Extracellular and Intracellular Magnesium-Calcium Interactions

J. Vormann, T. Günther

Zusammenfassung

Die physiologischen Wirkungen von Mg^{2+} und Ca^{2+} werden durch Interaktionen mit negativ geladenen Molekülen (ATP, ADP, Citrat, Isocitrat, DNA, RNA), Enzymen und anderen Proteinen (Ca^{2+} bindende Proteine, Kanal-Proteine, Struktur-Proteine etc.) hervorgerufen. Mg^{2+} und Ca^{2+} sind harte Lewis-Säuren. In Komplexen von harten Säuren mit harten Basen wird die Bindungsenergie durch die elektrostatische Anziehungskraft zwischen Metallion und Ligand bestimmt. Mg^{2+} und Ca^{2+} unterscheiden sich nur in ihrem Radius und in ihrer Polarisierbarkeit. Diese Unterschiede ergeben unterschiedliche Affinitäten zu Liganden und verschiedene Substitutionsraten für den Ersatz von H_2O-Molekülen aus der inneren Hydratationshülle. Einige Mg^{2+} und Ca^{2+} Komplexe haben die räumliche Anordnung eines Oktaeders, allerdings ist die räumliche Struktur der meisten Komplexe nicht geklärt. Die verschiedenen Mg^{2+}-Ca^{2+} Interaktionen, die hauptsächlich quantitativer Natur sind, lassen sich wie folgt klassifizieren:

1. Mg^{2+} und Ca^{2+} haben gleiche Wirkung, mit nur geringfügigen quantitativen Unterschieden, z. B. werden bestimmte Enzyme gleichermaßen durch Mg^{2+} und Ca^{2+} aktiviert (synergistischer, unspezifischer Effekt).
2. Ca^{2+} und Mg^{2+} werden mit unterschiedlicher Affinität an Proteine gebunden, wobei der Effekt von Mg^{2+} weniger ausgeprägt ist als der von Ca^{2+} (teilweise synergistischer, teilweise antagonistischer Effekt).
3. Die Wirkung eines Kations wird durch das andere, das in diesem Prozeß keine Wirkung hat, gehemmt (antagonistischer Effekt).
4. Der Effekt ist spezifisch von Mg^{2+} oder Ca^{2+} abhängig und wird durch das jeweils andere Kation innerhalb eines vernünftigen Konzentrationsbereichs nicht beeinflußt (spezifischer Effekt).

Die Interaktionen von Mg^{2+} und Ca^{2+} werden sowohl hinsichtlich des Transportes an verschiedenen Membranen (Intestinum, Niere, Plazenta, Plexus Chorioidei, Plasmamembran) als auch hinsichtlich ihrer Interaktionen an Enzymen, Proteinen und Phospholipiden analysiert.

Summary

The physiological effects of Mg^{2+} and Ca^{2+} are related to interactions with or binding to negatively charged molecules (ATP, ADP, citrate, isocitrate, DNA, RNA), enzymes and other proteins (Ca^{2+}-binding proteins, channel proteins, structure proteins etc.). Mg^{2+} and Ca^{2+} are hard Lewis acids. In complexes of hard acids with hard bases bond energy is dominated by the electrostatic attraction of the metal ion and the ligand. Mg^{2+} and Ca^{2+} differ only in their radius and polarizability. These differences yield different affinities to ligands and different rates of substitution of H_2O molecules from the inner hydration shell. The spatial structure of some Mg^{2+} and Ca^{2+} chelates is octahedral. However, the structure of most complexes has not been defined. The various Mg^{2+}-Ca^{2+} interactions which are primarily of quantitative nature can be qualified as following:

1. Mg^{2+} and Ca^{2+} can have the same effect. There may be only a small quantitative difference, e. g. enzymes are activated to about the same degree by Mg^{2+} and Ca^{2+} (synergistic, unspecific effect).
2. Ca^{2+} and Mg^{2+} are bound to proteins with different affinity. However, the effect of Mg^{2+} is less expressed than that of Ca^{2+} (partially synergistic, partially antagonistic).
3. The effect of one cation is inhibited by the other, which does not function in this process (antagonistic effect).
4. An effect is specifically dependent on Mg^{2+} or Ca^{2+} and the effect of one cation is not significantly affected by the other within a feasible concentration range (specific effect).

The interactions of Mg^{2+} and Ca^{2+} are analysed with respect to transport at various membranes (intestine, kidney, placenta, choroid plexus, plasma membrane) as well as their interactions at enzymes, proteins and phospholipids.

Institute of Molecular Biology and Biochemistry, Free University Berlin, Germany.

Résumé

Les effets physiologiques du Mg^{++} et du Ca^{++} sont liés à des interactions avec des molécules chargées négativement (ATP, ADP, citrate, isocitrate, ADN, ARN), des enzymes et d'autres protéines (protéines fixant le Ca^{++}, protéines canalaires, protéines de structure etc.). Le Mg^{++} et le Ca^{++} sont des acides forts de Lewis. Dans les complexes d'acides forts et de bases fortes, l'énergie de fixation dépend de la force d'attraction électrostatique entre l'ion métallique et le ligand. Le Mg^{++} et le Ca^{++} ne diffèrent que par leur rayon et leur polarisabilité. Ces différences déterminent des affinités différentes pour les ligands et des taux de substitution différents pour le remplacement des molécules d'eau à partir de la couche interne d'hydratation. Quelques complexes de Mg^{++} et de Ca^{++} offrent une configuration spatiale d'octaèdre, mais la structure spatiale de la plupart des complexes n'est pas encore élucidée. Les diverses interactions entre le Mg^{++} et le Ca^{++}, qui sont essentiellement quantitatives, peuvent être classées de la façon suivante:

1. Le Mg^{++} et le Ca^{++} exercent le même effet, avec seulement de discrètes différences quantitatives; par exemple le Mg^{++} et le Ca^{++} activent de la même façon certaines enzymes (effet synergique, non spécifique).
2. Le Ca^{++} et le Mg^{++} se fixent aux protéines avec une affinité différente, l'effet du Mg^{++} étant moins marqué que celui du Ca^{++} (effet tantôt synergique, tantôt antagoniste).
3. L'effet d'un cation est inhibé par l'autre, qui n'agit pas dans ce processus (effet antagoniste).
4. L'effet dépend spécifiquement du Mg^{++} ou du Ca^{++} et n'est pas modifié par l'autre cation, dans une fourchette de concentration raisonnable (effet spécifique).

Nous analysons ici les interactions entre le Mg^{++} et le Ca^{++} aussi bien du point de vue du transport dans diverses membranes (intestin, reins, placenta, plexus choroïde, membrane plasmique) que du point de vue des interactions sur les enzymes, protéines et phospholipides.

Introduction

The physiological effects of Mg^{2+} and Ca^{2+} are related to interactions with or binding to negatively charged molecules (ATP, ADP, citrate, isocitrate, DNA, RNA), enzymes and other proteins (Ca^{2+}-binding proteins, channel proteins, structure proteins etc.) (*Günther,* in press). Mg^{2+} and Ca^{2+} are hard lewis acids. Hard acids complex most strongly with donor atomes (O, N) which possess high electronegativity (hard bases). In complexes of hard acids with hard bases bond energy is dominated by the electrostatic attraction of the metal ion and ligand. Covalent binding, entropy and solvation effects are small.

Mg^{2+} and Ca^{2+} differ only in their radius and polarizability (Tab. 1) (*Pauling,* 1964; *Pearson,* 1963). These differences yield different affinities to ligands (*Martell* and *Calvin,* 1956; *Eigen* and *Hammes,* 1963) and different rates of substitution of H_2O molecules from the inner hydration shell. According to the electrostatic relationship (e^2/r), complex stability should be $Mg^{2+} > Ca^{2+}$. However, the sequence of stability is often reversed to $Mg^{2+} < Ca^{2+}$ (*Martell* and *Calvin,* 1956). The lower stability of the smaller cation Mg^{2+} can be explained by repulsion between negatively charged ligand groups due to their smaller distance, which leads to partial coordination with water molecules (*Williams,* 1959) and outer sphere complex binding. Complex and chelate formation with ligands representing various hard or soft bases may lead to different affinities and specificities. The spatial structure of some Mg^{2+} and Ca^{2+} chelates is octahedral. However, the structure of most complexes has not been defined. Therefore, Mg^{2+}-Ca^{2+} interactions can only be described from experimental results. So far, there is no exact systematic physico-chemical analysis of Mg^{2+}-Ca^{2+} interactions at the various target substances, but only single measurements or qualitative observations.

