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REGULATION OF CELLULAR MAGNESIUM

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1. ABSTRACT

The abundance of magnesium (Mg^{2+}) within mammalian cells is consistent with its relevant role in regulating tissue and cell functions. At the last count, more than three hundred and fifty enzymes, aside from metabolic cycles, appear to require and be regulated by concentrations of Mg^{2+} that are well within the physiological range observed in tissues and cells. The absence of detectable major changes in cellular free [Mg^{2+}], and the extremely slow turn-over of the cation across the cell plasma membrane under quiescent condition has supported for more than three decades the assumption that cellular Mg^{2+} content is kept constant at the level necessary for enzyme and channel function, and that its concentration does not require drastic and rapid changes to form complex with ATP and other phosphonucleotides.

In the last decade, a large body of new experimental observations has significantly reverted this way of thinking. Compelling evidence now suggests that large fluxes of Mg^{2+} can cross the cell plasma membrane in either direction following a variety of hormonal and non-hormonal stimuli, resulting in major changes in total and, to a lesser extent, free Mg^{2+} content within tissues, and in a marked variation in the opposite direction of circulating Mg^{2+} level. The present review will attempt to update our knowledge in this area and provide some insights on how changes in cellular Mg^{2+} content can result in a modification of the activity rate for several cellular enzymes.

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

For many decades the role of Mg^{2+} in biological systems has been hampered by the difficulty of measuring accurately and selectively Mg^{2+} in cell and biological system. This has been partly ameliorated by the introduction of atomic absorbance spectrophotometry in the early 1950s and, more recently, by additional analytical methods.

Mg^{2+} is now recognized being indispensable for enzyme activity and structural modification of phosphometabolites or channels. Yet, the general consensus from a large body of evidence indicates that Mg^{2+} concentration is relatively stable within the cell and that whilst Mg^{2+} presence is necessary for cell function, it will not modulate - like Ca^{2+} - cell function by changing concentrations within the cytsosol. In fact, studies attempting to equate Ca^{2+} and Mg^{2+} as signaling molecules for cytosolic enzymes have been disappointing. Ca^{2+} is a signaling molecule because of the following conditions: a) the free cytosolic concentration is extremely low; b) the concentrations in plasma and cytosolic reservoirs are very high, establishing a large concentration gradient across biological membranes; c) because of the low resting Ca^{2+} concentration, the movement of a few Ca^{2+} molecules can increase or decrease the concentration of Ca^{2+} by several orders of magnitude; d) the increase of Ca^{2+} concentrations results in specific Ca^{2+} binding to cytosolic proteins which are modified in their 3-D structure and function upon formation of a complex with Ca^{2+}.

These paradigms of operation are very different for Mg^{2+}. Mg^{2+} is kept both in the cytosol and in extracellular fluids in the millimolar or submillimolar concentration. Because of this initial large concentration, a total increase or decrease of Mg^{2+} in the cytosol equivalent to that occurring for Ca^{2+} will result in negligible free Mg^{2+} changes. Furthermore, due to the large difference between

the radius of hydrated and not hydrated Mg^{2+}, specific coordination to proteins is less likely than that of Ca^{2+}.

For the above reasons, the role of Mg^{2+} as transient regulator of cytosolic enzymes appears to be intrinsically denied by the coordination chemistry, the concentrations existing in the cytosol, and by experimental evidence. Nevertheless, fluxes of Mg^{2+} across the cell plasma membrane have been recently measured, leading to massive translocations that increase or decrease total cellular Mg^{2+} by an equivalent of 1-2 mM (approximately 5-10% of total cell content) within a few minutes. Yet, these fluxes result in minor or no changes in cytosolic free Mg^{2+} content.

Changes in total cellular Mg^{2+} in the absence of major changes in cytosolic free Mg^{2+} can only be explained assuming the fulfillment of the following tenets:

a) When massive total cellular Mg^{2+} release or uptake occurs, the source or destination of mobilized Mg^{2+} must be an intracellular compartment or a major binding site;

b) The plasma membrane must possess a *sensor* (which keeps cytosolic free Mg^{2+} relatively constant in spite of massive redistribution of the total) and/or a powerful *uptake/release mechanism* (which extrudes or accumulates the large amount of Mg^{2+} from the cell to the extracellular fluid, or viceversa, to maintain cytosolic free Mg^{2+} constant).

Hence, at variance from Ca^{2+}, regulation of cellular functions by Mg^{2+} should be expected to occur not in the cytosol but within organelles (or binding sites), where Mg^{2+} is being mobilized, and in the plasma, where Mg^{2+} concentration can rapidly increase or decrease more than 20%.

In the following pages we will focus mostly on what is known about hormonal modulation of extracellular Mg^{2+} concentration and fluxes of Mg^{2+} across the plasma membrane, and on the possible regulation by Mg^{2+} of metabolic parameters such as respiration, following changes of the cation within organelles.

3. CHANGES IN SERUM MG^{2+} LEVEL

Circulating Mg^{2+} level is 1.5-1.7 mEq/L in humans and in many mammals (1-3). A decrease in serum Mg^{2+} level has been reported to occur during several chronic diseases, both in humans and in animals (4-6). Yet, there is a remarkable lack of information, or contrasting result, as to whether magnesemia undergoes circadian fluctuations following the release of hormones or physiological stimuli (e.g. fasting or exercise). Studies conducted in conscious humans (7) or ovine (8) infused with catecholamine for a period of time varying between 30 min to 5 hours resulted in a varying level of hypomagnesemia and in a marked increase of Mg^{2+} excretion in the urine. In contrast, minimal or not changes in serum Mg^{2+} level were found in rats infused with epinephrine in the presence of alpha-adrenergic receptor blockade (9), or with isoproterenol (10), respectively.

This and other laboratories have reported that the administration of isoproterenol, epinephrine or norepinephrine to perfused rat hearts (11-13) and livers (14,15) results in an extrusion of Mg^{2+} from the organs into the perfusate. Consistent with this observation, the infusion of increasing doses of isoproterenol to anesthetized rat results in a marked dose- and time-dependent increase in circulating Mg^{2+} level. The increase is serum Mg^{2+} is already detectable within 10 min, reaches the maximum within 20 min after the agent administration (10,16), and remains unchanged up to 2 hours even in the absence of the agonist (10). This time course suggests that the increase in serum Mg^{2+} occurs independently of the hemodinamic changes (i.e. increase in heart rate and decrease in mean arterial pressure) induced by the beta-adrenergic agonist for the limited time of the infusion (16). Additional support to this hypothesis is provided by the inability of sodium nitroprusside to significantly change serum Mg^{2+} level despite the fact it can mimic the decrease in mean arterial pressure induced by isoproterenol (16). The persistent increase in serum Mg^{2+} also implies that the stimulation of beta-adrenergic receptor results in the activation of secondary mechanism(s) which account for the long-term persistence of this phenomenon. At its peak, the increase accounts for a net change of ~7 micromol Mg^{2+}/300 g b.w., or 10% above basal level, for an infused dose of 0.1 microgram isoproterenol/kg/min, and ~10 micromol Mg^{2+}/300 g b.w., or 20% above basal level, for a dose of 10 microgram/kg/min. This increase occurs via the specific activation of $beta_2$-adrenergic receptor. It can be mimicked by the administration of the selective $beta_2$-adrenergic agonist salbutamol, and inhibited by the specific $beta_2$-blocker ICI-118551, and not by the $beta_1$-adrenergic agonist prenalterol or the $beta_1$-blocker CGP-20712A, respectively (16). Most likely, this difference is attributable to the larger distribution of $beta_2$ versus $beta_1$ adrenergic receptors present in the all body (17,18), rather than to a different signaling pathway.

Based upon the observed percent change in serum Mg^{2+}, it can be estimated that the circulating level of the cation would increase from 0.75-0.8 mM to ~0.9-1 mM (16) as a result of tissue release into the bloodstream. Yet, the attempt to determine from which tissue(s) Mg^{2+} is mobilized into the circulation has not provided a conclusive answer (16). Because of the inhibitory effect of carbonic anhydrase inhibitor infused in an anesthetized rat, Gunther and co-workers (10) have proposed that bones may represent the primary source of Mg^{2+} mobilization following isoproterenol administration. However, as $beta_2$-adrenergic receptors are largely distributed throughout all the organs, it cannot be excluded that other tissues contribute, to a varying extent, to the observed increase in circulating Mg^{2+} level. It is worth to note that changes in renal excretion do not appear to contribute significantly to determine the initial increase in serum Mg^{2+} level. Based upon the pre-infusion level of serum Mg^{2+}, the glomerular filtration rate (1.62 mL/min (19)) and the fractional excretion (17% (19)), it can be 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 (16). More difficult to assess is the extent to which a reduced glomerular filtration, together with a beta-adrenergic stimulated, cAMP-mediated, increase in Mg^{2+} reabsorption at the level of the thick ascending portion of Henle's loop (20) contribute to the persisting elevated level of circulating Mg^{2+} up to 2 hours after the end of agonist infusion (10).

As expected, the infusion of epinephrine or norepinephrine to an anesthetized rat results in an increase in circulating Mg^{2+} of approximately 20%, comparable to that induced by isoproterenol. In this case, however, the increase occurs via activation of both alpha- and beta-adrenergic receptors (21), as it can be prevented by the use of pharmacological inhibitors for these two classes of adrenergic receptor (21). More interesting, however, is the observation that the administration of a bolus of insulin in the absence of glucose clamp induces a marked hypoglycemia which is followed, within 5-10 min, by an increase in serum Mg^{2+} that resembles, in onset and extent, that induced by catecholamine infusion (21). Because the increase in serum Mg^{2+} prompted by insulin does not occur in animals pre-treated with alpha- and beta-adrenergic receptor blockers, or with reserpine (21), it can be reasonably concluded that the increase in serum Mg^{2+} under these experimental conditions is attributable to the release of endogenous catecholamines triggered by hypoglycemic condition.

The discrepancy between the earliest studies, in which changes in serum Mg^{2+} were minimal or absent, and the most recent observations showing a considerable increase in magnesemia is not easy to explain. Several factors may contribute to the mentioned incongruity. The relative proportion of beta-adrenergic receptor subtypes in different experimental models, the ability of catecholamines and isoproterenol to stimulate with differing hierarchy alpha- and beta-adrenergic receptors or distinct beta-adrenoceptor subtypes, modality, rate and duration of drug infusion are but a few of the possibilities to be considered.

Although the most recent observations suggest that circulating Mg^{2+} level can increase following the release of endogenous catecholamine or their exogenous infusion, likely through the activation of common mechanism(s), the physiological significance of this increase still remains elusive. Also, it remains unanswered the question whether the increase in serum Mg^{2+} represents a signal for organs or tissues able to sense this change in circulating Mg^{2+}, and/or for the organs and tissues from which Mg^{2+} is released.

