Mg^{2+} modulates membrane sphingolipid and lipid second messenger levels in vascular smooth muscle cells

Gene A. Morrill[a], Raj K. Gupta[a], Adele B. Kostellow[a], Gui-Ying Ma[a], Amin Zhang[b], Bella T. Altura[b,c], Burton M. Altura[b,c,d,*]

[a] *Department of Physiology and Biophysics, Albert Einstein College of Medicine, Bronx, NY 10461, USA*
[b] *Department of Physiology, State University of New York, Health Science Center at Brooklyn, 450 Clarkson Avenue, Brooklyn, NY 11203, USA*
[c] *Department of Medicine, State University of New York, Health Science Center at Brooklyn, 450 Clarkson Avenue, Brooklyn, NY 11203, USA*
[d] *Center for Cardiovascular and Muscle Research, State University of New York, Health Science Center at Brooklyn, 450 Clarkson Avenue, Brooklyn, NY 11203, USA*

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Abstract In vitro studies with smooth muscle cells from rat aorta and dog cerebral blood vessels indicate that variation in free Mg^{2+}, within the pathophysiological range of Mg^{2+} concentrations, found in human serum, causes sustained changes in membrane phospholipids and lipid second messengers. Incorporation of [^{3}H]palmitic acid into phosphatidylcholine (PC) and sphingomyelin (SM) was altered within 15–30 min after modifying the extracellular Mg^{2+} ion level ($[Mg^{2+}]_o$). Decreased Mg^{2+} produced a fall in both [^{3}H]SM and [^{3}H]PC over the first 2 h. After an 18-h incubation, the [^{3}H]PC/[^{3}H]SM ratio changed from about 20:1 to about 50:1. Increased $[Mg^{2+}]_o$ resulted in a 2- to 3-fold increase in [^{3}H]SM compared to only a small increase in [^{3}H]PC over the same period. There was a reciprocal relationship between [^{3}H]ceramide and [^{3}H]1,2-DAG levels with highest [^{3}H]ceramide and lowest [^{3}H]-1,2-DAG levels seen at lowest $[Mg^{2+}]_o$. The results indicate that a fall in extracellular ionized Mg^{2+} concentration produces a rapid and sustained decrease in membrane sphingomyelin and a moderate rise in intracellular ceramide. A major effect of lowering $[Mg^{2+}]_o$ appears to be a down-regulation of SM synthase. The increased membrane SM content and a concomitant decrease in cell ceramide, in the presence of elevated $[Mg^{2+}]_o$, may be relevant to the apparent protective role of adequate Mg intake on vascular function in humans.

Key words: Magnesium; Phospholipid; Sphingomyelin; Ceramide; 1,2-Diacylglycerol; Smooth muscle

1. Introduction

Both animal and human studies indicate a statistical relationship between a reduced dietary intake of magnesium and cardiovascular disease [1–6]. It is known that the responsiveness of vascular smooth muscle to contractile stimuli in vitro is increased as extracellular ionized Mg^{2+} ($[Mg^{2+}]_o$) is lowered. Conversely, increased $[Mg^{2+}]_o$ relaxes vascular smooth muscle and improves cardiovascular performance [7,8]. These observations, plus clinical data indicating that patients presenting with severe cardiovascular problems frequently have low serum $[Mg^{2+}]_o$ [9,10], have formed the rationale for the therapeutic administration of Mg salts [6,11].

The range for total blood Mg in healthy human subjects extends from 0.70 to 0.96 mM but concentrations of free ionized Mg^{2+} in humans and many mammals [12] average about 0.6 mM, and the normal range is only about 0.54–0.65 mM [9,13]. Physiological effects of Mg deficiency can be fully explained only by identifying metabolic pathways that are particulary sensitive to small reductions in $[Mg^{2+}]_o$; large reductions would obviously cause widespread damage by downregulating a multitude of biochemical pathways which require Mg^{2+} as a cofactor.