The various Mg^{2+}-Ca^{2+} interactions which are primarily of quantitative nature can be qualified as following:

1. Mg^{2+} and Ca^{2+} can have the same effect. There may be only a small quantitative difference, e. g. enzymes are activated to about the same degree by Mg^{2+} and Ca^{2+} (F_0F_1 ATPase [*Ahlers* and *Günther,* 1975]) (synergistic, unspecific effect).
2. Ca^{2+} and Mg^{2+} are bound to ligands with different affinity (in the range of their physiologic concentration). However, the effect of one ion is less expressed than that of the other (e. g. troponin C, actin, calmodulin etc.) (partially synergistic, partially antagonistic). The latter effects may have regulatory significance (*Günther,* in press).
3. The effect of one cation is inhibited by the other, which has no effect in this process (e. g. Mg^{2+} inhibition of Ca^{2+} channels, Na^+Ca^{2+} exchange, therapeutic effect of Ca^{2+} injection in Mg^{2+} overdosage (antagonistic effect).
4. An effect is specifically dependent on Mg^{2+} or Ca^{2+} and the effect of one cation is not significantly affected by the other within a feasible concentration range (e. g. absorption of Mg^{2+} and Ca^{2+} in intestine and kidney, Na^+Mg^{2+} antiport, cellular Mg^{2+} uptake) (specific effect).

In the following chapters physiologic functions of Mg^{2+} and Ca^{2+} are analyzed with respect to Mg^{2+}-Ca^{2+} interactions.

Tab. 1: Physical properties of Mg^{2+} and Ca^{2+}.

	Mg^{2+}	Ca^{2+}	Reference
atomic radius (Å)	1.36	1.74	*Pauling,* 1964
ionic radius (Å)	0.65	0.99	*Pauling,* 1964
hydrated radius (Å)	3.44	3.05	*Kachmar* and *Boyer,* 1953
	4.67	3.21	*Padgham* et al., 1993
	7.70	6.78	*Nachod* and *Wood,* 1945
polarizability (Å^3)	0.072	0.8	*Jorgensen,* 1973
rate of H_2O substitution (sec^{-1})	10^5	10^8	*Eigen* and *Hammes,* 1963

Mg^{2+}-Ca^{2+} interactions in intact animals and humans

Hypermagnesemia

When Mg^{2+} was i. v. infused in animals or humans the following symptoms were observed, with increasing Mg^{2+} concentration in serum.

	$[Mg^{2+}]_o$ (mM)	effect
1.	1.5–2.5	hypotension
2.	2–4	nausea, vomiting, cutaneous flushing
3.	2.5–5	bradycardia, increased PR intervals
4.	5	decrease of tendon reflexes
5.	7	skeletal muscle paralysis
6.	7	coma
7.	7.5–12	respiratory arrest
8.	15–30	diastolic heart arrest.

Early stages of these symptoms can be reversed by i. v. injection of Ca^{2+} (*Mordes* and *Wacker,* 1978).

Parenteral Mg^{2+} is used in the treatment of preeclampsia and eclampsia. To achieve a therapeutic effect, $[Mg^{2+}]_o$ must be increased above 2 mM. The mechanism of action is a Ca^{2+}-antagonistic effect of Mg^{2+} and a Mg^{2+}-induced increased production of prostacyclin (*Watson* et al., 1986). Also in myocardial infarction parenteral treatment with Mg^{2+} exerted a beneficial effect, as has been proved by an extensive study (*Woods* et al., 1992).

Hypomagnesemia

The reciprocal test of Mg^{2+}-Ca^{2+} interactions in the intact animal may be hypomagnesemia due to Mg^{2+} deficiency. In Mg^{2+} deficiency, there is an increase of Ca^{2+} and a decrease of Mg^{2+} in some tissues (*Günther,* 1981). However, these experiments are long term experiments with complex interactions (leading to cell necroses, changed release of hormones, growth reduction) and can give no decision on the exact nature of Mg^{2+}-Ca^{2+} interactions. Therefore, these interactions can only be defined by analysing isolated physiological functions.

Mg^{2+}-Ca^{2+} interactions in intestinal absorption

Mg^{2+} and Ca^{2+} absorption in intestine involves two pathways for transfer:

1. Transcellular movement which is performed by a saturable active transport. Its amount depends on luminal Mg^{2+} and Ca^{2+} concentration. There is no interaction of Mg^{2+} and Ca^{2+} at the transcellular pathway.
2. Paracellular diffusion (*Karbach* et al., 1991; *Hardwick* et al., 1991; *Gilles-Baillien* and *Cogneau,* 1992; *Karbach* and *Rummel,* 1990).

High concentrations of one ion depress the absorption of the other ion along the paracellular pathway. The inhibitory effect of hypermagnesemia on intestinal Ca^{2+} transport has been attributed to diminution of the paracellular component of Ca^{2+} absorption (*Karbach,* 1989).

Mg^{2+}-Ca^{2+} interactions in renal reabsorption

The major part of ultrafiltrated Mg^{2+} (50–60%) is reabsorbed in the thick ascending limb of Henle's loop (TAL), whereas 20% is reabsorbed in the proximal tubulus. Mg^{2+} may move across the epithelium of the TAL through a transcellular and a paracellular pathway. The tight junctions of the paracellular pathway may alter Mg^{2+} transport by single file diffusion. Cation selectivity may be due to fixed negative charges on the tight junctions. The mechanism of transcellular pathway is not known as yet. It is possible that Mg^{2+} may be transported by Na^+/Mg^{2+} antiport, Mg^{2+} anion cotransport or by electrogenic Mg^{2+} channels (*Beyenbach,* 1986).

From ultrafiltrated Ca^{2+}, 60% is reabsorbed in the proximale tubulus and only 30–35% in Henle's loop.

Elevation of intraluminal Mg^{2+} did not influence Ca^{2+} absorption nor did intraluminal Ca^{2+} inhibit Mg^{2+} transport by TAL, indicating separate Mg^{2+} and Ca^{2+} pathways in the TAL. However, interactions of Mg^{2+} and Ca^{2+} occurs at the paracellular pathway by binding to tight junctions. Hypermagnesemia, hypercalcemia or luminal elevation of each divalent cation increases the resistance specifically to divalent cations and decreases voltage-dependent absorption through the paracellular pathway (for detailed review see: *Quamme* and *de Rouffignac,* 1993).

Mg^{2+}-Ca^{2+} interactions at the placenta

Mg^{2+} concentration in fetal serum is higher than in maternal serum, dependent on gestational age (*Vormann* and *Günther,* 1986). The enrichment of Mg^{2+} is due to placental active transport which is performed by transcellular active transport and probably by paracellular diffusion (*Lourdes Cruz* et al., 1992). Active transport of Mg^{2+} occurs via amilorid-sensitive Na^+/Mg^{2+} antiport and probably furosemide-sensitive Mg^{2+}/HCO_3^- cotransport (*Günther* et al., 1988). Whether Ca^{2+} affects Mg^{2+} transport has not been studied.

Ca^{2+} is similarly enriched in fetal serum (*Garel* and *Barlet,* 1976; *Bradbury* et al., 1972) as Mg^{2+}. However, there seems to be no significant paracellular diffusion of Ca^{2+}, since increasing maternal serum Ca^{2+} by Ca^{2+} infusion has no significant effect on fetal serum Ca^{2+} (*Bawden* and *Wolkoff,* 1967), although maternal-fetal $^{45}Ca^{2+}$ flux was increased (*Derewlany* and *Radde,* 1985). Probably, constancy of fetal serum Ca^{2+} at increased $^{45}Ca^{2+}$ flux is caused by increased $^{45}Ca^{2+}$-Ca^{2+} exchange. Ca^{2+} transport across the placenta was not affected when maternal serum Mg^{2+} was drastically reduced by Mg^{2+} deficiency (*Lourdes Cruz* et al., 1992).

Mg^{2+}-Ca^{2+} interactions at the choroid plexus

Mg^{2+} concentration in cerebrospinal fluid (CSF) is 45% higher than in serum (1.22 mM vs 0.84 mM) although protein concentration and protein-bound Mg^{2+} in CSF is low, indicating active transport of Mg^{2+} across the choroid plexus. On the other hand Ca^{2+} concentration in CSF amounts only to 50% of serum Ca^{2+} (1.21 mM vs 2.46 mM) representing an ultrafiltrate of serum (*Chutkow* and *Meyers,* 1968). In perfused choroid plexus reduction of Ca^{2+} concentration from 2.35 to 1.5 mM had no significant effect on Mg^{2+} transport either at 0.81 or 0.31 mM Mg^{2+}, indicating no Ca^{2+}-Mg^{2+} interaction at the membrane of the choroid plexus (*Allsop,* 1986).

Mg^{2+}-Ca^{2+} interactions at phospholipid membranes

The interaction of phospholipids (PL) with Mg^{2+} or Ca^{2+} was studied using pure PL, that form membraneous structures in water, e.g. liposomes. The surface-fixed charges at these membranes produce a surface potential, depending on the charge density, described by Grahame's equation.

The surface potential leads to an enrichment of ions in the aqueous phase at the surface, depending on the valence and concentration of ions in the bulk aqueous phase, described by Boltzmann's equation.

Because of the local enrichment of Mg^{2+} and Ca^{2+} ions, both are bound to negatively charged groups, according to the Langmuir adsorption isotherm (*Lau* et al., 1981; *Ohki* and *Sauve,* 1978). The extent of Mg^{2+} binding depends on the local activity coefficient. Mg^{2+} is bound to negatively charged groups by outer sphere complexes. The rate constant of this binding reaction is very high, limited only by diffusion of the ions. Binding of Ca^{2+} or Mg^{2+} reduces the surface potential. Only a few ions are bound to PL, the rest behave like an ion cloud at the charged surface. Generally, Ca^{2+} has a higher affinity to PL than Mg^{2+}.