The hypothesis that the increase in serum Mg^{2+} has systemic signaling activity implies that organs or tissues have the ability to sense changes in circulating Mg^{2+} level. Presently, no specific Mg^{2+} sensing mechanism has been identified. However, the Ca^{2+} sensing mechanism identified by Brown and collaborators as the physiological regulator of calcemia (22) appears to sense changes in circulating Mg^{2+} as well, though at higher concentrations than Ca^{2+} (23), that is in a range that would be consistent with the reported increase in serum Mg^{2+} level (10,16,21). Whether the Ca^{2+} sensing mechanism is a bi-functional regulator, or represents the epitome of a new class of sensors still to be identified, is topic for future studies. Regarding this possibility, it has to be mentioned that, in contrast to the original report by Brown *et al*. (22), Bapty *et al* have recently observed the operation of a Ca^{2+}-sensing mechanism with comparable sensitivity for extracellular Ca^{2+} and Mg^{2+} in mouse distal convoluted tubule cells (MDCT) (24). The activation of this sensor mechanism appears to inhibit the glucagone- or vasopressin-mediated entry of Mg^{2+} into the cell (25). This observation should explain the clinical and experimental evidence that hypermagnesemia and hypercalcemia can inhibit hormone-stimulated, cAMP-mediated, reabsorption of both Mg^{2+} and Ca^{2+} along the different segments of the nephron (26). Also, it may provide distal regulation to restore circulating Mg^{2+} to a physiological level following the increase observed in anesthetized animals infused with isoproterenol (10,16) or catecholamine (21), and opens an interesting and totally new area of investigation for hormonal regulation of cellular Mg^{2+} homeostasis.

The absence of showy physiological effects despite the magnitude of the increase in serum Mg^{2+} is a striking difference with a comparable increase in serum Ca^{2+}, which induces muscle weakness and arrhythmia. Overall, it appears that large variations in magnesemia are well tolerated *in vivo*. For example, rats infused with boluses of Mg^{2+} that increase magnesemia by 50% do not manifest significant systemic hemodynamic changes, but present a marked increase in coronary artery flow (27). Baboons that received pharmacological doses of Mg^{2+} sufficient to prevent cardiac arrhythmias induced by epinephrine presented a significant reduction in the epinephrine-induced increase in mean arterial pressure and systemic vascular resistance (28). Moreover, studies *in vitro* suggest that Mg^{2+} can regulate catecholamine release from both peripheral and adrenal sources (29), and that an elevated level of $[Mg^{2+}]_o$ has a significant modulatory effect on cardiac contractility (13). Taken together, these observations i) pose for a role of Mg^{2+} as an endogenous modulator of catecholamine release and activity, and ii) suggest that an increase in circulating Mg^{2+} following adrenergic stimulation may contribute to improve 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.

4. CHANGES IN CELLULAR MG^{2+} CONTENT

Concentrations of total Mg^{2+} ranging between 17-20 mM have been measured and estimated in the majority of mammalian cell types by a variety of technical approaches (see Table 1 in reference 30). Within the cell, total Mg^{2+}content is distributed almost homogeneously among nucleus, mitochondria and endo-(sarco)-plasmic reticulum (31,32). A considerable amount of Mg^{2+}, approximately 4-5 mM, is present in the cytosol as a complex with adenine trisphosphate and other

Table 1. Panel A. Tissues and cell types in which a cAMP-induced Mg^{2+} extrusion has been observed

Tissue	Agent	Reference
Rat cardiac cells	Norepinephrine, Forskolin, cAMP Analogs	11
Rat cardiac cells	Isoproterenol	12
Rat cardiac cells	Isoproterenol, Forskolin	13
Rat cardiac cells	Epinephrine, Forskolin, cAMP Analogs	84
Rat liver cells	Norepinephrine, Forskolin, cAMP analogs	14
Rat liver cells	Norepinephrine	15
Rat liver cells	Phenylephrine	40
Rat liver cells	Epinephrine, Isoproterenol, phenylephrine	42
Erythrocytes	Isoproterenol, Forskolin, cAMP analogs	49,62
Lymphocytes	cAMP analogs, PGE1, IFN-alpha	44
Erlich ascites cells	cAMP analogs, PGE1, PGE2	50
Erlich ascites cells	ATP, Arachidonic acid	54
HL-60 cells	dBcAMP, Forskolin	53
Thymocytes	dBcAMP analog	52
Sublingual mucous acini	Forskolin	51
A7r5 smooth muscle cells	Forskolin, cAMP analogs	84

Table 1. Panel B. Tissues and cell types in which an accumulation of Mg^{2+} has been observed

Tissue	Agent	Reference
Rat cardiac cells	Carbachol, TPA, OAG, SAG	11, 77
Rat cardiac cells	Vasopressin, TPA, OAG	84
Rat cardiac cells	Insulin	55
Rat cardiac cells	Mg^{2+} Depletion	85
Rat liver cells	Vasopressin, Carbachol, TPA	40,41,77
Sublingual mucous acini	Carbachol	51[1]
3T3 Fibroblasts	Insulin, Bombesin	72[1]
Pancreatic beta-cells	Carbachol, Insulin Secretagogues	73[1]
Renal Epithelial cells	Vasopressin, Glucagon	74[1]
Smooth muscle cells	Vasopressin, Angiotensin-II	75,76
A7r5 smooth muscle cells	vasopressin, TPA, OAG	84
Thymocytes	TPA	79
S49 lymphoma cells	TPA	80
MDCK cells	Mg^{2+} depletion	86
MDCT cells	Mg^{2+} depletion	87
MDCT cells	Intracellular [Pi]	88
MDCT cells	Intracellular [K^+]	89
MDCT cells	PGE2	90
MDCT cells	cAMP analogs	91
cTAL	Isoproterenol	20
cTAL	PTH, calcitonin	66
mTAL	PTH, calcitonin	66

[1] Mg^{2+} accumulation is inferred based upon an observed increase in $[Mg^{2+}]_i$.

phosphometabolites (33). Finally, with the exception of calmodulin (34) and S100 protein (35), no other protein is known to have specific binding sites for Mg^{2+} within the cell. The techniques for measuring cytosolic free Mg^{2+} are a far cry from those available to measure Ca^{2+}, H^+ or other cations. The approaches commonly used are ^{31}P-NMR, selective Mg^{2+}-electrode, ^{13}C-NMR citrate/isocitrate ratio or the fluorescent indicator Mag-Fura (see Table 1 in ref. 30), and each of them presents significant drawbacks in term of sensitivity and/or selectivity. Using these techniques, cytosolic *free* Mg^{2+} has been estimated to range between 0.5-1 mM (i.e. less than 5% of total cellular Mg^{2+}) in the majority of cells analyzed (see Table I in ref. 36). Under resting conditions no major changes in cytosolic *free* Mg^{2+} content are observed, and Mg^{2+} fluxes across the plasma membrane, measured by ^{28}Mg re-distribution (37), appear to be slow. For example, an early report by Page and Polimeni (37) indicates that radioisotope equilibrium is achieved within 72-80 hours in cardiac cells incubated at 37^{o}C, and an even longer period is required when the experimental temperature is reduced to 20^{o}C (37). These premises led to the conclusion that a Mg^{2+} transport mechanism with a slow rate of activity must be present in the cell plasma membrane (37).

In recent years, this perception has been largely reconsidered following reports by Vormann and Gunther (12,15), Jakob *et al* (38), Romani and Scarpa (11,14) and Howarth *et al* (13), which indicate that cardiac (11-13) and liver cells (14,15,38) extrude a considerable amount of

cellular Mg^{2+} within 5-6 min from the administration of an adrenergic agonist.

These first observations have been followed by a large number of similar reports, supporting the notion that different hormonal stimuli can induce significant changes in total and free Mg^{2+} content in a variety of cells. Based upon these studies, two main paradigms leading to Mg^{2+} extrusion or accumulation have emerged:

1) a condition in which Mg^{2+} is extruded from the cell via the stimulation of adrenergic receptors and the increase in cellular cAMP level, or by agents that decrease cellular ATP content, and

2) a condition in which Mg^{2+} is accumulated into the tissue following a decrease in cAMP level or the stimulation of protein kinase C pathway.

The tissues and organs in which these two processes have been observed are reported in panels A and B of Table 1.

4.1. Mg^{2+} extrusion following beta-adrenergic receptor stimulation, and role of cAMP

The administration of isoproterenol, epinephrine or norepinephrine results in a marked extrusion of Mg^{2+} from cardiac (11-13,39) or liver cells (14,15,40-42). This extrusion becomes evident within 1 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. Under perfusion conditions, after this period of time Mg^{2+} extrusion returns towards the basal level, independent of the persistence of the agonist in the perfusate (11,14,42). When a submaximal dose of agonist is added repeatedly to the perfusate, each addition results in an extrusion of Mg^{2+} progressively smaller than the previous one (11), a result which can be reasonably interpreted as a progressive depletion of intracellular Mg^{2+} store(s). As for the mechanism involved in the extrusion of Mg^{2+} from cardiac or liver cells, two distinct considerations can be made, regarding the intracellular signaling pathway and the transport mechanism involved at the plasma membrane, respectively. As for the *intracellular signaling* pathway, Mg^{2+} extrusion can be inhibited by the administration of non-selective beta-adrenergic receptor antagonist (propranolol or sotalol) (11,14) or, in the case of cardiac cells, by selective $beta_1$-adrenergic blocking agents (e.g. atenolol) (11). Furthermore, Mg^{2+} extrusion can be mimicked to a comparable extent by the administration of the cell permeant cyclic AMP analogs di-butyryl-cAMP, 8-Cl-cAMP or 8-Br-cAMP (11,14,40), or forskolin (11,14,40), an agent that activates in an irreversible manner adenylyl cyclase (43). Conversely, it can be inhibited by Rp-cAMPs (44), an isomer of cAMP able to cross the cell plasma membrane and prevent specifically protein kinase A activation by endogenous cAMP (45). These observations suggest that the activation of beta-adrenergic receptors (mainly $beta_1$ subtype in cardiac ventricular myocytes (46)) by catecholamine or isoproterenol increases the cytosolic level of cAMP via adenylyl cyclase, and induces an extrusion of Mg^{2+} from the organ into the perfusate or the extracellular compartment. As for the mechanism responsible for Mg^{2+} extrusion across the plasma membrane, it appears to require the presence of a physiological concentration of $[Na^+]_o$ (39,41) and $[Ca^{2+}]_o$ (39,41) (see following section for further details). In fact, the extrusion of Mg^{2+} induced by isoproterenol or by catecholamine can be inhibited by the removal of any of these two cations from the extracellular milieu (39,41), or by the administration of Na^+-transport inhibitors such as amiloride (12), imipramine (47) or quinidine (48), or Ca^{2+}-channel inhibitors such as verapamil (39) or nifedipine (39).