We have previously shown that as $[Mg^{2+}]_o$ is lowered within the pathophysiological range found in human serum (0.3–0.54 mM), fatty acid chain length and double bond content are progressively reduced in aortic and cerebrovascular smooth muscle lipids, indicating peroxidative degradation of fatty acid double bonds and truncation of the fatty acids in the *sn*-2 (middle carbon of glycerol) position [14,15]. We have now measured the uptake of [^{3}H]palmitic acid into the major phospholipid fractions of vascular smooth muscle as a function of $[Mg^{2+}]_o$. Palmitic acid (C 16:0) is the predominant fatty acid moiety in most mammalian sphingomyelin and a common fatty acid in the *sn*-1 position (outer carbon of the glycerol moiety) of most phospholipids (reviewed in [16]). We find that turnover of all the phospholipids increases when $[Mg^{2+}]_o$ levels are above 0.6 mM, and decreases when levels are below 0.6 mM. The greatest decrease is seen in sphingomyelin (SM), affecting the turnover of two lipid second messengers, ceramide and 1,2-diacylglycerol (1,2-DAG) which are related to SM synthesis.

The metabolic interrelationships between phosphatidylcholine (PC), SM, 1,2-DAG and ceramide are reviewed in [16,17]. SM synthesis, catalyzed by SM synthase, requires transfer of a choline phosphate moiety from PC to ceramide, generating 1,2-DAG. Conversely, sphingomyelinase (SMase) regenerates ceramide and choline phosphate. Our experiments indicate that as $[Mg^{2+}]_o$ is lowered from 0.6 mM to 0.3 mM (the pathophysiological range), the ratio of PC to SM nearly doubles; ceramide labeling increases, 1,2-DAG labeling falls. This indicates that low serum ionized Mg^{2+} may inhibit the synthesis of sphingomyelin.

2. Materials and methods

2.1. Materials

Phospholipid standards were obtained from Sigma, St. Louis, MO, USA, [9,10-^{3}H(N)]palmitic acid (60 Ci/mmol) from Amersham, Arlington Heights, IL, USA, and aminopropylsilyl minicolumns from Altec, Deerfield, IL, USA.

2.2. Tissue preparation and incubation

Adult male Wistar rats (16–20 weeks old, 275–325 g) were killed by decapitation and exsanguinated. Thoracic aortae were excised, imme-

*Corresponding author. Fax: (1) (718) 270-3103.

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diately placed in normal Krebs-Ringer bicarbonate (NKRB) solution at room temperature, cleaned of blood, loose connective tissue and fat, and the aortae cut into rings about 2–3 mm long [18]. Endothelial cells were rubbed off with wet filter paper. NKRB composition was (in mM): NaCl 118, KCl 4.7, $KHPO_4$ 1.2, $MgSO_4$ 1.2, $CaCl_2$ 2.5, glucose 10 and $NaHCO_3$ 25. Concentrations of ionized Mg^{2+} in culture media were determined using an ion selective electrode (NOVA Biomedical, MA, USA) [9].

Male mongrel dogs (15 ± 3 kg) were anesthetized with sodium pentobarbital (40 mg/kg, i.v.) and sacrificed by bleeding from the common femoral arteries. After craniotomy the brains were rapidly removed, placed in normal Krebs-Ringer bicarbonate solution (NKRB) at room temperature and basilar and middle cerebral arteries excised and cleaned of arachnoid membranes and blood elements. The vessels were cut into segments about 3–4 mm in length [19]. Control experiments showed that cerebrovascular or aortic smooth muscle tissue denuded of endothelial cells maintain normal ion gradients, a normal response to contractile stimuli and exclude trypan blue for at least 36 h under these conditions.

2.3. Incubation of tissue in ^3H-labeled medium

Up to 50 mg wet weight of tissue was incubated in 10 ml of NKRB solution containing [^{3}H]palmitic acid (4–20 μCi/ml) at 37°C for 3 h, rinsed with fresh NKRB solution and transferred to NKRB containing the Mg^{2+} concentrations described below. Isotope concentration in the medium was not rate-limiting and was used without carrier addition.