In mixed liposomes, binding of Mg^{2+} and Ca^{2+} to phosphatidylethanolamine (PE), phosphatidylserine (PS) and cardiolipine (CL) was the same as in pure liposomes (*McLaughlin* et al., 1981; *Macdonald* and *Seelig,* 1987).

Some authors reported Mg^{2+} and Ca^{2+} binding constants from $1.6 \times 10^3\ M^{-1}$ to $5.3 \times 10^4\ M^{-1}$ for PS (*Portis* et al., 1973; *Hendrickson* and *Fullington,* 1965) and $1 \times 10^5\ M^{-1}$ for the Ca^{2+} binding constant of CL (*Sokolove* et al., 1983). These high values represent "apparent binding constants". The surface potential of liposomes and ion enrichment at

the surface of the liposomes was not taken into account. The low Mg^{2+} and Ca^{2+} binding constants of PL agree with the low Mg^{2+} and Ca^{2+} binding constants when phosphate is bound to water-soluble residues that form no charged membranes.

The interaction of Ca^{2+} and Mg^{2+} with PS is more complicated. When the Ca^{2+} or Mg^{2+} concentration is increased to 1 or 3 mM, respectively, the PL vesicles aggregate because repulsing forces are reduced. With Ca^{2+}, the vesicles fuse because Ca^{2+} is bound to PS of neighbouring vesicles, Mg^{2+} is not bound to PS of neighbouring vesicles because of the stronger hydration of Mg^{2+} (*Wilschut* et al., 1981). In mixed PE-PS and PC-PS vesicles, Ca^{2+}, but not Mg^{2+} induces lateral diffusion of PS and phase separation (*Tokutomi* et al., 1981) or phase transition to hexagonal phase type II (*Tilcock* and *Cullis,* 1981). However, Mg^{2+} can induce hexagonal II phase in CL bilayers (*Vail* and *Stollery,* 1979) because CL is a more cone-shaped molecule. By hexagonal phase transition charged pores can be formed in PL membranes. Thus, divalent cations, particularly Ca^{2+}, are involved in membraneous phenomena such as permeability, exocytosis, secretion, or membrane receptor clustering (*Wilschut* et al., 1981).

Binding of Ca^{2+} to cell membranes reduces the fluidity by two mechanisms (*Storch* and *Schachter,* 1985):

1. Unspecific binding of Ca^{2+} to the polar head groups, as it is also produced by Mg^{2+}, reduces the motional freedom of PL.
2. Ca^{2+} decreases the arachidonic acid (AA) content and fluidity of membranes by a specific effect. The possible mechanism is the activation of the Ca^{2+}-dependent phospholipase A_2. Thus, AA is released and substituted by other fatty acids which results in a lower fluidity.

Mg^{2+}-Ca^{2+} interactions in hormone and neurotransmitter release

Release of some hormones e.g. catecholamines or insulin from their producing cells is preceded by influx of Ca^{2+}. It was found that the release of catecholamines is increased at reduced $[Mg^{2+}]_o$ as in Mg^{2+} deficiency (*Günther* et al., 1978; *Cadell* et al., 1986), whereas hypermagnesemia had no significant effect on secretion of catecholamines in men (*Zofkova* et al., 1993). ^{45}Ca uptake in pancreatic ß-cells and insulin secretion as well as secretion of other proteohormones in vitro or in perfusion experiments was inhibited by high $[Mg^{2+}]_o$ (*Zofkova* et al., 1988). Hypomagnesemia increased secretion of insulin (for literature see: *Durlach,* 1992). These results indicate Mg^{2+}-Ca^{2+} interactions in insulin secretion. However, also other mechanisms involved in the regulation of glucose metabolism are affected by high $[Mg^{2+}]_o$ (*Zofkova* et al., 1987).

Acetylcholin (ACh) release from nerve endings is brought about by Ca^{2+} entry. Mg^{2+} in the extracellular fluid competes with Ca^{2+} for active sites and depresses ACh output from motor nerve terminal and sympathetic ganglia (*Mantovani* and *Pepeu,* 1981).

The effect of Ca^{2+} on ACh release is specific. Substitution of Ca^{2+} with Mg^{2+} strongly reduced ACh release.

Mg^{2+}-Ca^{2+} interactions in release and action of parathyroid hormone (PTH)

$[Mg^{2+}]_o$ affects PTH secretion in vitro and in vivo. Mg^{2+} acted similar to Ca^{2+} in inhibiting the secretion of PTH. In most experiments Mg^{2+} was half as effective as Ca^{2+}. Either ion worked more effectively in the presence of minimum concentration of the other. This interdependence of Ca^{2+} and Mg^{2+} on the secretion of PTH would be consonant with both ions operating at separate biochemical sites, both of which must be occupied for hormone secretion (*Morrissey* and *Cohn,* 1978). However, other experiments indicated that in the absence of extracellular Ca^{2+} extracellular Mg^{2+} did not effect PTH release, whereas in the absence of Mg^{2+}, Ca^{2+} was effective, although to a less degree (*Brown* et al., 1984).

The Ca^{2+}-Mg^{2+} interaction in PTH release is complex and not completely understood. It was reported that in hereditary hypomagnesemia, administration of Mg^{2+} led to an increase in PTH secretion (for literature see: *Durlach,* 1992).

The second messenger in the inhibition of PTH release is $[Ca^{2+}]_i$, which is increased at elevated $[Ca^{2+}]_o$ and $[Mg^{2+}]_o$ (*Shoback* et al., 1983). However, influx of Ca^{2+} was required for PTH secretion (*Wallace* et al., 1983).

The action of PTH in bone is dependent on $[Mg^{2+}]_o$. In Mg^{2+}-deficient rats as well as in tissue culture at low $[Mg^{2+}]_o$ the action of PTH was reduced (*Heaton,* 1971). Probably, the function of osteoclasts was reduced at low $[Mg^{2+}]_o$.

Mg^{2+}-Ca^{2+} interactions in bone

About half of body Mg^{2+} is adsorbed to the surface of hydroxyapatite crystals in bone and part of them, dependent on age, can be mobilized. Thus bone Mg^{2+} represents a Mg^{2+} store. Mg^{2+} is adsorbed in hydroxyapatite according to Langmuir's adsorption isotherm. Mg^{2+} can compete with Ca^{2+} for the same adsorption sites at the crystal surface and thus can inhibit precipitation and crystal growth of Ca apatites (*Aoba* et al., 1992). Since the ionic radius of Mg^{2+} is two thirds that of Ca^{2+}, Mg^{2+} cannot replace Ca^{2+} isomorphously in the crystal lattice (*Messler* et al., 1990). Moreover, high $[Mg^{2+}]_o$ produced similar changes in the epiphyseal growth plate as organic Ca^{2+} antagonists by impairing Ca^{2+} influx into the cells. However, organic Ca^{2+} antagonists have no effect on mineralization (*Messler* et al., 1990).

Mg^{2+}-Ca^{2+} interactions in kidney stone formation

In in vitro experiments Mg^{2+} inhibits crystal growth rate of calcium oxalate in arteficial urine (*Achilles* and *Ulshöfer,* 1985). However, the effect of Mg^{2+} is relatively small compared to other urinary constituents e.g. Ca^{2+}, oxalate, citrate, H^+.

Mg^{2+}-Ca^{2+} interactions in membrane transport systems

1. *Na^+/Ca^{2+} antiport*
   Na^+/Ca^{2+} exchange occurs across the plasma membrane (in a ratio of 3 Na^+/1 Ca^{2+}), across the mitochondrial membrane and across the membrane of the sarcoplasmic (endoplasmic) reticulum (2 Na^+/1 Ca^{2+}).
   Mg^{2+} inhibits Na^+/Ca^{2+} exchange competitively to Ca^{2+} without being transported by this exchanger at the plasma membrane (*Lyu* et al., 1991), sarcoplasmic reticulum (*Trosper* and *Philipson,* 1983) and mitochondria (*Clark* and *Roman,* 1980). At the plasma membrane extracellular Mg^{2+} inhibits Ca^{2+} uptake via Na^+/Ca^{2+} exchange with a K_i of 80 to 100 µM (*Lyu* et al., 1991; *Bingham Smith* et al., 1987), whereas intracellular Mg^{2+} inhibits Ca^{2+} efflux via this system with a K_i of about 4 to 8 mM (*Ledvora* and *Hegyvary,* 1983; *Trosper* and *Philipson,* 1983). These different K_i are brought about by an additional influence of K^+ ions on the affinity of Mg^{2+} to the Ca^{2+} binding site in the Na^+/Ca^{2+} exchanger (*Bingham Smith* et al., 1987).
2. *Na^+/Mg^{2+} antiport*
   Na^+/Mg^{2+} antiport is not affected by intra- or extracellular Ca^{2+} (*Vormann* and *Günther,* 1993).
3. *Cellular Mg^{2+} uptake*
   Mg^{2+} uptake by isolated cardiomyocytes (*Quamme* and *Rabkin,* 1990), MDCK cells (*Quamme* and *Dai,* 1990) and hepatocytes (*Günther* and *Höllriegl,* 1993) was not significantly affected by $[Ca^{2+}]_o$ up to 5 mM.
4. *Mitochondrial Ca^{2+} uptake*
   Ca^{2+} is taken up by mitochondria via an electrogenic uniport. This transport system is inhibited by Mg^{2+}. K_i amounted to 10^{-3} M in kidney and heart mitochondriae and 1.8×10^{-3} M in liver mitochondriae (*Favaron* and *Bernardi,* 1985).