Following the first observations by Gunther and collaborators (12) and by this laboratory (11), the number of reports indicating the operation of the beta-adrenergic mediated Mg^{2+} extrusion mechanism in tissues or cells other than heart and liver has increased considerably. Erythrocytes (49), lymphocytes (44), Erlich ascites cells (50), sublingual mucous acini (51), thymocytes (52) and HL-60 promyelocytic leukemia cells (53) are just a few examples of tissues or cell types in which Mg^{2+} extrusion has been reported to occur via an increase in cellular cAMP level, independently of the pathway leading to cAMP increase. Cittadini and collaborators, for example, have observed that prostaglandin PGE_1 or PGE_2 and arachidonic acid are also able to increase cellular cAMP level and thus induce an extrusion of Mg^{2+} from freshly isolated spleen lymphocytes (44) or Erlich ascites cells (50,54), respectively. Therefore, a more general signaling pathway responsible for Mg^{2+} mobilization can be envisioned, whereby the increase in cellular cAMP level triggered by various exogenous stimuli can result in an extrusion of Mg^{2+} via a Na^+-dependent mechanism. Although the extrusion mechanism has not been purified or cloned, the published observations suggest that the increase in cAMP level likely increases the activity rate of the transporter via phosphorylation (52).

4.2. Is there a role for alpha-adrenergic receptors in Mg^{2+} extrusion?

The reports by Vormann and Gunther (12), Romani and Scarpa (11) and Howarth *et al*. (13) are consistent with the operation of a beta-adrenergic receptor induced, cAMP-mediated, Na^+-dependent Mg^{2+} extrusion mechanism in both cardiac and liver cells. In contrast, the data by Jakob *et al*. (38) indicate that Mg^{2+} can be extruded from liver cells by phenylephrine via activation of $alpha_1$-adrenergic receptor.

A recent report by Keenan *et al*. (42) further support the presence of two distinct signaling pathways for Mg^{2+} extrusion in liver cells. In search of hormones that can modulate cellular cAMP level and possibly counteract the effect of the second messenger on Mg^{2+} homeostasis and extrusion, our laboratory has recently focused its attention on insulin. The results obtained so far indicate that the infusion of insulin into heart (55) or liver (42) prior to isoproterenol or to cell permeant cAMP analogs (8-Br-cAMP or 8-Cl-cAMP) administration completely blocks the extrusion of Mg^{2+} induced by these agents. The replacement of isoproterenol with epinephrine or norepinephrine results in an extrusion of Mg^{2+} from liver cells that is minimally reduced (10-15%) by insulin pre-treatment (42). In contrast, insulin pre-treatment

does not affect the amplitude of Mg^{2+} extrusion from livers stimulated by phenylephrine administration (42).

Therefore, it can be hypothesized that both alpha- and beta-adrenergic receptor are able to induce Mg^{2+} extrusion from a tissue following the stimulation by specific alpha or beta agonists, or by mix agonists such as epinephrine or norepinephrine.

Because catecholamines stimulate both alpha- and beta-adrenergic receptors with a slightly different hierarchy (56), the observation by Jakob *et al*. (38) and our laboratory (42) pose several questions: Do $alpha_1$- and beta-adrenergic receptors activate the same Mg^{2+} extrusion mechanism? Which is the signaling mechanism used by $alpha_1$-adrenoceptors? From which intracellular pool(s) is Mg^{2+} mobilized following $alpha_1$ or beta-adrenergic stimulation? And why the tissue requires a redundancy of signaling and Mg^{2+} extrusion mechanisms? Are $alpha_1$ and beta-adrenergic receptors activated at the same time by catecholamine administration? Or does one class of receptors operate only when the other is inhibited?

Due to the relative novelty of this observation, no overall satisfactory answer is presently available, though certain observation can be explained. For example, because the stimulation of $alpha_1$-adrenergic receptors increases cytosolic Ca^{2+} (57), it is reasonable to infer that Ca^{2+} may activate directly the Mg^{2+} transporter. Yet, it is not fully defined whether Ca^{2+} is used to simply activate the transporter, or it is also utilized as a counter-ion for Mg^{2+} extrusion, or whether Ca^{2+} activates, in a synergistic or alternative manner, the Na^+-dependent mechanism activated by cAMP. Preliminary data from our laboratory would suggest that the administration of phenylephrine to liver cell results in a Mg^{2+} extrusion via a Ca^{2+}-dependent mechanism (approximately 15-20% of total Mg^{2+} extrusion) and via an Ca^{2+}-activated Na^+-dependent mechanism, that accounts for the majority (80-85%) of Mg^{2+} mobilization (58). Finally, by comparing the amount of Mg^{2+} mobilized by epinephrine, isoproterenol or phenylephrine, it appears that epinephrine can mobilize an amount of Mg^{2+} which is approximately double than the amount of Mg^{2+} mobilized by the administration of the selective alpha or beta agonist (42,58). In other words, epinephrine appears to mobilize Mg^{2+} by stimulating concomitantly both alpha and beta-adrenoceptor. Although preliminary and indirect, this observation would indicate that liver cells possess two distinct intracellular pools from where Mg^{2+} can be mobilized by the selective stimulation of the two classes of adrenergic receptors, respectively.

In Figure 1 the $alpha_1$and beta-adrenergic receptor activated signaling pathways and their modulatory effect on cellular Mg^{2+} homeostasis are schematically reported.

4.3. Other agents that induce Mg^{2+} extrusion

A marked extrusion of Mg^{2+} from liver cells (59-61), rat erythrocytes (62), and HL-60 promyelocytic leukemia cells (63) has also been observed following the administration of cyanide (59), FCCP or other mitochondria uncouplers (59,63), fructose (60,62), or ethanol (61). Because all these agents decrease cellular ATP content, it can be reasoned that, by depriving Mg^{2+} of its most abundant intracellular chelating component, these substances increase cytosolic free Mg^{2+}. This increase, in turn, results in an extrusion of Mg^{2+} from the cell via a Na^+-dependent mechanism. An afterthought of this consideration is how Mg^{2+} extrusion is activated under these conditions. The determination of cellular cAMP level following acute ethanol administration has failed to evidence an increase in second messenger content (61), thus excluding an involvement of this pathway in the extrusion of Mg^{2+}. Therefore, possibility is there that the increase in cytosolic free Mg^{2+} subsequent to the decrease in ATP content is sufficient *per se* to activate the transporter and induce the extrusion of Mg^{2+}. Alternatively, it can be hypothesized that Mg^{2+} is extruded as a compensatory mechanism for the altered Na^+ and K^+ distribution within cells depleted of ATP (64). An increase in cytosolic free Mg^{2+} has been observed by Lemasters and collaborators in cultured hepatocytes treated with cyanide (65), and by Hue and co-workers in liver cells incubated in the presence of fructose (60). However, the increase in free Mg^{2+} concentration is considerably lower than it would be expected based on the amplitude of ATP decrease in the cell. Although the possibility of a redistribution of Mg^{2+} among intracellular compartments cannot be excluded, the limited increase in cytosolic Mg^{2+} would be consistent with a Mg^{2+} extrusion, at least partial, across the cell plasma membrane, as reported by Dalal *et al* (59).

4.4. Hormones and stimulatory agents involved in Mg^{2+} accumulation

While adrenergic agonists stimulate cellular Mg^{2+} release, other hormones or agonists induce cellular Mg^{2+} accumulation.

The work by de Rouffignac and collaborators (66-68) and by Quamme and co-workers (reviewed in 2,68,69) suggests that the renal apparatus represent the major site where hormones operate to increase Mg^{2+} reabsorption and, consequently, decrease its hematic level. To avoid redundancy, we refer to that part of the monograph for a more exhaustive description about the role of the renal apparatus on Mg^{2+} handling, and limit our interest to hormones that modulate acutely, or within minutes, total and free Mg^{2+} content at the cell level.

Among the hormones that induce an accumulation of Mg^{2+} into the tissue or, at least, counteract the Mg^{2+} mobilizing effect of adrenergic agonists or catecholamine, insulin has a preeminent role. We have briefly mentioned some of the most recent data provided by our laboratory to this regard (see previous paragraph and also ref. 42). By phosphorylating the tyrosyl residues 350 and 354 at the C terminus of $beta_2$-adrenergic receptor, insulin would block the receptor (70), thus preventing its activation by isoproterenol or other agonists. Also, by increasing phosphodiesterase activity (71), insulin enhances the degradation of cAMP to AMP and hampers the signaling by the second messenger inside the cell.

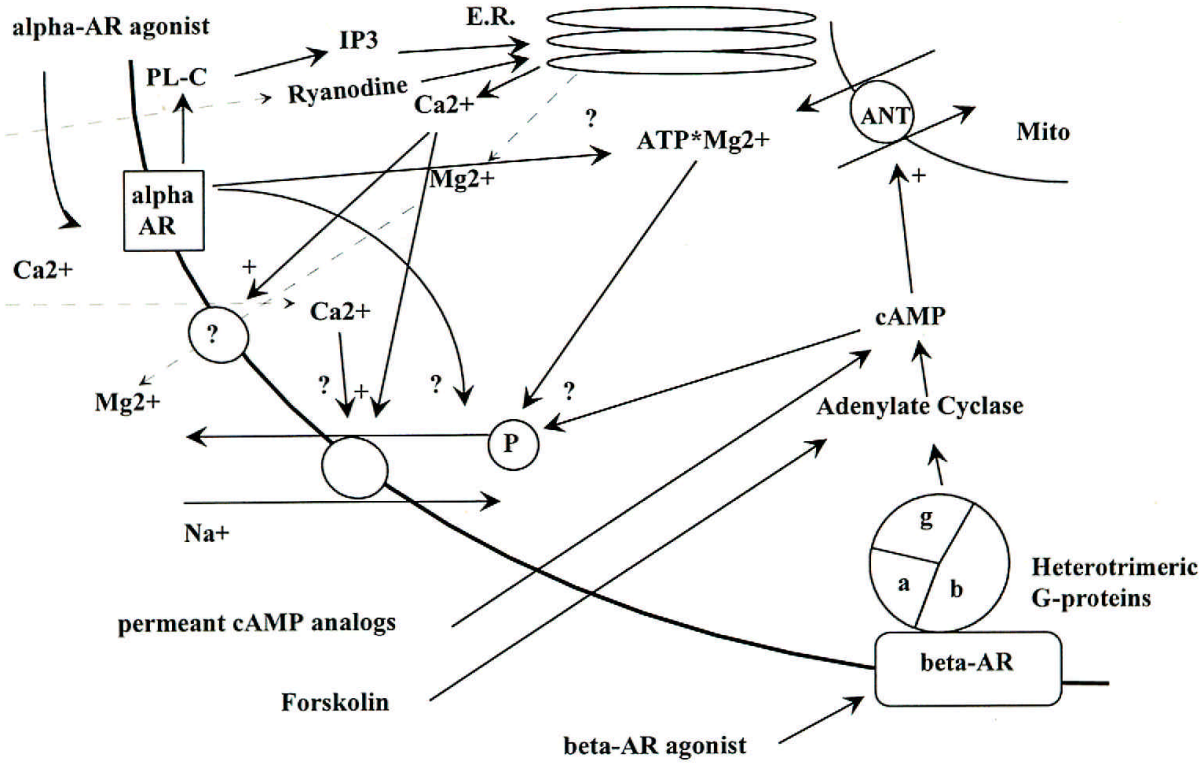

Figure 1. The intracellular signaling pathways activated following the stimulation of $alpha_1$- and beta- adrenergic receptor are schematically illustrated in the cartoon. The stimulation of *beta-adrenergic receptor* by proper agonists results in the activation of adenylyl cyclase to increase cytosolic cAMP level. The second messenger may interact at the mitochondrial level with the adenine nucleotide translocase (ANT) and induce an extrusion of Mg^{2+}*ATP from the organelle into the cytosol. Also, cAMP may directly, or indirectly, modulate the operation rate of the Na^+/Mg^{2+} exchanger at the plasma membrane level, determining an extrusion of cellular Mg^{2+} through this mechanism. The stimulation of *$alpha_1$-adrenergic receptor*, instead, induces the formation of IP_3 and diacyl-glycerol via phospholipase C activation. Inositol-trisphosphate interacts with a specific receptor at the endo-(sarco)-plasmic reticulum level and favors a release of Ca^{2+} from this pool into the cytosol, which – directly or indirectly – determines an entry of Ca^{2+} through the plasma membrane (capacitative Ca^{2+} entry). The activation of this signaling pathway also results in an extrusion of Mg^{2+} from the cell via the Na^+/Mg^{2+} exchanger and/or via a Na^+-independent, Ca^{2+}-modulated (dependent?) extrusion mechanism.