2.4. Primary culture of vascular smooth muscle cells

Aortic smooth muscle cells from adult Wistar rats (200–250 g) were isolated according to previous established methods and cultured in Dulbecco's modified Eagle's medium at 37°C in a humidified atmosphere composed of 95% air-5% CO_2 [20]. Coverslips containing a monolayer of primary cultured cells were labeled with [^{3}H]palmitic acid containing culture medium for 3 h under 95% air-5% CO_2. The labeled cells were washed for 5 min with unlabeled medium, transferred to culture medium containing differing $[Mg^{2+}]_o$ and incubated for 18 h under 95% air-5% CO_2.

2.5. Extraction of diacylglycerol and ceramide

Lipid extraction was performed using a modification of the procedure of Bocckino et al. [21]. Tissue samples were homogenized in 4 volumes of chloroform/methanol (1:2, v/v). Suspensions were quantitatively transferred to 15 ml glass-stoppered centrifuge tubes, using successive 0.3-ml aliquots of water and chloroform to rinse the homogenizer. The suspension was vortexed for 1 min, and centrifuged at $2000 \times g$ for 5 min. Upper aqueous and lower lipid-containing phases were separated, the interfacial material washed twice with 0.35 ml volumes of chloroform and the upper and lower phases separated and pooled with the first fractions. The lower chloroform phase was washed twice with fresh upper phase and taken to dryness under N_2. Crude lipids were dissolved in hexane and applied to aminopropylsilyl minicolumns and the neutral lipids, free fatty acids and N-containing phospholipids eluted successively with chloroform, methanol and ethanol. Lipids were stored in chloroform containing 0.05% BHT at −35°C under N_2.

2.6. Phospholipid analysis

Sphingomyelin, phosphatidylethanolamine, phosphatidylinositol and phosphatidylcholine in the lipid extracts were separated by TLC using LK5 silica gel 150A plates (Whatman Laboratory Division, Clifton, NJ, USA) and developed with $CHCl_3$/ethanol/water/tetraethylammonium (30:34:8:35, v/v) as described by Touchstone et al. [22], or by the two-dimensional system of Skidmore and Entemann [23]. Ceramide was separated on silica gel H plates (Analtech, Newark, DE, USA) and developed in diethylether/methyl alcohol (99:2). 1,2-DAG was separated on silica gel G plates developed in a solvent system of toluene/diethyl ether/ethanol/ammonium hydroxide (50:40:2:0.2, v/v). Lipids were visualized with I_2 vapor, scraped from the plate, and either transferred directly to counting vials containing BCS scintillant (Amersham, Arlington Heights, IL, USA) or eluted with chloroform/methanol (2:1) and aliquots counted in a Beckman LS 7500 Liquid Scintillation System. Sonication for 1 min facilitated extraction into the BCS scintillant. Recovery of total dpm applied to the TLC plates was greater than 96%.

2.7. Total lipid analysis by ^1H-NMR

^{1}H-NMR spectra were obtained on a Varian VXR-500 spectrometer, as previously described [14,15]. Total phospholipid content of tissue and cell extracts was determined using resonances unique to lipids [24].

Where appropriate, means ± SD were calculated and examined for statistical significance by means of ANOVA. Values of $P < 0.05$ were considered to be statistically significant.

3. Results

3.1. Low $[Mg^{2+}]_o$ changes membrane phospholipid and lipid second messenger turnover in vascular smooth muscle cells within minutes

Segments of cerebrovascular smooth muscle tissue, denuded of endothelial cells, were preincubated in NKRB solution containing 0.6 mM ionized Mg^{2+} plus [^{3}H]palmitic acid for 3 h at 37°C. About 25% of the total labeled palmitic acid taken up into the tissue was recovered in the free fatty acid pool. For comparison, about 45% was recovered in the neutral lipid fraction (triglyceride, 1,2-DAG, ceramide) and about 25% was taken up into the basic phospholipids (PC, phosphatidylethanolamine (PE), SM). Only about 5% of the total palmitic acid-derived label was recovered in the acidic phospholipid fraction (phosphatidylinositol (PI), phosphatidic acid).