Mg^{2+}-Ca^{2+} interactions at cardiac Ca^{2+} channels

Cardiac cells contain two distinct inward Ca^{2+} currents, T-type and L-type, that can be distinguished by their voltage dependence and differential sensitivity to various substances. Mg^{2+} decreased T and L current amplitudes. The I-V (current-voltage) relationships for T and L current were shifted to more positive voltages.
T current was inhibited to a greater extent than L current by Mg^{2+}. The inhibition of T and L currents by Mg^{2+} was increased at reduced $[Ca^{2+}]_o$ (*Wu* and *Lipsius,* 1990) indicating competition between Ca^{2+} and Mg^{2+}.
Ca^{2+} channels are permeable for Ba^{2+} and Sr^{2+} but not for Mg^{2+} (*Wu* and *Lipsius,* 1990). Therefore, the competitive inhibition of Ca^{2+} current by Mg^{2+} can be explained by interaction of Mg^{2+} with charged groups of the membrane. As reported above (Mg^{2+}-Ca^{2+} interaction with PL) with increasing $[Mg^{2+}]_o$, Mg^{2+} is screened and partially bound to negatively charged groups of the cell membrane, thus reducing the surface potential. Hence, 1. less Ca^{2+} is screened near the surface of the membrane and 2. the potential shift across the membrane, which is given by the difference of the membrane and surface potential, is reduced. Consequently, voltage-gating of Ca^{2+} channels is reduced.
Additionally, Ca^{2+} and Mg^{2+} may compete for binding sites within the Ca^{2+} channel.

Mg^{2+}-Ca^{2+} interactions with vasoactive substances and organic Ca^{2+} antagonists

Increasing $[Ca^{2+}]_o$ in Krebs-Ringer bicarbonate incubation medium from 10^{-5} to 10^{-2} M induced an increase in isometric tension of some – not all – blood vessels.
When $[Mg^{2+}]_o$ was simultaneously increased from 0 to 9.6 mM a higher $[Ca^{2+}]_o$ threshold was necessary to induce contraction and at the same time maximum isometric tension was reduced in sensitive blood vessels. When $[Mg^{2+}]_o$ was lowered, ^{45}Ca influx and intracellular Ca^{2+} content were increased. High $[Mg^{2+}]_o$ decreased exchangeable and membrane-bound Ca^{2+} indicating competition between Mg^{2+} and Ca^{2+} for some functional sites (*Altura* and *Altura,* 1982).
These blood vessel preparations react to catecholamines, angiotensins, acetylcholine, serotonin, vasoactive peptides, prostaglandins, and others. The action of these substances was increased by lowering $[Mg^{2+}]_o$ and was reduced by increasing $[Mg^{2+}]_o$. Since these substances exhibit their function via Ca^{2+} influx, the Mg^{2+} interaction is mainly due to Mg^{2+}-Ca^{2+} competition. Besides this mechanism, Mg^{2+} can change the affinity of effectors to their membrane receptors (*Günther,* in press). The Mg^{2+} – Ca^{2+} interacting sites are different from the membrane sites (or channels) affected by blockers. High extracellular Mg^{2+} markedly potentiated the inhibitory effect of verapamil (10^{-6} M) on Ca^{2+}-induced contraction of rat aorta and canine coronary arteries but not those of portal vein. The synergistic effect could be due to the effect of Mg^{2+} and verapamil at two different sites. The lack of synergism in the portal vein could indicate that Mg^{2+} and verapamil might inactivate the same membrane channels involved in Ca^{2+} influx (*Altura* and *Altura,* 1984).

Mg^{2+}-Ca^{2+} interactions at smooth muscle cells

Ca^{2+} current through Ca^{2+} channels of smooth muscle cells from Taenia caeci can be fitted with a Langmuir curve with an apparent dissociation constant of 1.2 mM Ca^{2+}. Mg^{2+} was not transported by these Ca^{2+} channels, but reduced Ca^{2+} current by competition. In the presence of 5 mM Mg^{2+} the apparent dissociation constant of Ca^{2+} was increased from 1.2 to 2.9 mM (*Ganitkevich,* 1988). The negatively charged groups of the cell membrane create a surface potential. Surface charges in the vicinity (1–2 nm) of Ca^{2+} channels are essential for gating. Increasing $[Mg^{2+}]_o$ (or other divalent cations) results in reduction of the surface potential by screening and binding, as reviewed above. Besides a direct Mg^{2+}-Ca^{2+} competition at the smooth muscle cell membrane, Mg^{2+} exerts an indirect effect on vascular smooth muscle tone

via competition with Ca^{2+} at the endothelial cells, thereby changing the synthesis of endothelium-derived nitric oxide (EDNO). Whereas the direct effect of reduced $[Mg^{2+}]_o$ at the smooth muscle cells results in increased contraction through enhanced Ca^{2+} influx, the indirect effect results in relaxation, via Ca^{2+}-dependent formation and release of EDNO (*Gold* et al., 1990).

Mg^{2+}-Ca^{2+} interactions in cell adhesion

Cell – cell and cell – matrix adhesive interactions are essential to development, inflammation, hemostasis, and immune recognition. The integrins are a broadly distributed family of cell surface receptors, that contribute to these adhesive reactions by recognition of a multiplicity of extracellular matrix protein ligands including laminin, collagens, fibrinogen, fibronectin, intercellular adhesion molecules (ICAMs) and others (*Hynes,* 1992). Divalent cations are essential for receptor functions. Vitronectin binding to $\alpha_v\beta_3$ integrin was supported by Ca^{2+} and Mg^{2+}, whereas binding of fibronectin to $\alpha_v\beta_1$ was only supported by Mg^{2+}, addition of Ca^{2+} led to an inhibition of fibronectin binding (*Kirchhofer* et al., 1991).
Binding of T-lymphoblasts to ICAM-1 required the presence of either Ca^{2+} or Mg^{2+}, Mg^{2+} being more effective. Addition of both ions acted more than additively to produce maximal binding, indicating a synergistic effect of Mg^{2+} and Ca^{2+} (*Marlin* and *Springer,* 1987). Because Ca^{2+} is held very constant in the extracellular fluids, physiologically occurring fluctuations of the extracellular Mg^{2+} concentration might be important for the process of cell adhesion.

Mg^{2+}-Ca^{2+} interactions at intracellular organelles

Ca^{2+} is stored and released from compartments of sarcoplasmic and endoplasmic reticulum and mitochondria. Ca^{2+}-induced Ca^{2+} release from Ca^{2+}-loaded sarcoplasmic reticulum vesicles is almost completely inhibited by 0.3 mM Mg^{2+}. When the Mg^{2+} concentration in the medium was increased, higher Ca^{2+} concentrations were needed for activation, indicating competition of Mg^{2+} with Ca^{2+} in releasing stored Ca^{2+}. However, Ca^{2+} acts in releasing stored Ca^{2+} by binding to two or more interacting sites and it is not known whether Mg^{2+} competes at both sites (*Meissner* et al., 1986). Similarly, IP_3-induced Ca^{2+} release from mitochondria and microsomes was inhibited by Mg^{2+}. A 10-fold increase of free Mg^{2+} from 0.03 to 0.3 mM reduced the extent of Ca^{2+} release 2- to 3-fold and increased the apparent Michaelis-Menten constant of IP_3 from 0.5 to 0.8 µM. A further increase of Mg^{2+} had no additional significant effect on Ca^{2+} release (*Volpe* et al., 1990). On the other hand, Ca^{2+} uptake by rat liver microsomes was activated by Mg^{2+}, being maximal at a concentration of free Mg^{2+} of 1 mM (*Zhang* and *Kraus-Friedmann,* 1990).
Thus, $[Mg^{2+}]_i$ has a level at which Ca^{2+} release is almost maximally inhibited whereas Ca^{2+} storage is almost maximally activated. Consequently, together with the inhibition of Ca^{2+} influx by extracellular Mg^{2+}, $[Mg^{2+}]_i$ establishes a minimal $[Ca^{2+}]_i$, so that effectors can induce high changes in $[Ca^{2+}]_i$. Additionally, it was suggested that varying the extent of Mg^{2+} inhibiton e. g. by reducing the Mg^{2+} affinity and thus Mg^{2+} binding to a regulatory site of Ca^{2+} channels may be a mechanism to regulate Ca^{2+} release from Ca^{2+} stores (*Lamb* and *Stephenson,* 1992; *Zhang* and *Kraus-Friedmann,* 1990; *Jacquemond* and *Schneider,*1992).
The effect of increased $[Mg^{2+}]_i$ on Ca^{2+} induced Ca^{2+} influx and release was tested in rat thymocytes (in prep.). When $[Mg^{2+}]_i$ was increased by 0.3 mM, thapsigargin-induced increase of $[Ca^{2+}]_i$ was not influenced. On the other hand, increasing $[Ca^{2+}]_i$ from about 100 to about 400 nM (through incubation with thapsigargin) did not lead to significant changes in $[Mg^{2+}]_i$.