Not only insulin prevents the Mg^{2+} extrusion induced by isoproterenol or cell permeant cAMP in cardiac (55) and liver cells (42), but also induces a detectable accumulation of Mg^{2+} in 3T3 fibroblasts (72) and other cell types (73). Vasopressin and angiotensin-II also induce Mg^{2+} accumulation in cells. Our laboratory has reported that the administration of vasopressin to hepatocytes (40,41) results in an accumulation of Mg^{2+} in these cells via a Na^+-dependent mechanism. Similar results have been observed by Dai *et al* in renal epithelial cells stimulated by vasopressin (74), and by Touyz and Schiffrin (75) and by Okada *et al* (76) in smooth muscle cells from mesenteric artery stimulated by vasopressin (75,76) or angiotensin-II (75). The main difference between these data is that we measured changes in total Mg^{2+} by atomic absorbance spectrophotometry, whereas the other groups detected an increase in cytosolic free Mg^{2+} by using the fluorescent dye Mag-Fura (74-76). The use of this technique allowed Touyz and Schiffrin (75) to notice that the change in cytosolic free Mg^{2+} induced by vasopressin appears to be biphasic. A first, transient, increase in cytosolic free Mg^{2+} content is rapidly (within 2 min) followed by a decrease. Whether the second phase indicates redistribution into an intracellular compartment, or a belated extrusion across the plasma membrane is undefined. However, because under our experimental conditions (40,41,77) Mg^{2+} accumulation persists for about 6 min after the drug administration, the former possibility appears to be more likely.

In all the experimental models mentioned above, however, the hormones appear to operate via activation of protein kinase C pathway. In fact, under conditions in which protein kinase C pathway is inhibited by calphostin C (75), or is down regulated by exposure to a supra-maximal dose of phorbol myristate acetate (77), the accumulation of Mg^{2+} does not occur. On the other hand, the effect of vasopressin can be mimicked, to a comparable extent, by the administration of diacyl-glycerol analogs

(e.g. oleoyl-acetyl-glycerol or stearoyl-arachidonoyl-glycerol) or phorbol-myristate acetate analogs (e.g. PMA or PDBU) that permeate the cell plasma membrane and interact directly with protein kinase C (78). Accumulation of Mg^{2+} by phorbol myristate acetate in thymocytes or S49 lymphoma cell lines has also been reported by Csermely *et al* (79) and Maguire and Grubbs (80), respectively. Additional agents that induce Mg^{2+} accumulation are bombesin, which increases cytosolic free Mg^{2+} concentration in 3T3 fibroblasts (72), and carbachol, which appears to mediate a similar effect in pancreatic islets (73), cardiac ventricular myocytes (11,39), hepatocytes (14,39,77), and sublingual mucosa acini (51), most likely via a decrease in cytosolic cAMP level (81).

As the administration of vasopressin or angiotensin-II induces also changes in cytosolic Ca^{2+}, the possibility that this cation plays a relevant role in modulating Mg^{2+} uptake cannot be dismissed. Several experimental evidences support this hypothesis. Hepatocytes loaded with BAPTA-AM as an intracellular Ca^{2+} chelating agent do not accumulate Mg^{2+} when stimulated by vasopressin or phorbol myristate acetate (41). Also, when hepatocytes are stimulated by vasopressin within two minutes from thapsigargin administration, the amplitude of Mg^{2+} accumulation declines with time, up to a point in which an extrusion of Mg^{2+}, rather than an accumulation, is observed (41). These observations are difficult to be interpreted because changes in cellular Ca^{2+} and Mg^{2+} occur on different time-scales and have different amplitudes (82,83). Cytosolic *free* Ca^{2+} can increase several orders of magnitude within seconds or minutes, returning towards basal level thereafter. Cytosolic *free* Mg^{2+}, which is already in the millimolar range, can increase in absolute amount far more than Ca^{2+}, though the concentration only increases by approximately 10-15% (84). Thus, it is reasonable to postulate that the rapid increase in cytosolic Ca^{2+} induced by hormones like vasopressin is required to activate an entry of Mg^{2+} across the cell plasma membrane and its redistribution within intracellular compartments.

An indication that the cell is able to *sense* the intracellular concentration of Mg^{2+} and modulate it dynamically is provided by the observation by Quamme and co-workers that an accumulation of Mg^{2+} occurs in cardiac ventricular myocytes (85), MDKC cells (86), or MDCT cells (87-90) as a consequence of a prolonged exposure (~16 hours) of the cells to 0 mM $[Mg^{2+}]_o$. Under these experimental conditions, the cytosolic free Mg^{2+} concentration decreases to about half the normal level and remains unaltered as long the cells are maintained in an incubation medium with 0 mM $[Mg^{2+}]_o$. When $[Mg^{2+}]_o$ is increased, intracellular free Mg^{2+} level returns to the normal level (87), at a rate of 170-180 nM/sec, within a time frame that is directly proportional to $[Mg^{2+}]_o$ (87). The process occurs within a few minutes and is prevented by the presence of L-type Ca^{2+}-channel inhibitors (i.e. verapamil, nifedipine) or La^{3+} in the incubation medium (85-87). Because no significant changes in cytosolic $[Ca^{2+}]_i$ are observed under these experimental conditions, it appears that these inhibitory agents affect directly the Mg^{2+} entry mechanism (85-90).

Mg^{2+} accumulation can also occur as a consequence of ion redistribution. In fact, additional observation from Quamme and collaborators indicates that renal epithelial cells accumulate Mg^{2+} as a result of phosphate (88) or potassium (89) redistribution across the cell plasma membrane. The latter point is particularly interesting and worth further investigation because it implies that the change in membrane potential determined by a redistribution of K^+ across the plasma membrane may result in an activation of Mg^{2+} transport for charge compensation. Finally, it is interesting to note that the same agents that induce Mg^{2+} extrusion in other cell types or tissues (e.g. PGE_2 (90), isoproterenol (20), cAMP (91)) all induce a Mg^{2+} uptake at the level of the thick ascending limb of the Henle's loop.

Considering altogether the ability of several tissues to release or accumulate Mg^{2+} as a result of varying stimulatory conditions, two distinct considerations can be drawn: a) that the cell *senses* the cytosolic free Mg^{2+} concentration and adjusts it according to its physiological requirement as a result of changes in energy content or other cations distribution, and b) that the hormones stimulating Mg^{2+} extrusion from different organs or tissues, thereby increasing plasma Mg^{2+}, also increase Mg^{2+} reabsorption at the renal level to prevent a net loss of the cation.

5. Mg^{2+} transport across the cell plasma membrane

Heretofore, the Mg^{2+} transporter(s) in the plasma membrane has(have) not been purified or cloned, and its(their) presence is inferred based upon data provided by Mg^{2+} fluxes. Not only uncertainty exists about the number of Mg^{2+} extrusion mechanisms present in the cell plasma membrane but it is also undefined whether Mg^{2+} release and accumulation are accomplished by the same transporters operating in either direction or by distinct transport mechanism(s).

Gunther and Vormann first reported the operation of a Mg^{2+} extrusion mechanism in the plasma membrane of chicken and turkey erythrocytes (92,93). The authors observed that in these cells the extrusion mechanism operates as an electroneutral Na^+/Mg^{2+} exchanger (2 Na^+_{in} for 1 Mg^{2+}_{out}) (93) inhibited by amiloride (93). Since their report, an increasing number of observations suggests that a similar mechanism operates in the majority of mammalian cells. Table 2 lists the cell types in which the operation of a Na^+/Mg^{2+} exchanger has been observed. Over the years, the list has extended to comprehend a broad variety of normal cells, including cardiac myocytes (12,39,94), smooth muscle cells (95), hepatocytes (15,41,96), erythrocytes (48,92,93,97,100), lymphocytes (44), sublingual mucous acini (101), as well as pathological cell lines (HL-60 promyelocytic leukemia cells (44) or Erlich ascites cells (50)). Nor the list is limited to mammalian cells, as the operation of a similar Mg^{2+} transport mechanism has also been observed for decades in non-

Table 2. Tissues in which the operation of a Na^+/ Mg^{2+} exchanger has been observed or hypothesized

Tissue	Reference
Rat cardiac cells	12,39,94, 105
Rat liver cells	15,41,58,61,96,123[1]
Smooth muscle cells (tenia cecum)	95
Smooth muscle cells (mesenteric artery)	75
Thymocytes	52
Lymphocytes	44
HL-60	53,54
Erlich ascites cells	50,63
Human erythrocytes	47,48, 93, 99
Chicken erythrocytes	93
Turkey erythrocytes	93
Rat erythrocytes	97
Ferret erythrocytes	98
Hamster erythrocytes	100
Sublingual mucous acini	101
Rabbit ileum brush border cells	120[1]
Squid axon	102
Barnacle muscle	103
Leech neuron	104
Paramecium tetraurelia	108[2]

[1]Operation of Na^+/Mg^{2+} exchanger evidenced in plasma membrane vesicles from these organs. [2]In Paramecium, Na^+ and Mg^{2+} currents have been recorded at the cell plasma membrane.

mammalian cell types such as squid axon (102) or barnacle muscle (103), and – more recently - in leech neuron (104).