After a 3-h uptake period in 0.6 mM $[Mg^{2+}]_o$ and [^{3}H]palmitate, cerebrovascular smooth muscle segments pooled from 4 dogs were rinsed in fresh NKRB containing 0.6 mM $[Mg^{2+}]_o$ and transferred to palmitate-free NKRB containing either low (0.17 mM) or elevated (1 2 mM) $[Mg^{2+}]_o$. Fig. 1A compares the changes in [^{3}H]phosphatidylcholine (PC) as a function of time during the 2-h period following transfer. ^{3}H-labeled PC decreased by about 25% following transfer to low (0.17 mM) $[Mg^{2+}]_o$ whereas a slight rise in ^{3}H-labeled PC occurred following transfer to elevated

Table 1
[^{3}H]Palmitic acid incorporation[a] into rat aortic smooth muscle cell lipids as a function of extracellular Mg^{2+} ($[Mg^{2+}]_o$) during an 18-h incubation period

Mg^{2+} status[b]	Mg^{2+} (mM)	PC	SM	PE	PI
Low	0.17	754 (83%)	14.6 (1.7%)	41.1 (4.5%)	2.40 (0.27%)
Normal	0.6	848 (80%)	35.8 (3.3%)	60.1 (5.7%)	6.70 (0.63%)
High	1.2	958 (79%)	45.6 (3.7%)	75.1 (6.2%)	8.20 (0.67%)
Mg^{2+} therapy dosage	4.8	1007 (76%)	57.0 (4.4%)	91.1 (6.9%)	18.9 (1.4%)

[a]dpm per mg tissue wet wt. 64, 75, 86 and 93% of the total labeled palmitate taken up into the tissue was recovered in the lipid-bound fraction in 0.17, 0.6, 1.2 and 4.8 mM $[Mg^{2+}]_o$, respectively. The increasing lipid incorporation of radioactivity from palmitate reflects increased incorporation of this fatty acid into lipids with increasing $[Mg^{2+}]_o$. The numbers in parentheses refer to % of lipid-bound radioactivity; these account for approximately 90% of the total lipid-bound radioactivity. The remainder, approximately 10% of the lipid-bound activity, was resident in components not assayed here.

[b]Relative to human serum ionized Mg^{2+} levels.

