cytoplasmic side of the inwardly rectifying K^+ channel blocked the outward currents without affecting the inward currents. The Mg^{2+} block was achieved at a half-saturation concentration of 1.7 μM. When the Mg^{2+} concentration was elevated to 2-10 μM, the outward current fluctuated between two intermediate sublevels in addition to the fully open and closed configuration. However, these concentrations of Mg^{2+} are far from being physiological and it is difficult to envision the occurrence of similar regulatory effects under normal conditions unless Mg^{2+} microcompartmentation is invoked. A regulatory role of intracellular Mg^{2+} on K_v channels in vascular smooth muscle cells has been observed by Tammaro *et al.* (237). These authors have observed that an increase in intracellular Mg^{2+} in a range consistent with the physiological variation of the cation can slow down the kinetic of activation of the Kv channel, cause inward rectification at positive membrane potentials, and shift the voltage-dependent inactivation (237), thus representing a novel mechanism for the regulation of this channel in the vasculature. Intracellular Mg^{2+} also modulates large-conductance (BK type) Ca^{2+}-dependent K^+ channels by either blocking the pore of BK channels in a voltage-dependent manner, or by activating the channels independently of Ca^{2+} and voltage changes by preferentially binding to the channel open conformation at a site different from Ca^{2+} sites. Interestingly, Mg^{2+} may also bind to Ca^{2+} sites and competitively inhibit Ca^{2+}-dependent activation (238).

The inhibitory effect of Mg^{2+} is not restricted to cell membrane channels. Experimental evidence by Bednarczyk *et al.* (239) indicates that Mg^{2+} in the mitochondrial matrix can modulate gating and conductance of mitochondrial K_{ATP} channels, which play a key role under ischemia/reperfusion conditions.

7.2. Mg^{2+} as a regulator of mitochondrial dehydrogenases

Mitochondria represent one of the major cellular pools for Mg^{2+}, its concentration being 14-16 mM (3,14,20), and circumstantial evidence from this (70,82,140) and other laboratories (61, 240) has suggested that Mg^{2+} can be mobilized from mitochondria following hormone-mediated increase in cytosolic cAMP level through a mechanism(s) that has not been fully elucidated. Several reviews have analyzed in detail how Mg^{2+} homeostasis is regulated in the organelle (1,3,20), and we refer to those reviews for further information. In this section, instead, we will indicate some recent evidence about a role of intra- and extra-mitochondrial Mg^{2+} on the activity of specific mitochondrial proteins.

While it is commonly accepted that changes in matrix Ca^{2+} can affect the rate of mitochondrial dehydrogenases and therefore the rate of respiration (241,242), limited evidence is currently available about a similar role for Mg^{2+}. Yet, the activity of several mitochondrial dehydrogenases increases within minutes despite the absence of a detectable increase in mitochondrial Ca^{2+} (243,244). In addition, mitochondrial Mg^{2+} content significantly changes during transition between state 3 and state 4 (245), affecting at the same time the amplitude of mitochondria respiration. Lastly, the activity of purified α-ketoglutarate dehydrogenase is regulated *in vitro* by both Ca^{2+} and Mg^{2+} (246).

The role of matrix Mg^{2+} as regulator of dehydrogenases and consequently mitochondrial respiration has been investigated by measuring the activity of several dehydrogenases in mitochondria under conditions in which matrix Ca^{2+} and/or Mg^{2+} concentration were varied. From those data, it appears that α-ketoglutarate dehydrogenase and pyruvate dehydrogenase are not regulated by changes in mitochondrial Mg^{2+}, whereas Mg^{2+} removal increases several fold the activity of succinate and glutamate dehydrogenases (247). This evidence would therefore indicate that changes in matrix Mg^{2+} content (in combination with or in alternative to changes in mitochondrial Ca^{2+}) can control mitochondria respiration, at least under well defined conditions. In a broader perspective, the effect of catecholamine on increasing respiration could be explained in part by a cAMP-mediated modulation of mitochondrial Mg^{2+}, which - in turn - will stimulate directly some dehydrogenases while rendering

others more susceptible to the Ca^{2+} concentrations present in the mitochondrial matrix.

The regulatory effect of Mg^{2+} on mitochondrial function is not restricted to the dehydrogenases activity but encompasses also the organelle volume. This effect is achieved *via* direct regulation of the K^+/H^+ antiporter (see ref. 1 for more detail) and anion channel present in the mitochondrial membrane (248) and by indirectly modulating the opening of the permeability transition pore (249). The mitochondrial inner membrane anion channel (IMAC) transports various anions and is involved in regulating the organelle volume in conjunction with the K^+/H^+ antiporter. Although its fine regulation is still not completely elucidated, evidence has been provided that this channel is inhibited by matrix Mg^{2+} and proton, the process being required to keep the channel closed and/or limit its activity under physiological conditions (248). Kinetic studies by Beavis and collaborators suggest that the main role of Mg^{2+} is to maintain the channel in a condition that would allow fine modulation by small changes in pH and proton distribution under physiological conditions.

As for the permeability transition pore (PTP), this structure is a proteinaceous pore that opens in the inner mitochondrial membrane following a marked decrease in membrane potential and results in the rapid equilibration and redistribution of matrix and extramitochondrial solutes down their concentrations gradient. While it is still debated exactly which proteins participate and how the pore opens, it is well established that an increase in mitochondrial Ca^{2+} content facilitates the opening whereas an increase in mitochondrial Mg^{2+} antagonizes it. Recently, Wallimann and collaborators have provided evidence about a new and indirect role of Mg^{2+} in modulating PTP opening (249). These authors have shown that creatine kinase can regulate the opening of the permeability transition pore by tightly associating to the mitochondrial membrane and remaining in an active state. Both these processes are Mg^{2+} dependent, in that removal of Mg^{2+} from the extramitochondrial environment results in a decline in creatine kinase activity and opening of the permeability transition pore (249).

Considering the effect of Mg^{2+} on mitochondrial function, including the operation of specific channels (*Section 6.1*), it would appear that Mg^{2+} plays more than one role within this organelle by regulating mitochondrial volume, ion composition, ATP production and metabolic interaction with the hosting cell .

8. CONCLUSIONS

In the last 3-4 years, our understanding of cellular and whole body Mg^{2+} homeostasis has significantly advanced. While it still lags behind as compared to the knowledge available for other ions such as Ca^{2+}, H^+, K^+ or Na^+, yet the identification of putative Mg^{2+} channels and transport mechanisms in the membrane of the cell or mitochondria, as well as an improved understanding of the signaling pathways and conditions regulating Mg^{2+} transport is providing new tools to address essential questions about the physiological role Mg^{2+} plays inside the cell and in the whole body.

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