The modality of activation and operation of this transport mechanism are not fully defined, to the point that its presence in certain cells (e.g. cardiac ventricular myocytes) is questioned (see ref. 105 for more detail). The data available in literature point to an involvement of this Mg^{2+} transport mechanism in the Mg^{2+} extrusion induced by beta-adrenergic receptor stimulation, likely through a cAMP-mediated phosphorylation process (52), or by cyanide, fructose or ethanol administration, likely via the increase in cytosolic free Mg^{2+} content subsequent to the decrease in ATP content determined by these agents (59-63). The inability to measure accurately Mg^{2+} fluxes across the plasma membrane does not permit to confirm or exclude whether this exchanger is responsible for the slow turnover of cellular Mg^{2+} observed under basal condition (37). Also, the stoichiometry of operation of the exchanger appears to vary according to the cell type considered. For example, an electroneutral ratio 2:1 has been observed in chicken and turkey erythrocytes (93), but not in hamster (100) or human red blood cells (99,106), in which an exchange of one to three Na^+ for one Mg^{2+} has been documented. An electrogenic operation of the exchanger would imply at least one of the following possibilities: a) that other ions have to be transported across the plasma membrane for charge compensation, or b) that changes in membrane potential would have a marked effect on the Na^+/Mg^{2+} exchanger.

The latter possibility, though it cannot completely dismissed, appears to be the less likely. Attempts to induce modest changes in membrane potential have results in negligible or no change in cellular Mg^{2+} content (102,107). To this respect, it has to be kept in mind that the results provided by Quamme and collaborators (88-91) have been obtained in Mg^{2+} deficient cells, as no changes in $[Mg^{2+}]_i$ were observed in cells that maintained a physiological level of cytosolic free Mg^{2+} of ~0.5 mM (74,89). On the other hand, experimental results have been provided by several laboratories about the operation of a Mg^{2+} extrusion mechanism that does not requires Na^+ and therefore termed Na^+-independent (41,48, 108,109). In terms of ion counter-transported for Mg^{2+}, the Na^+-independent mechanism does not appear to be extremely selective. A Mg^{2+} extrusion in exchange for extracellular Ca^{2+} (39,41), Mn^{2+} (108,110), Cl^- (41,109), or HCO_3^- (96,111), has been described in erythrocytes (108-110), cardiac myocytes (39), hepatocytes (41,96) and other cell types as well. The operation of this transport mechanism is facilitated by the presence of a low concentration of $[Na^+]_o$, as increasing concentrations of $[Na^+]_o$ as well as quinidine, ATP depletion, or cAMP administration (96,108) markedly inhibit its functioning. The extrusion mediated by the Na^+-independent mechanism appears to occur on one-to-one ratio, at least when Mn^{2+} is exchanged for Mg^{2+} (108), and can also operate in a reverse mode, thus favoring an entry on Mg^{2+} into the cell (108,112). A particular Na^+-independent transport mechanism is the Mg^{2+}/Mg^{2+} exchanger, whose operation has been hypothesized to occur in erythrocytes (113) and cardiac cells (114), as well as in non-mammalian cell types (115). The data, obtained by monitoring the movement of ^{24}Mg and ^{28}Mg radioisotopes in opposite directions across the plasma membrane of red blood cells (113), indicate a one-to-one transport of Mg^{2+} across the cell membrane, with not net gain or loss in terms of total cellular Mg^{2+} content. Kinetic evaluation provides a Km value for the putative Mg^{2+}/Mg^{2+} exchanger which is not too dissimilar from that obtained for the Na^+/Mg^{2+} exchanger (93), thus rising the question whether these two transport mechanisms are separate entities or two mode of operation of the same transporter, as proposed by Gunther (116).

The possibility that Mg^{2+} may cross the plasma membrane via a selective channel is appealing but not sufficiently supported by available experimental evidence. Preston and co-workers have provided evidence for the operation of a selective Mg^{2+}-channel in *Paramecium* (117) that becomes operative following the administration of extracellular GTP (118). Yet, no comparable observation is available for mammalian cells. The possibility that Mg^{2+} may enter MDCT cells (88-91), cardiac myocytes (85) or MDCK cells (86) via a channel is indirectly supported by the inhibitory effect of Ca^{2+}-channel blockers (verapamil or nifedipine) or La^{3+} (85-91, 119) on Mg^{2+} accumulation in these cell types.

Finally, it has to be mentioned the relevant role that intracellular ATP plays at regulating these Mg^{2+} extrusion pathways. While the results obtained in squid axon (102) indicate that the Na^+-dependent Mg^{2+} efflux requires cellular ATP and is inhibited by procedures that

decrease ATP content (102), the data obtained in mammalian cells provided a far more complex scenario. As mentioned previously, the Na^+-independent pathway, irrespective of the cation used, appears to be inhibited by the removal of ATP, at least in erythrocytes (109) or hepatocytes (96). As no evidence exists that Mg^{2+} is extruded from the cell via the operation of an outwardly oriented Mg^{2+}-ATPase, it has been hypothesized that ATP is required to maintain the transporter in a phosphorylated, active state (96-109). On the other hand, under conditions in which ATP level is permanently (chemical hypoxia, (59,65)), or transiently (acute ethanol treatment, (61)) decreased, the extrusion of Mg^{2+} via a Na^+-dependent mechanism inhibited by amiloride appears to be enhanced (59,61). The latest scenarios are more consistent with ATP being the major ligand for Mg^{2+} in the cytosol of the cell, so that – when ATP content decreases – the consequent increase in free Mg^{2+} concentration results in an extrusion across the plasma membrane (59,61).

Recently, to better characterize the Mg^{2+} transport mechanism(s), different research groups have undertaken the more direct approach of purifying plasma membrane vesicle from brush border cells of rabbit ileum (120), of duodenum and jejunum of rat (121,122) and from rat hepatocytes (123).

The transport of Mg^{2+} in vesicles from rabbit ileum has been investigated by using the fluorescent dye Mag-Fura2 both as cell impermeant and permeant (acetoxymethylester) derivatives, whereas ^{28}Mg or atomic absorbance spectrophometry were used to detect Mg^{2+} fluxes in plasma membrane from small intestine or liver cells, respectively. The result obtained in ileum and liver plasma membrane support the operation of two Mg^{2+} transport mechanisms, one of which modulated by an established Na^+ gradient across the plasma membrane. Yet, some marked differences can be observed between the two experimental models.

In the case of liver plasma membrane (123), a reversible exchange of Na^+ for Mg^{2+} occurs with a varying stoichiometry. The extrusion mechanism is quite specific, in that Na^+ cannot be replaced by other monovalent cations (e.g. Li^+ or K^+), becomes saturated at a concentration of $[Na^+]_o$ = 25-30 mM, and is inhibited, though not completely, by amiloride, quinidine or imipramine. In addition, the vesicles possess another Mg^{2+} transport mechanism that extrudes intravesicular Mg^{2+} for extravesicular Ca^{2+}. This pathway, which operates only in one direction ($Ca^{2+}{}_{in}$:$Mg^{2+}{}_{out}$), is activated by micromolar concentrations of Ca^{2+} or other divalent cations (Ca^{2+}>>Co^{2+}=Mn^{2+}>Sr^{2+}>>Ba^{2+}>Cu^{2+}>>Cd^{2+}), and is also blocked, to a variable extent, by amiloride. Finally, intravesicular ATP does not modulate the activity of these two transporters, though some inhibitory effect by ATP-gamma-S on both mechanisms is observed.

In the case of membrane vesicles from rabbit ileum (120), a saturable Mg^{2+} uptake mechanism is observed when $[Na^+]_i$>$[Na^+]_o$, and not when $[Na^+]_i$<$[Na^+]_o$ or $[Na^+]_i$=$[Na^+]_o$ or in the absence of external Na^{2+}. This pathway is not reversible, and is not inhibited by the amiloride analogs DMA or EIPA, or by Ca^{2+}-channel inhibitors. A major effect of intravesicular anions, especially Cl^- and SCN^-, on Mg^{2+} transport is observed, and antagonist of anion transport (e.g. H_2-DIDS) exerts a stimulatory effect on Mg^{2+} accumulation into the vesicles. The transport appears to be electroneutral and has a Km for Na^+ of 16 mM, a value not dissimilar from the Km that can be calculated from the data of Cefaratti *et al* (123), or that has been reported by Tashiro and Konishi (95) in smooth muscle cells from guinea pig tenia caecum, and by Gunther and Vormann in chicken erythrocytes (93, 116).

A Mg^{2+} uptake mechanism has also been described in plasma membrane vesicles from rat duodenum and jejunum (121,122). The vesicles from the duodenum appear to possess a single Mg^{2+} uptake mechanism with an apparent K(t) of 0.8 mM. Kinetic analysis of Mg^{2+} uptake in vesicles from jejunum suggest, instead, the presence of two distinct transport mechanisms with a K(t) of 0.15 and 2.4 mM, respectively. The accumulation of Mg^{2+} is not affected by verapamil, but is reduced by 30-40% by the use of L-leucine or L-phenylalanine as inhibitors of intestinal alkaline phosphatase (121). Under the same experimental conditions $^{45}Ca^{2+}$ uptake is unaffected, thus suggesting that Ca^{2+} and Mg^{2+} are transported via distinct pathways. Furthermore, the Mg^{2+} transport mechanisms have an optimal temperature at 28^{o}C and are stimulated by an electronegative potential inside the vesicle (122). When Mg^{2+} accumulation is performed at 0.1 mM $[Mg^{2+}]_o$, the uptake is stimulated by an alkaline pH_o and by a difference in Mg^{2+} concentration across the plasma membrane, and is inhibited by amiloride or by the presence of other divalent cations (e.g. Co^{2+}, Ni^{2+} and Ba^{2+}) whereas $[Na^+]_o$ or alkaline pH_i are ineffective (121). When Mg^{2+} accumulation is performed at 1 mM $[Mg^{2+}]_o$, the uptake is insensitive to changes in pH_o or pH_i, is not stimulated by a difference in Mg^{2+} concentration across the plasma membrane, but is still inhibited, though to a lesser extent, by amiloride (121). Under these conditions, the presence of Na^+ or K^+ in the extravesicular space strongly enhances Mg^{2+} accumulation. In contrast, the presence of divalent cations inhibits Mg^{2+} uptake to a varying extent (Co^{2+}>Mn^{2+}>Ca^{2+}>Ni^{2+}>Ba^{2+}>Sr^{2+}) (122).

Figure 2 summarizes the different Mg^{2+} transport mechanism identified in plasma membrane vesicles from mammalian cells.