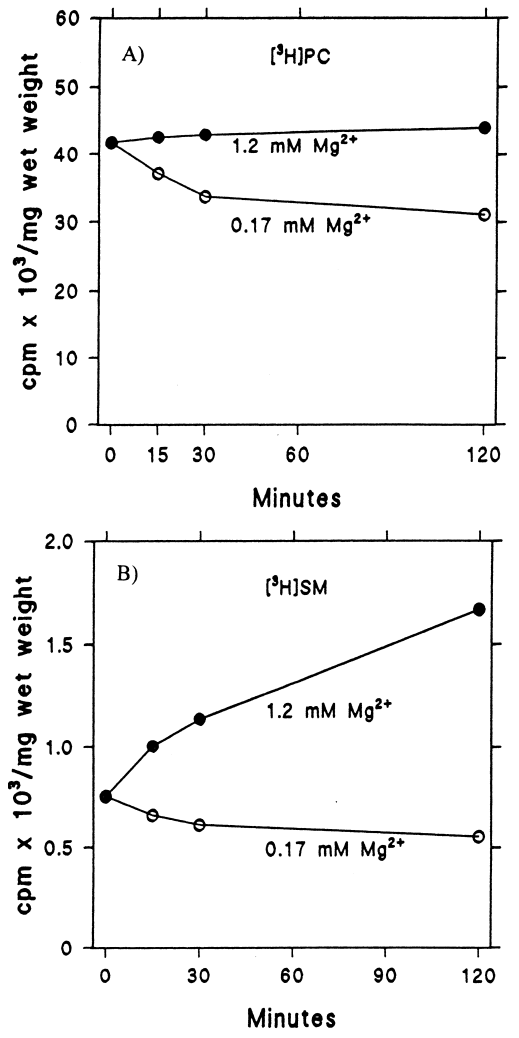

Fig. 1. A and B: Changes in [^{3}H]phosphatidylcholine (PC) in dog cerebral blood vessel segments as a function of time after transfer to Krebs-Ringer bicarbonate (KRB) medium containing 0.17 or 1.2 mM Mg^{2+} (A). Segments pooled from 4 dogs were preincubated in normal KRB containing [^{3}H]palmitic acid and 0.6 mM ionized Mg^{2+} for 3 h at 37°C. Segments were then rinsed with fresh normal KRB, transferred to KRB containing low (0.17 mM) or high (1.2 mM) Mg^{2+} and extracted at the times indicated. B: Changes in [^{3}H]sphingomyelin (SM) in dog cerebral blood vessel segments as a function of time after transfer to Krebs-Ringer bicarbonate (KRB) medium containing 0.17 or 1.2 mM Mg^{2+}. Values were obtained from analysis of the samples described in Section 2. For details see Section 2. $N = 6$.

(1.2 mM) $[Mg^{2+}]_o$. As shown in Fig. 1B, sphingomyelin (SM) labeling in the same samples also decreased by about 25% over the first 2 h in low $[Mg^{2+}]_o$ but increased 2–3-fold over the same time period after transfer to elevated $[Mg^{2+}]_o$. A similar time course of PC and SM labeling was seen for rat aortic smooth muscle cells in culture (data not shown).

3.2. *Prolonged exposure to elevated or lowered $[Mg^{2+}]_o$ results in marked changes in membrane lipid composition*

Rat aortic smooth muscle cells were preincubated in NKRB solution containing physiological levels (0.6 mM) of Mg^{2+} plus [^{3}H]palmitic acid for 3 h at 37°C. The tissue was then rinsed in fresh NKRB for a few minutes, and transferred to NKRB solutions containing 0.17, 0.6, 1.2 or 4.8 mM Mg^{2+} for 18 h at 37°C. At the end of an 18-h incubation period, the tissue was removed and the total lipids extracted. Table 1 illustrates [^{3}H]palmitic acid incorporation into PC, SM, PE and PI as a function of $[Mg^{2+}]_o$. The total lipid-bound radioactivity varied with the concentration of $[Mg^{2+}]_o$ and was in the range of 64–93% of the total labeled palmitate taken up into the tissue. About 80% of the total phospholipid counts were recovered in the PC fraction, and about 10% in the PE and SM fractions. The remainder, approximately 10% of lipid-bound activity, was resident in components not assayed in this study. Less than 1% of the phospholipid-associated [^{3}H]palmitate was recovered with the PI fraction, probably because PI often contains almost exclusively stearic acid in the *sn*-1 position and arachidonic acid in the *sn*-2 position. Thus, the [^{3}H]palmitic acid labeling protocol used here is probably not the best choice for labeling PI. Counts also migrated with phosphatidylmonomethylethanolamine, phosphatidyldimethylethanolamine and phosphatidylglycerol. Minimal counts were recovered with the phosphatidylserine component on TLC.

[^{3}H]Palmitic acid incorporation decreased in the four major classes of phospholipids with decreasing extracellular Mg^{2+} concentrations, with the largest decreases in SM, PI and PE. [^{3}H]PC increased 33% with increasing $[Mg^{2+}]_o$, whereas [^{3}H]SM and [^{3}H]PE increased by 390 and 220%, respectively. The 8-fold increase in PI ^{3}H-labeling with increased $[Mg^{2+}]_o$ (to 4.8 mM) may reflect changes in a minor PI component and not be indicative of changes in the total PI pool.