Mg^{2+}-Ca^{2+} interactions at Ca^{2+}-binding proteins

Ca^{2+}-binding proteins may be subdivided into two groups with distinct structural features:

1. The Ca^{2+}-modulated proteins include: calmodulin, troponin C, parvalbumin, oncomodulin, S-100 proteins, calbindins, myosin light chains, calpain, calcineurin etc. There are about 170 proteins, belonging to this family (*Heizmann* and *Hunziker,* 1991; *Strynadka* and *James,* 1989).
2. Annexins, also known as lipocortins, calcimedins, calpactins etc., interact with phospholipids and cellular membranes in a Ca^{2+}-dependent manner (*Heizmann* and *Hunziker,* 1991; *Villereal* and *Palfrey,* 1989).

Calmodulin, troponin C and the other members of this family exhibit a common structural motif, the EF-hand. The various Ca^{2+}-binding proteins of this family contain 2–8 of these domains. Each of these domains consists of a loop of 12 amino acids (a variant loop with 14 amino acids is present in the S-100 protein subfamily), 5 of which have a carboxyl (or a hydroxyl) group in their side chain and are precisely spaced to coordinate a Ca^{2+} ion or, with less affinity, a Mg^{2+} ion. The 6th ligand is a water molecule. Each loop is flanked by two α-helices.
No sequences homologous to the EF-hand sequence of Ca^{2+}-binding proteins can be found in the primary structure of the annexins, suggesting that they contain a different Ca^{2+}-binding structure. Calmodulin can bind 4 Ca^{2+} or with less affinity (K_d, $10^{-3} - 10^{-2}$ M) 4 Mg^{2+}. The four binding sites bind Ca^{2+} and Mg^{2+} competitively and show cooperativity when either Ca^{2+} or Mg^{2+} is bound (*Iida* and *Potter,* 1986).
Troponion C exhibits 6 divalent cation binding sites (*Potter* and *Gergely,* 1975; *Levine* et al., 1978): two high-affinity Ca^{2+} binding sites that also bind Mg^{2+} competitively, two sites with lower affinity for Ca^{2+} that do not bind Mg^{2+}, and two sites that bind Mg^{2+} but not Ca^{2+}.
Parvalbumins (α, β) contain 2 Ca^{2+}-Mg^{2+} loops. Ca^{2+} and Mg^{2+} compete for common binding sites (*Haiech* et al., 1979; *Strynadka* and *James,* 1989).
Oncomodulin is a parvalbumin-like protein in fetal placenta and tumor cells. Contrarily to parvalbumin, oncomodulin possesses a single high-af-

finity Ca^{2+}/Mg^{2+} site and a single low-affinity Ca^{2+}-specific site (*Hapak* et al., 1989). Mg^{2+} and Ca^{2+} compete for the high-affinity site (*Cox* et al., 1990).
Calbindin contains several Ca^{2+}-binding sites which can also bind Mg^{2+} (*Heizmann* and *Hunziker,* 1991).
The reviewed Ca^{2+}-binding proteins of this family have similar properties with respect to Ca^{2+}- and Mg^{2+}-binding. Therefore, also other members of this family expressing the EF-hand motif, which have not yet been investigated for Mg^{2+}-binding and Mg^{2+}-Ca^{2+} competition, may have similar binding properties.
After metal ion binding to these proteins, the structure is changed to a more hydrophobic surface and in consequence these proteins are bound to target proteins, thus regulating their biological activity.
Similar properties with respect to Ca^{2+}-Mg^{2+} interactions were reported for tubulin (*Mejillano* and *Himes,* 1991), actin (*Estes* et al., 1987), and gelsolin (*Doi* et al., 1990).

Mg^{2+}-Ca^{2+} interactions in enzymes

There are some hundreds of enzymes being activated by Mg^{2+} or Ca^{2+}. A systematic investigation of the Mg^{2+}-Ca^{2+} interactions in these reactions has only been done for a small number of enzymes. Therefore, only a few examples are given. In all reactions involving ATP, the true intracellular substrate is MgATP and, therefore, all ATPase- or kinase-catalysed reactions have an intrinsic need for Mg^{2+}. In the following only enzymes are considered, which are influenced by Mg^{2+} independently from the need of MgATP as a substrate. The enzymes were classified as defined in the introduction.
1. Rat heart SR (*Mahey* and *Katz,* 1990) and guinea pig pancreatic acinar plasma membrane (*Mahey* et al., 1991) Ca^{2+}-ATPase could be fully activated either by Ca^{2+} ($K_{d,rat}$, 50 μM and $K_{d,\,guinea\,pig}$, 1 μM) or Mg^{2+} ($K_{d,rat}$, 50 μM and $K_{d,\,guinea\,pig}$, 4 μM) in the complete absence of the other cation. 5 mM Ca^{2+} or Mg^{2+} activated ecto-ATPase-ADPase of rat placental tissue to about the same degree (*Pieber* et al., 1991). Ceramid kinase in HL-60 cells was half-maximally activated by either 0.3 mM Ca^{2+} or Mg^{2+} (*Kolesnick* and *Hemer,* 1990). 0.25 mM Ca^{2+} stimulated rat brain synaptic membrane phospholipase D to the same extent as 1 mM Mg^{2+} (*Chalifa* et al., 1990).
These enzymes are activated to about the same degree at similar concentration of either Ca^{2+} or Mg^{2+}. Thus, in these enzymes Ca^{2+} and Mg^{2+} have identical functions.
However, there is another mode of Ca^{2+}-Mg^{2+} synergism, in which both ions are essentially working together by operating from different sites.
The sarcoplasmic reticulum Ca^{2+}-ATPase of skeletal muscle is half-maximally activated at 0.5 mM Mg^{2+} (*Moutin* and *Dupont,* 1991; *Michelangeli,* 1990). Ca^{2+} and Mg^{2+} are simultaneously needed in a complicated, not fully understood mechanism, leading to Ca^{2+} uptake into SR. Mg^{2+} binding to the enzyme allows a conformational change of the protein, subsequently Mg^{2+} is exchanged with Ca^{2+}, which then can be transported by the ATPase.
The phospholipase A_2 of liver macrophages could not be activated by physiological concentrations of Ca^{2+} or Mg^{2+} alone, both ions together, however, led to full activation of the enzyme (K_d Ca^{2+} = 0.3 μM, in the presence of 10 mM Mg^{2+}, K_d Mg^{2+} = 1 mM in the presence of 1 μM Ca^{2+}) (*Krause* et al., 1991). Phospholipase A_2 of human platelets required 1 mM Ca^{2+} and 5 mM Mg^{2+} for full activity (*Silk* et al., 1989). Also, nuclease of rat liver, thymus and spleen were fully activated by 1 mM Ca^{2+} and 5 mM Mg^{2+} but only to a small amount by Ca^{2+} or Mg^{2+} alone (*Giannakis* et al., 1991). In a complex mechanism, activation of rat brain protein kinase C requires four essential activators: Ca^{2+}, Mg^{2+}, phosphatidylserine and diacylglycerol (*Hannun* and *Bell,* 1990). The enzyme first associates with the membrane in the presence of Ca^{2+} and phosphatidylserine, in a second step the enzyme becomes activated upon diacylglycerol and Mg^{2+} (about 1 mM) binding.
2. There are no enzymes which can be classified in this category.
3. The phosphotyrosine protein phosphatase of bovine brain is a Mg^{2+}-dependent enzyme (K_d = 20 μM). Ca^{2+} inhibits the enzyme competitively to Mg^{2+} with an IC_{50} of 0.6 μM (*Singh,* 1990). RNA-dependent DNA polymerase activity of HIV reverse transcriptase is maximally activated at 1–2 mM Mg^{2+} (*Tan* et al., 1991). Ca^{2+} competes with Mg^{2+} for a metal-binding site on this enzyme, inhibiting the activity (IC_{50} = 20 μM). Also smooth muscle phosphatase-II is a Mg^{2+}-dependent enzyme (K_d = 0.3 mM) that is competitively inhibited by Ca^{2+} (IC_{50} at 1 mM Mg^{2+} = 0.2 mM) (*Pato* and *Kerc,* 1991). Growth hormone-releasing factor-sensitive adenylate cyclase of somatotrophs requires Mg^{2+} (K_d = 3.4 mM) for basal but less amounts of Mg^{2+} (K_d = 0.5 mM) for hormone stimulated activity. Basal and stimulated activity was inhibited by Ca^{2+} (IC_{50}, basal activity = 4.2 μM, stimulated = 2.9 μM) (*Narayanan* et al., 1989).
In these examples, only Mg^{2+} acts as an activator, whereas Ca^{2+} is not able to stimulate the enzymatic activity, but, on the contrary, leads to inhibition by displacing Mg^{2+} from its binding site.
4. The Ca^{2+}-dependent α 1,2-mannosidase of rabbit liver is dependent on Ca^{2+}. Mg^{2+} neither activates nor inhibits the enzyme (*Schutzbach* and *Forsee,* 1990). Inositol 1,4,5-trisphosphate 5-phosphomonoesterase from rabbit peritoneal neutrophils is half-maximally activated by 0.28 mM Mg^{2+} (*Kennedy* et al., 1990). Ca^{2+} in concentrations up to 0.5 mM cannot activate or inhibit the enzyme.
These examples show that the effect of one ion is specific and cannot be mimicked or influenced by the other.