Because of the difference in organs and animals, the diversities in Mg^{2+} transport cannot be conscribed to a common denominator. Yet, a few general concepts can be defined. Organs like liver appear to utilize Na^+ as the primary driving force for Mg^{2+} transport in either direction across the plasma membrane. Most likely, this transport mechanism is the pathway that different hormones activate to induce Mg^{2+} extrusion or accumulation. A secondary mechanism, utilizing Ca^{2+} or other cations, may

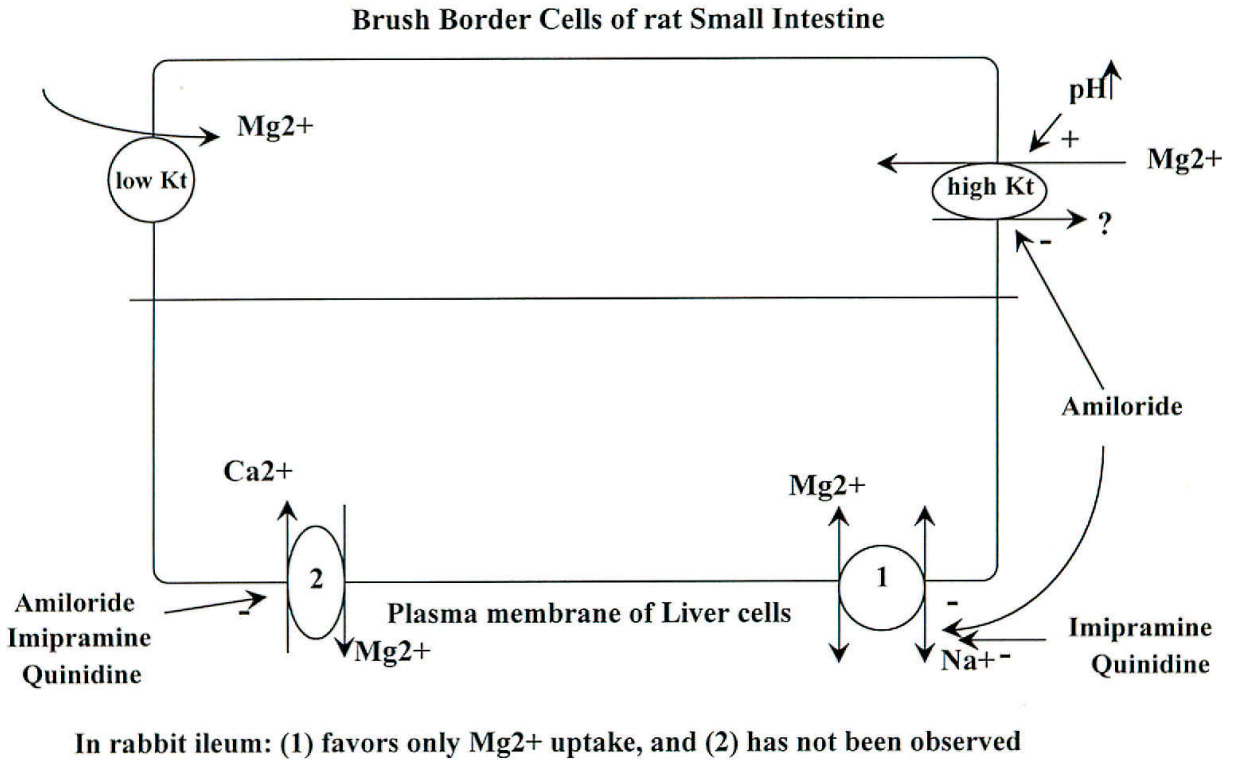

Figure 2. The cartoon depicts the Mg^{2+} transport mechanism recently characterized in plasma membrane vesicles from brush border cells of the small intestine (top part), liver cells and brush border cells of rabbit ileum (bottom part). See section 4 for more detail.

play a subsidiary role under more varying situation. Whether this second mechanism is the same transporter activated by $alpha_1$-adrenergic stimulation is, at present, matter of speculation. As for the intestine, due to the absorption function of the organ, it is reasonable to envisage that the Mg^{2+} transport mechanism(s) here respond(s) primarily to the gradient of Mg^{2+} present across the brush border of the small intestine and, to a varying extent, to the presence of both mono- and di-valent cations and to changes in pH across the plasma membrane, until a certain level of intracellular accumulation is achieved. The lack of information about which hormones are able to modulate the accumulation through these mechanisms renders more difficult to define under which condition one route of Mg^{2+} accumulation is preferentially utilized versus the other. However, the operation of two transporters in both liver cells and brush border cells from small intestine with different regulation and sensitivity can be interpreted as the presence of a *coarse* and a *fine tuning* mechanism to regulate cellular Mg^{2+} homeostasis under the most diverse physiological situations.

6. PHYSIOLOGICAL SIGNIFICANCE OF MG^{2+} CHANGES WITHIN MITOCHONDRIA

Within the cell mitochondria contain large amount of Mg^{2+} (40,124), some of which can be rapidly mobilized following the increase in cytosolic cAMP level (39,40). While the overall mechanism affected by cAMP may be far more complex than what initially reported, many conditions leading to cAMP increase are accompanied by an efflux of Mg^{2+} from the mitochondria (39,40,51). Little is known as to whether changes in matrix Mg^{2+} can affect the rate of mitochondrial dehydrogenases and therefore the rate of respiration.

There is significant evidence that changes in Ca^{2+} matrix can control respiration (125, 126). Those observations, however, have been questioned by other studies showing:

a) that several mitochondrial dehydrogenases can increase activity within minutes in the absence of an increase in mitochondrial Ca^{2+} (127,128);

b) that mitochondrial Mg^{2+} changes during state 3 to 4 transition (129) and in the presence of cAMP (39,40);

c) that the activity of the isolated alpha-ketoglutarate dehydrogenase is regulated *in vitro* by both Ca^{2+} and Mg^{2+} (130).

The role of matrix Mg^{2+} as regulator of dehydrogenases and overall mitochondrial respiration has

been recently investigated by measuring the activity of several dehydrogenases in mitochondria whose matrix Ca^{2+} and/or Mg^{2+} varied. From the data, it appears that alpha-ketoglutarate dehydrogenase and pyruvate dehydrogenase are not regulated by changes in mitochondrial Mg^{2+}. By contrast, removal of Mg^{2+} stimulates several fold succinate and glutamate dehydrogenases (131). This and other experimental evidence clearly indicate that changes in matrix Mg^{2+} (in addition to those of Ca^{2+}) could control respiration and metabolic pathways regulated by changes in concentration of matrix substrates.

Hence, in principle, the effect of catecholamine on increasing respiration could be explained as the decrease in mitochondrial Mg^{2+} resulting from an increase in cellular cAMP. This in turn will stimulate some dehydrogenases directly and render others more susceptible to the concentrations of Ca^{2+} present in the mitochondrial matrix.

7. CONCLUSIONS

It is clear that the understanding of cell and body Mg^{2+} homeostasis is presently at a primordial stage, and it is a far cry with respect to that existing in the literature for other ions such as Ca^{2+}, H^+, K^+ or Na^+. The list of questions is far greater than the list of answers:

a) What is the effect of increasing plasma Mg^{2+} concentrations on body functions? While there is a large body of evidence, often contradictory on several physiological responses, a common denominator (a Rosetta's stone) to classify these observations in a logical framework is still missing.

b) What is the role of various distinct organs and tissues (i.e. bone, muscle and kidney) in redistributing plasma and parenchymal Mg^{2+} in response to stimuli?

c) What is the nature, or even the operation and regulation, of the plasma membrane transporter(s)?

d) How is Mg^{2+} redistributed among cytosolic organelles or structures?

e) What is the role of Mg^{2+} changes within organelles on their function and metabolism?

For Mg^{2+} research it is clearly a new frontier, interesting and challenging!

8. ACKNOWLEDGEMENT

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9. REFERENCES

1. G H Mudge: Agents affecting volume and composition of body fluids. In: The Pharmacological Basis of Therapeutics. Eds.: Goodman Gilman A, Godman LS, Rall TW, Murad F, MacMillian, NY (1989)

2. G.A Quamme: Magnesium Homeostasis and renal magnesium handling. *Miner Electrolyte Metab* 19, 218-225 (1993)

3. Geigy Scientific Tables. Ed.: Lentner C., Ciba-Geigy, Basel, Switzerland (1984)

4. R. Taylor, L Agius: The biochemistry of diabetes. *Biochem J* 250, 625-640 (1988)

5. L M Resnick: Ionic basis of hypertension, insulin resistance, vascular disease, and related disorders. The mechanism of "syndrome X". *Am J Hypertens* 6, 123S-134S (1993)

6. S Wallach, R L Verch: Tissue magnesium content in diabetic rats. *Magnesium* 6, 302-306 (1987)

7. H Joborn, G Akerstrom, S Ljunghall: Effects of exogenous catecholamines and exercise on plasma magnesium concentrations. *Clin Endocrinol* 23:219-226 (1985)

8. Y Rayssiguier: Hypomagnesemia resulting from adrenaline infusion in ewes: its relation to lipolysis. *Horm Metab Res* 9:309-314 (1977)

9. G Guideri: Catecholamine modulation of Mg^{2+} plasma levels in the rat. *Arch Intern Pharmacodyn Therap* 14, 122-125 (1992)

10. T Gunther, J Vormann: Mechanism of beta-agonist-induced hypermagnesemia. *Magnes Bull* 14, 122-125 (1992)

11. A Romani, A Scarpa: Hormonal control of Mg^{2+} in the heart. *Nature* 346, 841-844 (1990)

12. J Vormann, T Gunther: Amiloride sensitive net Mg^{2+} efflux from isolated perfused rat hearts. *Magnesium* 6, 220-224 (1987)

13. F C Howarth, J Waring, B I Hustler, J Singh: Effects of extracellular magnesium and beta adrenergic stimulation on contractile force and magnesium mobilization in the isolated rat heart. *Magnes Res* 7, 187-197 (1994)

14. A Romani, A Scarpa: Norepinephrine evokes a marked Mg^{2+} efflux from liver cells. *FEBS Lett* 269, 37-40 (1990)

15. T Gunther, J Vormann, V Hollriegl: Noradrenaline-induced Na^+-dependent Mg^{2+} efflux from rat liver. *Magnes Bull* 13, 122-124 (1991)

16. D Keenan, A Romani, A Scarpa: Differential regulation of circulating Mg^{2+} in the rat by $beta_1$- and $beta_2$-adrenergic receptor stimulation. *Circ Res* 77, 973-983 (1995)

17. P B Molinoff: Alpha- and beta-adrenergic receptor subtypes, properties, distribution and regulation. *Drugs* 28, 1-14 (1984)

18. P J Barnes: Beta-adrenergic receptors and their regulation. *Am J Respir Crit Care Med* 152, 838-860 (1995)

19. I M Shafik, G A Quamme: Early adaptation of renal Mg^{2+} reabsorption in response to magnesium restriction. *Am J Phyiol* 257, F974-F977 (1989)

20. C Bailly, M Imbert-Teboul, N Roinel, C Amiel: Isoproterenol increases Ca, Mg, and NaCl reabsorption in mouse thick ascending limb. *Am J Physiol* 258, F1224-F1231 (1990)

21. A K Konstantakos, A Romani, A Scarpa: Catecholamine-induced hypermagnesemia: Effect of epinephrine in pharmacological and physiological conditions. *Am J Physiol*, submitted (1999)