Clearly, a variation in intracellular free Mg^{2+} has a greater effect on SM turnover than on turnover of any of the other major membrane phospholipids (PC, PE) (Table 1). Since SM is synthesized at the expense of PC, the PC/SM ratio was calculated from Table 1. Fig. 2 illustrates the [^{3}H]PC/[^{3}H]SM and the [^{3}H]PC/[^{3}H]PE ratios over a range of

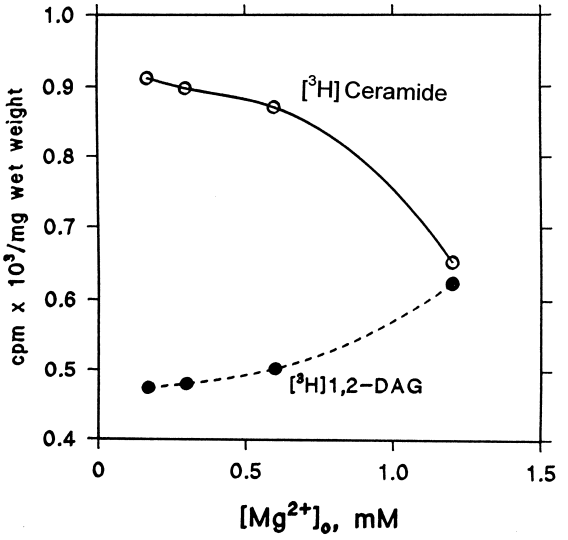

Fig. 2. Left hand ordinate: Changes in PC/SM and PC/PE ratios in [^{3}H]palmitic acid-labeled rat aortic cells as a function of $[Mg^{2+}]_o$. Values shown are calculated from the data shown in Table 1. Right hand ordinate: Change in total phospholipid content of rat aortic cells as a function of $[Mg^{2+}]_o$. Total phospholipid content of triplicate samples was determined by ^{1}H-NMR at 3.99 ppm as described in Section 2. Vertical dotted and solid lines indicate human serum ionized Mg^{2+} ranges: from left to right, pathological (0.3–0.54 mM), normal (0.55–0.65 mM), and after Mg^{2+} therapy (1.2–4.8 mM).

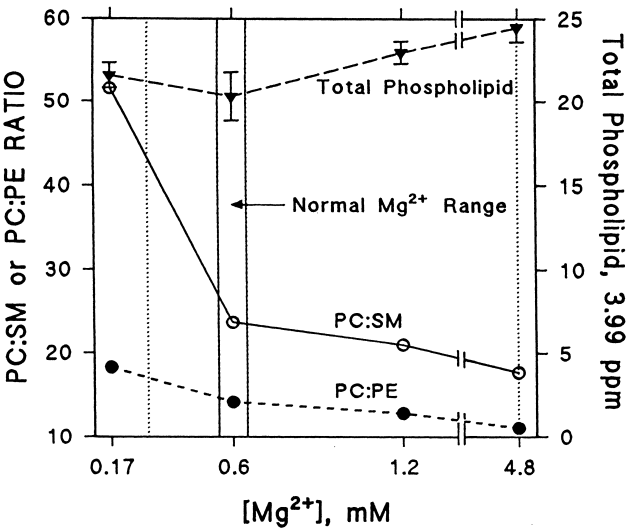

Fig. 3. Changes in [^{3}H]ceramide and 1,2-[^{3}H]diacylglycerol (1,2-DAG) in [^{3}H]palmitic acid-labeled rat aortic cells as a function of $[Mg^{2+}]_o$. Values are from the experiment described in Table 1.

$[Mg^{2+}]_o$. When the [^{3}H]PC/[^{3}H]SM ratios are plotted as a function of $[Mg^{2+}]_o$, it can be seen that the ratio of labeled PC/SM in vascular smooth muscle cells increased from about 17:1 at the lowest $[Mg^{2+}]_o$ to about 52:1 at the highest extracellular Mg^{2+} ion concentration. For comparison, the PC/[^{3}H]PE ratio rose only from about 11:1 to 18:1. The solid vertical lines indicate the rather narrow range for normal extracellular ionized Mg^{2+} (0.55–0.65 mM). The vertical dotted line at 0.3 mM indicates the lowest reported serum ionized Mg^{2+} concentration in patients, whereas that at 4.8 mM indicates the upper limit reported in patients after Mg^{2+} therapy [9]. It can be seen that the greatest change in the PC/SM ratio occurred within the range observed in patients with hypomagnesemia.