Concluding Remarks

The actions of Mg^{2+} are produced by binding to negatively charged ligands of substrates (ATP^{4-}, ADP^{3-}, $citrate^{3-}$), by binding to polyvalent anions (RNA, DNA) or by binding to proteins (enzymes, ion channels). In enzyme reactions, Mg^{2+} or the Mg^{2+} substrate complex is bound to the active center.

In all binding reactions Mg^{2+} and Ca^{2+} form octahedral complexes or chelates with more or less H_2O, and Mg^{2+} and Ca^{2+} compete according to their binding constants.

So far, only in a few cases, the exact structure of the octahedral Mg^{2+} substrate enzyme complex is known (*Günther*, in press). The structural and spatial relationship by which Mg^{2+}-Ca^{2+} interactions can be differentiated to the four described categories is not defined. Since the rate of substitution of H_2O molecules from the inner hydration shell by other ligands is highly different (3000 times) between Ca^{2+} and Mg^{2+}, this mechanism seems to be most probable to cause specificity for Mg^{2+} or Ca^{2+} (category 4).

Outer sphere complex formation with H_2O molecules of the inner hydration shell may yield unspecificity between Mg^{2+} and Ca^{2+} (category 1).

Taking together, there is a wide variety of Ca^{2+}-Mg^{2+} interactions in the body. Physiologically, intracellular interactions are of minor importance for regulative purposes. Intracellular Mg^{2+} is kept constant even at pathophysiological conditions, and also pharmacologically, intracellular Mg^{2+} cannot be changed.

Interactions at extracellular sites, however, are important in respect to regulative or pathophysiological processes. Extracellular Ca^{2+} ist regulated very well, whereas extracellular Mg^{2+} may fluctuate. A marked increase of the normal serum Mg^{2+} content cannot be achieved by high dietary Mg^{2+} intake but will occur during renal failure or during Mg^{2+} infusion. This Mg^{2+} increase produces Ca^{2+} antagonistic effects.

On the other hand, a reduced extracellular Mg^{2+} content occurs at insufficient dietary supply or increased renal loss of Mg^{2+} (for example during treatment with diuretics, aminoglycosides, amphotericin B, cyclosporin A, cisplatin) and will lead to symptoms that are characterized by a predominant action of Ca^{2+}.

References

Achilles, W.; Ulshöfer, B.: The effect of magnesium on the crystal growth rate of calcium oxalate in human urine. Magnesium-Bulletin 7 (1985) 114–116.

Ahlers, J.; Günther, T.: Activation and inhibition of the Mg-Ca-ATPase from E. coli by Mg^{2+} and Ca^{2+}. Z. Naturforsch. **30** (1974) 412–416.

Akerman, K. E.: Effect of Mg^{2+} and spermine on the kinetics of Ca^{2+} transport in rat-liver mitochondria. J. Bioenerg. Biomembr. **9** (1977) 65–72.

Allsop, T. F.: Transfer of magnesium across the perfused choroid plexus of sheep. Aust. J. Biol. Sci. **39** (1986) 161–169.

Altura, B. M.; Altura, B. T.: Magnesium-Calcium interaction and contraction of arterial smooth muscle: In ischemic heart diseases, hypertension, and vasoplastic disorders. In: *Wester, P. O.* (ed.): Electrolytes and the heart. TransMedica, New York 1982, pp. 41–56.

Altura, B. M.; Altura, B. T.: Microcirculatory actions and uses of naturally-occurring (magnesium) and novel synthetic calcium channel blockers. Microcirc. Endothel. Lymphatics **1** (1984) 185–220.

Aoba, T.; Moreno, E. C.; Shimoda, S.: Competitive adsorption of magnesium and calcium ions into synthetic and biological apatites. Calcif. Tissue. Int. **51** (1992) 143–150.

Bawden, J. W.; Wolkoff, A. S.: Fetal blood calcium response to maternal calcium infusion in sheep. Am. J. Obst. Gynec. **99** (1967) 55–60.

Beyenbach, K. W.: Unresolved questions of renal magnesium homeostasis. Magnesium **5** (1986) 234–247.

Bingham Smith, J.; Cragoe, Jr. E. J.; Smith, L.: Na^+/Ca^{2+} antiport in cultured arterial smooth muscle cells. J. Biol. Chem. **262** (1987) 11988–11994.

Bradbury, M. W. B.; Crowder, J.; Desai, S.; Reynolds, J. M.; Reynolds, M.; Saunders, N. R.: Electrolyte and water in the brain and cerebrospinal fluid of the foetal sheep and guinea pig. J. Physiol. **227** (1972) 591–610.

Brown, E. M.; Thatcher, J. G.; Watson, E. J.; Leombruno, R.: Extracellular calcium potentiates the inhibitory effects of magnesium on parathyroid function in dispersed bovine parathyroid cells. Metabolism **33** (1984) 171–175.

Caddell, J.; Kupiecki, R.; Proxmire, D. L.; Satoh, P.: Plasma catecholamines in acute magnesium deficiency in weanling rats. J. Nutr. **116** (1986) 1896–1901.

Chalifa, V.; Möhn, H.; Liscovitch, M.: A neutral phospholipase D activity from rat brain synaptic plasma membranes. J. Biol. Chem. **265** (1990) 17512–17519.

Chutkow, J. G.; Meyers, S.: Chemical changes in the cerebrospinal fluid and brain in magnesium deficiency. Neurology **18** (1968) 963–974.

Clark, A. F.; Roman, I. J.: Mg^{2+} interaction of Na^+ stimulated Ca^{2+} release from brain mitochondria. J. Biol. Chem. **255** (1980) 6556–6588.

Cox, J. A.; Milos, M.; McManus, J. P.: Calcium- and magnesium-binding properties of oncomodulin. J. Biol. Chem. **265** (1990) 6633–6637.

Derewlany, L. O.; Radde, I. C.: Transplacental ^{45}Ca and ^{32}P flux in the guinea pig: effect of maternal hypercalcemia and hypocalcemia. Can. J. Physiol. Pharmacol. **63** (1985) 1577–1580.

Doi, Y.; Kim, F.; Kido, S.: Weak binding of divalent cations to plasma gelsolin. Biochem. **29** (1990) 1392–1397.

Durlach, J.: Magnesium in der klinischen Praxis. Gustav Fischer Verlag, Jena, Stuttgart 1992, S. 37–42.

Eigen, M.; Hammes, G. G.: Elementary steps in enzyme reactions. In: *Nord, F.* (ed.): Adv. Enzymol. 25, John Wiley & Sons, New York, London 1963, pp. 1–38.

Estes, J. E.; Selden, L. A.; Gershman, L. C.: Tight binding of divalent cations to monomeric actin. J. Biol. Chem. **262** (1987) 4952–4957.

Favaron, M.; Bernardi, P.: Tissue specific modulation of the mitochondrial calcium uniporter by magnesium ions. FEBS Lett. **183** (1985) 260–264.

Garel, J.-M.; Barlet, J.-P.: Calcium metabolism in newborn animals: The interrelationship of calcium, magnesium, and inorganic phosphorus in newborn rats, foals, lambs, and calves. Pediat. Res. **10** (1976) 749–754.

Ganitkevich, V. A.; Shuba, M. F.; Smirnov, S. V.: Saturation of calcium channels in single isolated smooth muscle cells of guinea-pig taenia caeci. J. Physiol. **399** (1988) 419–436.

Giannakis, C.; Forbes, I. J.; Zalewski, P. D.: Ca^{2+}/Mg^{2+}-dependent nuclease: Tissue distribution, relationship to internucleosomal DNA fragmentation and inhibition by Zn^{2+}. Biochem. Biophys. Res. Comm. **181** (1991) 915–920.

Gilles-Baillien, M.; Cogneau, M.: Magnesium uptake by intestinal brush-border membranes of spontaneously hypertensive rats. Proc. Soc. Exp. Biol. Med. **201** (1992) 119–124.

Gold, M. E.; Buga, G. M.; Wood, K. S.; Byrns, R. E.; Chaudhuri, G.; Ignarro, L. J.: Antagonistic modulatory roles of magnesium and calcium on release of endothelium-derived relaxing factor and smooth muscle tone. Circ. Res. **66** (1990) 355–366.

Günther, T.: Biochemistry and pathobiochemistry of magnesium. Magnesium-Bulletin **1** (1981) 91–101.

Günther, T.: Magnesium-protein interactions. In: *Berthon, G.* (ed.): Handbook of metal-ligand interactions in biological fluids. Marcel Dekker, New York and Basel, in press.