22. E M Brown, G Gamba, D Riccardi, M Lombardi, R Butters, O Kifor, A Sun, M A Hediger, J Lytton, S C Herbert: Cloning and characterization of an extracellular Ca^{2+}-sensing from bovine parathyroid. *Nature* 366, 575-580 (1993)
23. E F Nemeth, A Scarpa: Rapid mobilization of cellular Ca^{2+} in bovine parathyroid cells by external divalent cations. *J Biol Chem* 262, 5188-5196 (1987)
24. B W Bapty, L J Dai, G Ritchie, F Jirik, L Canaff, G N Hendy, G A Quamme: Extracellular Mg^{2+}- and Ca^{2+}-sensing in mouse distal convoluted tubule cells. *Kidney Intern* 53, 583-592 (1998)
25. B W Bapty, L J Dai, G Ritchie, L Canaff, G N Hendy, G A Quamme: Mg^{2+}/Ca^{2+} sensing inhibits hormone-stimulated uptake in mouse distal convolute tubule cells. *Am J Physiol* 275, F353-F360 (1998)
26. G A Quamme, J H Dirks: Effect of intraluminal and contraluminal magnesium on magnesium and calcium transfer in the rat nephron. *Am J Physiol* 238, F187-F198 (1980)
27. D J Dipette, K Simpson, J Guntupalli: Systemic and regional hemodynamic effect of acute magnesium administration in the normotensive and hypertensive state. *Magnesium* 6, 136-149 (1987)
28. M F M James, R C Cork, G M Harlen, J F White: Interaction of adrenaline and magnesium in the cardiovascular system of the baboon. *Magnesium* 7, 37-43 (1988)
29. J B Stanbury: The blocking action of magnesium on sympathetic ganglia. *J Pharmacol Exp Ther* 93, 52-62 (1948)
30. A Romani, C Marfella, A Scarpa: Mg^{2+} homeostasis in cardiac ventricular myocytes. In: Biochemistry of Cell Membranes: a compendium of selected topics. Eds.: Papa S, Tager J M, Birkhauser-Verlag, Base, Switzerland (1995)
31. T Gunther: Functional compartmentation of intracellular magnesium. *Magnesium* 5, 53-59 (1986)
32. R L Griswold, N Pace: The intracellular distribution of metal ions in rat liver. *Exp Cell Res* 11, 362-367 (1956)
33. L Garfinkel, R A Altschuld, D Garfinkel: Magnesium in cardiac energy metabolism. *J Mol Cell Cardiol* 18, 1003-1013 (1986)
34. S Oki, M Ikura, M Zhang: Identification of Mg^{2+} binding sites and role of Mg^{2+} on target recognition by calmodulin. *Biochemistry* 36, 4309-4316 (1997)
35. Y Ogoma, H Kobayashi, T Fujii, Y Kondo, A Hachimori, T Shimizu, M Hatano: Binding study of metal ions to S100 protein: ^{43}Ca, ^{25}Mg, ^{67}Zn and ^{39}K N.M.R.. *Int J Biol Macromol* 14, 279-286 (1992)
36. A Romani, A Scarpa: Regulation of cell magnesium. *Arch Biochem Biophys* 298, 1-12 (1992)
37. P I Polimeni, E Page. In: Recent Advances in study in cardiac cells and metabolism. Ed.: Dhalle N S, vol IV, pp. 217-232, University Park Press, Baltimore (1974)
38. A Jakob, J Becker, G Schottli, G Fritzsch: $alpha_1$-adrenergic stimulation causes Mg^{2+} release from perfused rat liver. FEBS Lett 246, 127-130 (1989)
39. A Romani, C Marfella, A Scarpa: Regulation of magnesium uptake and release in the heart and in isolated ventricular myocytes. *Circ Res* 72, 1139-1148 (1993)
40. A Romani, E Dowell, A Scarpa: Cyclic AMP-induced Mg^{2+} release from rat liver hepatocytes, permeabilized hepatocytes, and isolated mitochondria. *J Biol Chem* 266, 24376-24384 (1991)
41. A Romani, C Marfella, A Scarpa: Hormonal regulation of Mg^{2+} uptake in hepatocytes. *J Biol Chem* 268, 15489-15495 (1993)
42. D Keenan, A Romani, A Scarpa: Regulation of Mg^{2+} homeostasis by insulin in perfused rat livers and isolated hepatocytes. *FEBS Lett* 395, 241-244 (1996)
43. R D Huang, M F Smith, W L Zahler: Inhibition of forskolin-activated adenylate cyclase by ethanol and other solvents. *J Cyclic Nucleot Res* 8, 385-394 (1982)
44. F I Wolf, A Di Francesco, V Covacci, A Cittadini: Regulation of magnesium from rat spleen lymphocytes. *Arch Biochem Biophys* 344, 397-403 (1997)
45. J D Rothermel, L H Parker Botelho: A mechanistic and kinetic analysis of the interactions of the diastereisomers of adenosine 3',5'-(cyclic)phosphorothioate with purified cyclic AMP-dependent protein kinase. *Biochem J* 251, 757-762 (1988)
46. P Stemmer, P L Wisler, A M Watanabe: Isolated myocytes in experimental cardiology. In: The heart and cardiovascular system. Scientific Foundations. Eds. Fozzard H A Haber E, Jennings R B, Katz A M, Morgan H E, Raven Press, New York (1992)
47. J-C Feray, R Garay: Demonstration of a Na^+:Mg^{2+} exchange in human red cells by its sensitivity to tricyclic antidepressant drugs. *Naunyn-Schmied Arch Pharmacol* 338, 332-337(1988)
48. J-C Feray, R Garay: An Na^+-stimulated Mg^{2+} transport system in human red blood cells. *Biochim Biophys Acta* 856, 76-84 (1986)
49. T Matsuura, Y Kanayama, T Inoue, T Takeda, I Morishima: cAMP-induced changes of intra-cellular Mg^{2+} levels in human erythrocytes. *Biochim Biophys Acta* 1220, 31-36 (1993)
50. F I Wolf, A Di Francesco, V Covacci, A Cittadini: cAMP activates magnesium efflux via the Na/Mg antiporter in ascite cells. Biochem Biophys Res Commun 202, 1209-1214 (1994)
51. G H Zhang, J E Melvin: Secretagogue-induced mobilization of an intracellular Mg^{2+} pool in rat sublingual mucous acini. *J Biol Chem* 267, 20721-20727 (1992)
52. T Gunther, J Vormann: Activation of Na+/Mg2+ antiport in thymocytes by cAMP. *FEBS Lett* 297, 132-134 (1992)
53. F I Wolf, V Covacci, N Bruzzese, A Di Francesco, A Sachets, D Cord, A Cittadini: Differentiation of HL-60 promyelocytic leukemia cells is accompanied by a modification of magnesium homeostasis. *J Cell Biochem* 71, 441-448 (1998)
54. F I Wolf, A Di Francesco, V Covacci, D Corda, A Cittadini: Regulation of intracellular magnesium in ascites cells: Involvement of different regulatory pathways. *Arch Biochem Biophys* 331, 194-200 (1996)
55. A Romani, V Matthews, A Scarpa: Synergism for Mg^{2+} and glucose transport in heart following insulin administration. *Biophys J* 72, W210 (1997)
56. G Milligan, D Svoboda, C Brown: Why there are so many adrenoceptor types? *Biochem Pharmacol* 48, 1059-1071 (1994)
57. K P Minneman: $Alpha_1$-adrenergic receptor subtypes, inositol phosphates, and sources of cell Ca^{2+}. *Pharmacol Rev* 40, 87-119 (1988)