Total phospholipid was measured in crude lipid extracts of rat aorta and dog cerebrovascular tissue using ^{1}H-NMR techniques. The protons at the C3 position of glycerol in phospholipids appear as a multiplet at 3.99 ppm [25] and can be used to quantitate total phospholipid in either crude extracts or aminopropylsilyl column fractions. As shown in Fig. 2, the total phospholipid content of aortic smooth muscle cells increased less than 5% with increasing $[Mg^{2+}]_o$. Similarly, no marked change in total phospholipid was detected in total lipid extracts of dog cerebral blood vessel smooth muscle tissue (data not shown).

3.3. *Prolonged exposure to elevated or lowered $[Mg^{2+}]_o$ leads to reciprocal changes in 1,2-DAG and ceramide levels in vascular smooth muscle cells*

Ceramide and 1,2-DAG pools were measured in rat aortic smooth muscle cells labeled with [^{3}H]palmitic acid following an 18-h incubation in NKRB media containing 0.17, 0.3, 0.6 or 1.2 mM $[Mg^{2+}]_o$. As $[Mg^{2+}]_o$ was decreased, [^{3}H]ceramide levels increased sharply with a concomitant fall in 1,2-DAG, showing a reciprocal relationship between DAG and ceramide levels (Fig. 3). Since PC and ceramide undergo an exchange reaction (via SM synthase) to form 1,2-DAG and SM, the changes in ceramide and 1,2-DAG levels, together with the drop in SM, indicate that a reduction in ionized Mg^{2+} may inhibit SM synthase activity in smooth muscle.

4. Discussion

Our data directly indicate that the incorporation and turnover of palmitate radioactivity into lipids is clearly $[Mg^{2+}]_o$ dependent. Our in vitro studies with aortic and cerebrovascular smooth muscle show that reduction in $[Mg^{2+}]_o$ below the normal serum value of 0.6 mM down-regulates synthesis of some of the key phospholipids. The decrease in PC, the major glycerophospholipid, is, however, small compared to that of SM, the major sphingophospholipid. The ratio of PC to SM nearly doubles as $[Mg^{2+}]_o$ is reduced from 0.6 mM to 0.3 mM (Fig. 2), the latter occurring in the serum of patients with hypomagnesemia. These samples also show a small but significant rise in ceramide, and a reciprocal drop in 1,2-DAG (Fig. 3). Since SM is synthesized by a transfer reaction between ceramide and PC (thereby releasing 1,2-DAG), and since 1,2-DAG does not increase, but ceramide does, it appears that low $[Mg^{2+}]_o$ somehow results in down-regulation of SM synthase activity. In contrast, increasing $[Mg^{2+}]_o$ within the range observed in patients after therapeutic administration of Mg salts caused only a slight reduction in the PC/SM ratio, but a large reduction in ceramide and an increase in 1,2-DAG (Fig. 2).

There are several possible consequences of a reduction in SM. Most SM is confined to the plasma membrane [16], where it is a source of lipid-derived second messengers. Ligands which bind to receptors for tumor necrosis factor α (TNF-α), interleukin-1, Fas, and interferon-γ are among the intracellular activators of SMase [26,27]. The ceramide generated may either accumulate or be further metabolized to sphingosine or sphingosine-1-phosphate, which have also been proposed as lipid mediators (reviewed in [27]). Many effects of ligand binding to these receptors have been duplicated using short chain cell-permeable ceramides. Ceramide and its downstream products activate different serine/threonine protein kinase cascades and protein phosphatases which regulate the function of several transcription factors, such as NF-κB, which coordinately control genes encoding inflammatory cytokines and cell adhesion molecules. Ceramide-activated protein kinase initiates pro-inflammatory activity in response to TNF-α stimulation, probably by phosphorylating and activating Raf-1. The multiple effects of ceramide (reviewed in [26,27]) suggest that it mediates a variety of cell responses to stress, initiated by signals from different plasma membrane receptors. In a preliminary report we described a linear increase in the p65 subunit of NF-κB when cell cultures of aortic vascular smooth muscle were incubated with decreasing $[Mg^{2+}]_o$ [28]. We presume that this was due not to receptor activation but to the increased intracellular ceramide and/or its downstream products. If low $[Mg^{2+}]_o$ does in fact promote the transcription of inflammatory proteins and adhesion molecules [29], it could be a contributing factor in atherogenesis, which has inflammatory and leukocyte components.