Günther, T.; Höllriegl, V.: Na^+- and anion-dependent Mg^{2+} influx in isolated hepatocytes. Biochem. Biophys. Acta **1149** (1993) 49–54.

Günther, T.; Ising, H.; Merker, H. J.: Elek-

trolyt- und Kollagengehalt von Rattenherzen bei chronischem Magnesium-Mangel und Streß. J. Clin. Chem. Clin. Biochem. **16** (1978) 293–297.

Günther, T.; Vormann, J.; Höllriegl, V.: Effects of amiloride and furosemide on ^{28}Mg transport into fetuses and maternal tissues of rats. Magnesium-Bulletin **10** (1988) 34–37.

Haiech, J.; Derancourt, J.; Pechre, J.-F.; Demaille, J. G.: Magnesium and calcium binding to parvalbumins: Evidence for differences between parvalbumins and an explanation of their relaxing functions. Biochem. **18** (1979) 2752–2758.

Hannun, Y. A.; Bell, R. M.: Rat brain protein kinase C. J. Biol. Chem. **265** (1990) 2962–2972.

Hapak, R. C.; Lammers, P. J.; Palmisano, W. A.; Birnbaum, E. R.; Henzl, M. T.: Site-specific substitution of glutamate for aspartate at position 59 of rat oncomodulin. J. Biol. Chem. **264** (1989) 18751–18760.

Hardwick, L. L.; Jones, M. R.; Brautbar, N.; Lee, D. B. N.: Magnesium absorption: Mechanism and the influence of vitamin D, calcium and phosphate. J. Nutr. **121** (1991) 13–23.

Heaton, F. W.: The physiological relation between magnesium and the skeleton. In: *Durlach, J.* (ed.): 1st International symposium on magnesium deficit in human pathology. SGEMV Publisher, Vittel 1971, pp. 165–174.

Heizmann, C. W.; Hunziker, W.: Intracellular calcium-binding proteins: more sites than insights. Trends. Biochem. Sci. **16** (1991) 98–103.

Hendrickson, H. S.; Fullington, J. G.: Stabilities of metal complexes of phospholipids: Ca(II), Mg(II), and Ni(II) complexes of phosphatidylserine and triphosphoinositide. Biochem. **4** (1965) 1599–1605.

Hynes, R. O.: Integrins: Versatility, modulation, and signaling in cell adhesion. Cell **69** (1992) 11–25.

Iida, S.; Potter, J. D.: Calcium binding to calmodulin. Cooperativity of the calcium-binding site. J. Biochem. **99** (1986) 1765–1772.

Jacquemond, V.; Schneider, M. F.: Low myoplasmic Mg^{2+} potentiates calcium release during depolarization of frog skeletal muscle fibers. J. Gen. Physiol. **100** (1992) 137–154.

Jorgensen, C. K.: Electric polarizability, innocent ligands and spectroscopic oxidation states. In: *Pearson, R. G.* (ed.): Hard and soft acids and bases. Dowden, Hutchinson & Ross, Inc., Stroudsburg, Pennsylvania 1973, pp. 135–149.

Kachmar, J.; Boyer, P. D.: Kinetic analysis of enzyme reactions. J. Biol. Chem. **200** (1953) 669–682.

Karbach, U.: Magnesium transport across colon ascendens of the rat. Dig. Dis. Sci. **34** (1989) 1825–1831.

Karbach, U.; Rummel, W.: Cellular and paracellular magnesium transport across the terminal ileum of the rat and its interaction with the calcium transport. Gastroent. **98** (1990) 985–992.

Karbach, U.; Schmitt, A.; Hakan Saner, F.: Different mechanism of magnesium and calcium transport across rat duodenum. Dig. Dis. Sci. **36** (1991) 1611–1618.

Kennedy, S. P.; Ramadan, I. S.; Becker, E. L.: Purification and characterization of soluble inositol 1,4,5-trisphosphate 5-phosphomonoesterase from rabbit peritoneal neutrophils. J. Leukoc. Biol. **47** (1990) 535–544.

Kirchhofer, D.; Grzesiak, J.; Pierschbacher, M. D.: Calcium as a potential physiological regulator of integrin-mediated cell adhesion. J. Biol. Chem. **266** (1991) 4471–4477.

Kolesnick, R. N.; Hemer, M. R.: Characterization of a ceramide kinase activity from human leukemia (HL-60) cells. J. Biol. Chem. **265** (1990) 18803–18808.

Krause, H.; Dieter, P.; Schulze-Specking, A.; Ballhorn, A.; Ferber, E.; Decker, K.: Synergistic effect of magnesium and calcium ions in activation of phospholipase A_2 of liver macrophages. Biochem. Biophys. Res. Comm. **175** (1991) 532–536.

Lamb, G. D.; Stephenson, D. G.: Importance of Mg^{2+} in excitation-contraction coupling in skeletal muscle. NIPS 7 (1992) 270–274.

Lau, A.; McLaughlin, A.; McLaughlin, S.: The adsorption of divalent cations to phosphatidylglycerol bilayer membranes. Biochim. Biophys. Acta **645** (1981) 279–292.

Ledvora, R. F.; Hegyvary, C.: Dependence of Na^+/Ca^{2+} exchange and Ca^{2+}-Ca^{2+} exchange on monovalent cations. Biochim. Biophys. Acta **729** (1983) 123–136.

Levine, B. A.; Thornton, J. M.; Fernandes, R.; Kelly, C. M.; Mercola, D.: Comparison of the calcium- and magnesium-induced structural changes of troponin-C. Biochim. Biophys. Acta **535** (1978) 11–24.

Lourdes Cruz, M.; Mimouni, F.; Tsang, R. C.; Hammond, G.: Effect of chronic maternal dietary magnesium deficiency on placental calcium transport. J. Am. Coll. Nutr. **11** (1992) 87–92.

Lyu, R.-M.; Smith, L.; Bingham Smith, J.: Sodium-calcium exchange in renal epithelial cells: Dependence on cell sodium and competitive inhibition by magnesium. J. Membrane Biol. **124** (1991) 73–83.

Macdonald, P. M.; Seelig, J.: Calcium binding to mixed cardiolipin-phosphatidylcholine bilayers as studied by deuterium nuclear magnetic resonance. Biochem. **26** (1987) 6292–6298.

Mahey, R.; Bridges, M. A.; Katz, S.: Relationship between Ca^{2+}-transport and ATP hydrolytic activities in guinea-pig pancreatic acinar plasma membranes. Mol. Cell Biochem. **105** (1991) 137–147.

Mahey, R.; Katz, S.: A non-specific Ca^{2+}(or Mg^{2+})-stimulated ATPase in rat heart sarcoplasmic reticulum. Mol. Cell Biochem. **96** (1990) 175–182.

Mantovani, P.; Pepeu, G.: Interactions between ionophores, Mg^{2+} and Ca^{2+} on acetylcholine formation and release in brain slices. Pharmacol. Res. Comm. **13** (1981) 175–184.

Marlin, S. D.; Springer, T. A.: Purified intercellular adhesion molecule-1 (ICAM-1) is a ligand for lymphocyte function-associated antigen 1 (LFA-1). Cell **51** (1987) 813–819.

Martell, A. E.; Calvin, M.: Chemistry of the Metal Chelate Compounds. Prentice-Hall Inc., Englewood Cliffs, N. J. 1956.

McLaughlin, S.; Mulrine, N.; Gresalfi, T.; Vaio, G.; McLaughlin, A. C.: Adsorption of divalent cations to bilayer membranes containing phosphatidylserine. J. Gen. Physiol. **77** (1981) 445–473.

Meissner, G.; Darlind, E.; Eveleth, J.: Kinetics of rapid Ca^{2+} release by sarcoplasmic reticulum. Effect of Ca^{2+}, Mg^{2+}, and adenine nucleotides. Biochem. **25** (1986) 236–244.

Mejillano, M. R.; Himes, R. H.: Binding of guanine nucleotides and Mg^{2+} to tubulin with a nucleotide-depleted exchangeable site. Arch. Biochem. Biophys. **291** (1991) 356–362.

Messler, H. H.; Koch, W.; Münzenberg, K. J.: Analogous effects of organic calcium antagonists and magnesium on the epiphyseal growth plate. Clin. Orthopaed. Rel. Res. **258** (1990) 135–141.

Michelangeli, F.; Colyer, J.; East, J. M.; Lee, A. G.: Effect of pH on the activity of the Ca^{2+} + Mg^{2+}-activated ATPase of sarcoplasmic reticulum. Biochem. J. **267** (1990) 423–429.

Mordes, J. P.; Wacker, W. E. C.: Excess magnesium. Pharmacol. Rev. **29** (1978) 273–300.

Morrissey, J. J.; Cohn, D. V.: The effects of calcium and magnesium on the secretion of parathormone and parathyroid secretory protein by isolated porcine parathyroid cells. Endocrinol. **103** (1978) 2081–2090.

Moutin, M.-J.; Dupont, Y.: Interaction of potassium and magnesium with the high affinity calcium-binding sites of the sarcoplasmic reticulum calcium-ATPase. J. Biol. Chem. **266** (1991) 5580–5586.