58. T E Fagan, A Romani: Activation of a Na^+- and a Ca^{2+}-dependent Mg^{2+} extrusion mechanism by alpha- and beta-adrenergic agonists in perfused rat liver. *FASEB J* 13(4), (1999)
59. P Dalal, A Romani, A Scarpa: Redistribution of Mg^{2+} in isolated rat hepatocytes upon chemical hypoxia. *Biophys J* 74, A193 (1998)
60. V Gaussin, P Gailly, J-M Gillis, L Hue: Fructose-induced increase in intracellular free Mg^{2+} ion concentration in rat hepatocytes: relation with the enzymes of glycogen metabolism. *Biochem J* 326, 823-827 (1997)
61. P A Tessman, A Romani: Acute effect of EtOH on Mg^{2+} homeostasis in liver cells: Evidence for the activation of an Na^+/Mg^{2+} exchanger. *Am J Physiol* 275, G1106-G1116 (1998)
62. J Hwa, A Romani, C Marfella, A Scarpa: Mg^{2+} transport in erythrocytes: Regulation by intra-cellular pH or cAMP. *Biophys J* 63, A307 (1993)
63. F I Wolf, A Di Francesco, A Cittadini: Characterization of magnesium efflux from Erlich ascites tumor cells. *Arch Biochem Biophys* 308, 335-341 (1994)
64. A Gasbarrini, A B Borle, H Farghali, C Bender, A Francavilla, D van Thiel: Effect of anoxia on intracellular ATP, Na^+_i, Ca^{2+}_i, Mg^{2+}_i, and cytotoxicity in rat hepatocytes. *J Biol Chem* 267, 6654-6663 (1992)
65. A W Harman, A-L Nieminen, J J Lemasters, B Herman: Cytosolic free magnesium, ATP, and blebbing during chemical hypoxya in cultured rat hepatocytes. *Biochem Biophys Res Commun* 170, 477-483 (1990)
66. A Di Stefano, M Wittner, R Nitchke, R Braitsch, R Greger, C Bailly, C Amiel, N Roniel, C De Rouffignac: Effects of parathyroid hormone and calcitonin on Na^+, Cl^-, K^+, Mg^{2+} and Ca^{2+} transport in cortical and medullary thick ascending limbs of mouse kidney. *Pflugers Arch* 417, 161-167 (1990)
67. C De Rouffignac, B Mandon, M Wittner, A Di Stefano: Hormonal control of renal magnesium handling. *Miner Electrolyte Metab* 19, 226-231 (1993)
68. C De Rouffignac, G A Quamme: Renal magnesium handling and its hormonal control. *Physiol Rev* 74, 305-322 (1994)
69. G A Quamme: Renal magnesium handling: New insights in understanding old problems. *Kidney Inter* 52, 1180-1195 (1997)
70. V Karoor, K Baltensperger, H Paul, M C Czech, C C Malbon: Phosphorylation of tyrosyl residues 350/354 of the beta-adrenergic receptor is obligatory for counterregulatory effects of insulin. *J Biol Chem* 270, 25305-25308 (1995)
71. J A Smoake, G-M M Moy, B Fang, S S Solomon: calmodulin-dependent cyclic AMP phospho-diesterase in liver plasma membranes: Stimulated by insulin. *Arch Biochem Biophys* 323, 223-232 (1995)
72. S Ishijima, M Tatibana: Rapid mobilization of intracellular Mg2+ by bombesin in Swiss 3T3 cells: Mobilization through external Ca2+- and tyrosine kinase-dependent mechanism. *J Biochem* 115, 730-737 (1994)
73. E Gylfe: Insulin secretagogues induce Ca2+-like changes in cytoplasmic Mg2+ in pancreatic beta cells. *Biochim Biophys Acta* 1055, 82-86, (1990)
74. L-J Dai, B Bapty, G Ritchie, G A Quamme: Glucagone and arginine vasopressin stimulate Mg2+ uptake in mouse distal convoluted tubule cells. *Am J Physiol* 274, F328-F335 (1998)
75. R M Touyz, E L Schiffrin: Angiotensin II and vasopressin modulate intracellular free magnesium in vascular smooth muscle cells through Na^+-dependent protein kinase C pathways. *J Biol Chem* 271, 24353-24358 (1996)
76. K Okada, S-E Ishikawa, T Saito: Cellular mechanisms of vasopressin and endothelin to mobilize $[Mg^{2+}]_i$ in vascular smooth muscle cells. *Am J Physiol* 263, C873-C878 (1992)
77. A Romani, C Marfella, A Scarpa: Regulation of Mg^{2+} uptake in isolated rat myocytes and hepatocytes by protein kinase C. *FEBS Lett* 296, 135-140 (1992)
78. M Castagna, Y Takai, K Kabuchi, K Sano, U Kikkawa, Y Nishizuka: Direct activation of calcium-activated phospholipid-dependent protein kinase by tumor promoting phorbol esters. *J Biol Chem* 257, 7848-7851 (1982)
79. P Csermely, P Fodor, J Somogyi: The tumor promoter tetradecanoylphorbol-13-acetate elicits the redistribution of heavy metal in subcellular fractions of rabbit thymocytes as measured by plasma emission spectroscopy. *Carcinogenesis* 8,1663-1666 (1987)
80. R D Grubbs, M E Maguire: Regulation of magnesium but not calcium by phorbol esters. *J Biol Chem* 261, 12550-12554 (1986)
81. K H Jakobs, K Aktories, G Schultz: GTP-dependent inhibition of cardiac adenylate cyclase by muscarinic cholinergic agonists. *Naunyn-Schmiedeberg's Arch Pharmacol* 310, 113-119 (1979)
82. X Zhu and L Birnbaumer: Calcium channels formed by mammalian Trp homologues. *News in Physiol Sci* 13, 211-217 (1998)
83. R D Grubbs, M E Maguire: Magnesium as a regulatory cation: Criteria and evaluation. *Magnesium* 6, 113-127 (1987)
84. M M Fatholahi, A Scarpa: Cytosolic free magnesium changes in isolated rat cardiomyocytes and smooth muscle cell line A7r5. *Biophys J* 76, A305
85. G A Quamme, S W Rabkin: Cytosolic free magnesium in cardiac myocytes: Identification of a Mg^{2+} influx pathway. *Biochem Biophys Res Commun* 167, 1406-1412 (1990)
86. G A Quamme, L-S Dai: Presence of a novel influx pathway for Mg^{2+} in MDCK cells. *Am J Physiol* 259, C521-C525 (1990)
87. L-J Dai, G A Quamme: Intracellular Mg^{2+} and magnesium depletion in isolated renal thick ascending limb cells. *J Clin Invest* 88, 1255-1264 (1991)
88. L-J Dai, P A Friedman, G A Quamme: Phosphate depletion diminishes Mg^{2+} uptake in mouse distal convoluted tubule cells. *Kidney Inter* 51, 1710-1718 (1997)
89. L-J Dai, P A Friedman, G A Quamme: Cellular Mechanism of chlorothiazide and cellular potassium depletion on Mg^{2+} uptake in mouse distal convoluted tubule cells. *Kidney Inter* 51, 1008-1017 (1991)
90. L-J Dai, B Bapty, G Ritchie, G A Quamme: PGE2 stimulates Mg^{2+} uptake in mouse distal convoluted tubule cells. *Am J Physiol* 275, F833-F839 (1998)
91. L-J Dai, G A Quamme: Cyclic nucleotides alter intracellular free Mg^{2+} in renal epithelial cells. *Am J Physiol* 262, F1100-F1104 (1992)
92. T Gunther, J Vormann, R Forster: Regulation of intracellular magnesium by Mg^{2+} efflux. *Biochem Biophys Res Commun* 119, 124-131 (1984)

93. T Gunther, J Vormann: Mg^{2+} efflux is accomplished by an amiloride-sensitive Na^+/Mg^{2+} antiport. *Biochem Biophys Res Commun* 130, 540-545 (1985)
94. R D Handy, I F Gow, D Ellis, P W Flatman: Na-dependent regulation of intracellular free magnesium concentration in isolated rat ventricular myocytes. *J Mol Cell Cardiol* 28, 1641-1651 (1996)
95. M Tashiro, M Konishi: Na^+ gradient-dependent Mg^{2+} transport in smooth muscle cells of guinea pig tenia cecum. *Biophys J* 73, 3371-3384 (1997)
96. T Gunther, V Hollriegl: Na^+- and anion-dependent Mg^{2+} influx in isolated hepatocytes. *Biochim Biophys Acta* 114949-54 (1993)
97. T Gunther, J Vormann, V Hollriegl: Characterization of Na^+-dependent Mg^{2+} efflux from Mg^{2+}-loaded rat erythrocytes. Biochim Biophys Acta 1023, 455-461 (1990)
98. P W Flatman, L M Smith: Magnesium transport in ferret red cells. *J Physiol* 431, 11-25 (1990)
99. H Ludi, H J Schatzmann: Some properties of a system for sodium-dependent outward movement of magnesium from metabolizing human red blood cells. *J Physiol* 390, 367-382 (1987)
100. W Xu, J S Willis: Sodium transport through the amiloride-sensitive Na-Mg pathway of hamster red cells. *J Membr Biol* 141, 277-287 (1994)
101. G H Zhang, J E Melvin: Na^+-dependent release of Mg^{2+} from an intracellular pool in rat sublingual mucous acini. *J Biol Chem 271*, 29067-29072 (1996)
102. R Di Polo, L Beauge': An ATP-dependent Na^+/Mg^{2+} countertransport is the only mechanism for Mg^{2+} extrusion in squid axons. *Biochim Biophys Acta* 946, 424-428 (1988)
103. P F Baker, A C Crawford: Mobility and transport of magnesium in squid giant axons. J Physiol 227, 855-874 (1972)
104. D Gunzel, W-R Schlue: Sodium-magnesium antiport in Retzius neurons of the leech *Hirudo medicinalis*. J Physiol 491 595-608 (1996)
105. E Murphy, C C Freudenrich, M Lieberman: Cellular magnesium and Na/mg exchange in heart cells. *Annu Rev Physiol* 53, 273-287 (1991)
106. H J Schatzmann: Asymmetry of the magnesium sodium exchange across the human red cell membrane. *Biochim Biophys Acta* 1148, 15-18 (1993)
107. P W Flatman: Mechanisms of magnesium transport. *Annu Rev Physiol* 53, 259-271 (1991)
108. J-C Feray, R Garay: One-to-one Mg^{2+}:Mn^{2+} exchange in rat erythrocytes. J Biol Chem 262, 5763-5768 (1987)
109. T Gunther, J Vormann: Characterization of Na^+-independent Mg^{2+} efflux from erythrocytes. *FEBS Lett* 271, 149-151 (1990)
110. T Gunther, J Vormann E J Cragoe Jr: Species-specific Mn^{2+}/Mg^{2+} antiport from Mg^{2+}-loaded erythrocytes. *FEBS Lett* 261, 47-51 (1990)
111. T Gunther: Mechanisms and regulation of Mg^{2+} efflux and Mg^{2+} influx. *Miner Electrolyte Metab* 19, 250-265 (1993)
112. T Gunther, J Vormann: Induction of Mn^{2+}/H^+ antiport in chicken erythrocytes by intracellular Mg^{2+} and Mn^{2+}. *FEBS Lett* 265, 55-58 (1990)
113. T Gunther, J Vormann: Characterization of Na^+/Mg^{2+} antiport by simultaneous $^{28}Mg^{2+}$ influx. Biochem Biophys Res Commun 148, 1069-1074 (1987)
114. M E Maguire, R Crome, D J Hearse, A S Mannings: Efflux of ^{28}Mg from isolated rat heart: Effects of ischaemia, reperfusion, temperature and extracellular magnesium concentration. *J Mol Cell Cardiol* 16, 34 (1984)
115. F J Brinley: Calcium and magnesium transport in single cells. *Fed Proc* 32, 1735-1739 (1973)
116. T Gunther: Putative mechanism of Mg^{2+}/Mg^{2+} exchange and Na^+/Mg^{2+} antiport. *Magnes Bull* 18, 2-6 (1996)
117. R R Preston: A magnesium current in *Paramecium*. *Science* 250, 285-288 (1990)
118. K D Clark, R R Preston: Extracellular GTP causes membrane-potential oscillations through the parallel activation of Mg^{2+} and Na^+ currents in Paramecium tetraurelia. *J Membr Biol* 157, 159-167 (1997)
119. L-J Dai, P A Friedman, G A Quamme: Acid base changes alter Mg^{2+} uptake in mouse distal convolute tubule cells. *Am J Physiol* 272, F759-F766 (1997)
120. R Juttner, H Ebel: Characterization of Mg^{2+} transport in brush border membrane vesicles of rabbit ileum studied with mag-fura-2. *Biochim Biophys Acta* 1370, 51-63 (1998)
121. M Baillien, H Wang, M Cogneau: Uptake of ^{28}Mg by duodenal and jejunal brush border membrane vesicle in the rat. *Magnesium* 8, 315-329 (1995)
122. M Baillien, M Cogneau: Characterization of two mechanisms of ^{28}Mg uptake in rat jejunal brush border membrane vesicles. *Magnesium* 8, 331-339 (1995)
123. C Cefaratti, A Romani, A Scarpa: Characterization of two Mg^{2+} transporters in sealed plasma membrane vesicles from rat liver. *Am J Physiol* 275, C995-C1008 (1998)
124. P W Flatman: Magnesium transport across cell membrane. *J Membr Biol* 80, 1-14 (1984)
125. J G McCormack, A Halestrap, R M Denton: Role of calcium ions in regulation of mammalian intramitochondrial metabolism. *Physiol Rev* 70, 391-425 (1990)
126. R G Hansford: Physiological role of mitochondrial Ca^{2+} transport. *J Bioenerg Biomembr* 26, 495-508 (1994)
127. C S Moravec, M Bond: Calcium is released from the junctional sarcoplasmic reticulum during cardiac muscle contraction. *Am J Physiol* 260, H989-H997 (1991)
128. C S Moravec, M Bond: Effect of inotropic stimulation on mitochondrial calcium in cardiac muscle. *J Biol Chem* 267, 5310-5316 (1992)
129. G P Brierley, M Davis, D W Jung: Respiration-dependent uptake and extrusion of Mg^{2+} by isolated heart mitochondria. *Arch Biochem Biophys* 253, 322-332 (1987)
130. A Panov, A Scarpa: Independent modulation of the activity of alpha-ketoglutarate dehydrogenase complex by Ca^{2+} and Mg^{2+}. *Biochemistry* 35, 427-432 (1996)
131. A Panov, A Scarpa: Mg^{2+} control of respiration in isolated rat liver mitochondria. *Biochemistry* 35, 12849-12856 (1996)

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