Nachold, F. C.; Wood, W.: The reaction velocity of ion exchange. J. Am. Chem. Soc. **67** (1945) 629–631.

Narayanan, N.; Lussier, B.; French, M.; Moor, B.; Kraicer, J.: Growth hormone-releasing factor-sensitive adenylate cyclase system of purified somatotrophs: Effects of guanine nucleotides, somatostatin, calcium and magnesium. Endocrinol. **124** (1989) 484–495.

Ohki, S.; Sauve, R.: Surface potential of phosphatidylserine monolayers. Biochim. Biophys. Acta **511** (1978) 377–387.

Padgham, C.; Birch, N. J.; Davie, R. J.; Phillips, J. D.: Magnesium uptake, transport and pharmacological effects. In: *Birch, N. J.* (ed.): Magnesium and the cell. Academic press, London 1993, pp. 263–279.

Pato, M. D.; Kerc, E.: Regulation of smooth muscle phosphatase-II by divalent cations. Mol. Cell Biochem. **101** (1991) 31–41.

Pauling, L.: Struktur der chemischen Bindung. 2. Aufl., Verlag Chemie, Weinheim 1964.

Pearson, R. G.: Hard and soft acids and bases. J. Am. Chem. Soc. **85** (1963) 3533–3539.

Pieber, M.; Valenzuela, M. A.; Kettlun, A. M.; Mancilla, M.; Aranda, E.; Collados, L.; Traverso-Cori, A.: ATPase-ADPase activities of rat placental tissue. Comp. Biochem. Physiol. **100 B** (1991) 281–285.

Portis, A.; Newton, C.; Pangborn, W.; Papahadjopoulos, D.: Studies on the mechanism of membrane fusion: Evidence for an intermembrane Ca^{2+}-phospholipid complex, synergism with Mg^{2+}, and inhibition by spectrin. Biochem. **18** (1979) 780–790.

Potter, J. D.; Gergely, J.: The calcium and magnesium binding sites in troponin and their role in the regulation of myofibrillar adenosine triphosphase. J. Biol. Chem. **250** (1975) 4628–4633.

Quamme, G. A.; Dai, L.-J.: Presence of a novel influx pathway for Mg^{2+} in MDCK cells. Am. J. Physiol. **259** (1990) C521–C525.

Quamme, G. A.; Rabkin, S. W.: Cytosolic free magnesium in cardiac myocytes: Identification of a Mg^{2+} influx pathway. Biochem. Biophys. Res. Commun. **167** (1990) 1406–1412.

Quamme, G. A.; de Rouffignac, C.: Transport of magnesium in renal epithelial cells. In: *Birch, N. J.* (ed.): Magnesium and the cell. Academic press, London 1993, pp. 235–262.

Schutzbach, J. S.; Forsee, W. T.: Calcium ion activation of rabbit liver α1,2-mannosidase. J. Biol. Chem. **265** (1990) 2546–2549.

Shaw, A. J.; Mughal, M. Z.; Maresh, M. J.; Sibley, C. P.: Sodium-dependent magnesium transport across in situ perfused rat placenta. Am. J. Physiol. **261** (1991) R369–R372.

Shoback, D.; Thatcher, J.; Leombruno, R.; Brown, E.: Effects of extracellular Ca^{2+} and Mg^{2+} on cytosolic Ca^{2+} and PTH release in dispersed bovine parathyroid cells. Endocrinol. **113** (1983) 424–426.

Silk, S. T.; Cleja, S.; Witkom, K.: Evidence of GTP-binding protein regulation of phospholipase A_2 activity in isolated human platelet membranes. J. Biol. Chem. **264** (1989) 21466–21469.

Singh, T. S.: Characterization of a bovine brain magnesium-dependent phosphotyrosine protein phosphatase that is inhibited by micromolar concentrations of calcium. Biochem. Biophys. Res. Comm. **167** (1990) 621–627.

Sokolove, P. M.; Brenza, J. M.; Shamoo, A. E.: Ca^{2+}-cardiolipin interaction in a model system. Selectivity and apparent high affinity. Biochim. Biophys. Acta **732** (1983) 41–47.

Storch, J.; Schachter, D.: Calcium alters the acyl chain composition and lipid fluidity of rat hepatocytes plasma membranes in vitro. Biochim. Biophys. Acta **812** (1985) 473–484.

Strynadka, N. C. J.; James, M. N. G.: Crystal structure of the helix-loop-helix calcium binding proteins. Ann. Rev. Biochem. **58** (1989) 951–998.

Tan, C.-K.; Zhang, J.; Li, Z.-Y.; Tarpley, W. G.; Downey, K. M.; So, A. G.: Functional characterization of RNA-dependent DNA polymerase and RNase H activities of a recombinant HIV reverse transcriptase. Biochem. **30** (1991) 2651–2655.

Tilcock, C. P. S.; Cullis, P. R.: The polymorphic phase behaviour of mixed phosphatidylserine-phosphatidylethanolamine model systems as detected by ^{31}P-NMR. Biochim. Biophys. Acta **641** (1981) 189–201.

Tokutoni, S.; Lew, R.; Onishi, S.: Ca^{2+}-induced phase separation in phosphatidylserine phosphatidylethanolamine and phosphatidylcholine mixed membranes. Biochim. Biophys. Acta **643** (1981) 271–282.

Trosper, T. L.; Philipson, K. D.: Effects of divalent and trivalent cations on Na^+/Ca^{2+} exchange in cardiac sarcolemmal vesicles. Biochim. Biophys. Acta **731** (1983) 63–68.

Vail, W. J.; Stollery, J. G.: Phase changes of cardiolipin vesicles mediated by divalent cations. Biochim. Biophys. Acta **551** (1989) 74–84.

Villereal, M. L.; Palfrey, H. C.: Intracellular calcium and cell function. Ann. Rev. Nutr. **9** (1979) 347–376.

Volpe, P.; Alderson-Lang, B. H.; Nickols, G. A.: Regulation of inositol 1,4,5-trisphosphate-induced Ca^{2+} release. I. Effect of Mg^{2+}. Am. J. Physiol. **258** (1990) C1077–C1085.

Vormann, J.; Günther, T.: Development of fetal mineral and trace element metabolism in rats with normal as well as magnesium- and zinc-deficient diets. Biol. Trace. Elem. Res. **9** (1986) 37–59.

Vormann, J.; Günther, T.: Magnesium transport mechanisms. In: *Birch, N. J.* (ed.): Magnesium and the cell. Academic press, London 1993, pp. 137–155.

Wallace, J.; Pintado, E.; Scarpa, A.: Parathyroid hormone secretion in the absence of extracellular free Ca^{2+} and transmembrane Ca^{2+} influx. FEBS Lett. **151** (1983) 83–88.

Watson, K. V.; Moldow, C. F.; Ogburn, P. L.; Jacob, H. S.: Magnesium sulfate: Rationale for its use in preeclampsia. Proc. Natl. Acad. Sci. **83** (1986) 1075–1078.

Williams, R. J. P.: The enzymes. Vol. 1, Academic Press, New York 1959, pp. 392–441.

Wilschut, J.; Düzgünes, N.; Papahadjopoulos, D.: Calcium/magnesium specificity in membrane fusion: Kinetics of aggregation and fusion of phosphatidylserine vesicles and the role of bilayer curvature. Biochem. **20** (1981) 3126–3133.

Woods, K. L.; Fletcher, S. F.; Roffe, C.; Haider, Y.: Intravenous magnesium sulphate in suspected acute myocardial infarction: Results of the second Leicester intraveneous magnesium intervention trial (LIMIT-2). Lancet **339** (1992) 1553–1558.

Wu, J.; Lipsius, S. L.: Effects of extracellular Mg^{2+} on T- and L-type Ca^{2+} currents in single atrial myocytes. Am. J. Physiol. **259** (1990) H1842–H1850.

Zhang, G. H.; Kraus-Friedmann, N.: The effects of Mg^{2+} on rat liver microsomal Ca^{2+} sequestration. Cell Calcium **11** (1990) 397–403.

Zofkova, I.; Zamrazil, V.; Simeckova, A.: Acute hypermagnesemia retards glucose assimilation and inhibits insulin secretion during intravenous glucose tolerance test (IVGTT). Horm. Metabol. Res. **20** (1988) 120–121.

Zofkova, I.; Lamberg-Allardt, C.; Kancheva, R. L.; Starka, L.: Effect of hypermagnesemia on the adenohypophyseal-gonadal function, parathyroid hormone secretion and some other hormonal indicators. Horm. Metab. Res. **25** (1993) 29–33.

Zofkova, I.; Nedvidkova, J.; Zamrazil, V.; Simeckova, A.: Influence of magnesium on glucose tolerance. Acute hypermagnesemia reduces the glucose tolerance independently on hormonal indicators. Horm. Metabol. Res. **19** (1987) 228–229.

Correspondence to:
PD Dr. *J. Vormann,* Institut für Molekularbiologie und Biochemie, Freie Universität Berlin, Arnimallee 22, D-14195 Berlin, Germany.