We have previously reported that low $[Mg^{2+}]_o$ led to increased fatty acid saturation and plasmalogen (α,β-unsaturated ether) content as well as to a decreased average fatty acid chain length in vascular smooth muscle cells [15]. The alterations in phospholipid composition and lipid second messengers may be relevant to the increased contractility [7,8] and

changes in Ca^{2+} permeability [20,30] that are known to occur in low extracellular Mg^{2+}. Taken in toto, extracellular Mg^{2+} appears to modulate the basal levels of at least two lipid messengers as well as SM synthesis, fatty acid saturation and the acyl ether content of different types of the membrane phospholipids of vascular smooth muscle cells.

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References

[1] Altura, B.M. and Altura, B.T. (1985) Magnesium 4, 226–244.
[2] Sjögren, A., Edvinsson, I. and Fallgren, B. (1989) J. Intern. Med. 226, 213–222.
[3] Altura, B.T., Brust, M., Bloom, S., Barbour, R.L., Stempak, J.G. and Altura, B.M. (1990) Proc. Natl. Acad. Sci. USA 87, 1840–1844.
[4] Eisenberg, M.J. (1992) Am. Heart J. 124, 544–549.
[5] Rasmussen, H.S. (1993) Dan. Med. Bull. 40, 84–99.
[6] Purvis, J.R. and Movahed, A. (1992) Clin. Cardiol. 15, 556–568.
[7] Altura, B.M. and Altura, B.T. (1974) Microvasc. Res. 7, 145–155.
[8] Turlapaty, P.D.M.V. and Altura, B.M. (1980) Science 208, 198–200.
[9] Altura, B.T., Bertschat, F., Jeremias, A., Ising, H. and Altura, B.M. (1994) Scan. J. Clin. Lab. Invest. 54 (Suppl. 217), 77–82.
[10] Altura, B.M. and Altura, B.T. (1995) Sci. Am. Sci. Med. 2, 28–37.
[11] Sadeh, M. (1989) Stroke 20, 1273–1275.
[12] Altura, B.M., Altura, B.T. and Gupta, R.K. (1992) Magnesium Trace Elem. 10, 122–135.
[13] Altura, B.T., Shirey, T.C., Young, C.C., Dell'Orfano, K., Hiti, J., Welsh, F., Yeh, O., Barbour, R.L. and Altura, B.M. (1994) Scan. J. Clin. Lab. Invest. 54 (Suppl 217), 21–36.
[14] Kostellow, A.B., Morrill, G.A., Ma, G.-Y., Zhang, A., Altura, B.T., Altura, B.M. and Gupta, R.K. (1995) Proc. Soc. Magn. Reson. 3, 1622–1623.
[15] Morrill, G.A., Gupta, R.K., Kostellow, A.B., Ma, G.-Y., Zhang, A., Altura, B.T. and Altura, B.M. (1997) FEBS Lett. 408, 191–194.
[16] Vance, D.E. and Vance, J. (Eds.) (1991) Biochemistry of Lipids; Lipoproteins and Membranes, 2nd Edn., Elsevier, New York, NY.
[17] Merrill, A.H. and Wang, E. (1992) in: Methods in Enzymology, Vol. 209, pp. 427–446, Academic Press, New York, NY.
[18] Zhang, A., Altura, B.T. and Altura, B.M. (1992) Br. J. Pharmacol. 105, 305–310.
[19] Zhang, A., Altura, B.T. and Altura, B.M. (1993) Eur. J. Pharmacol. 248, 229–236.
[20] Zhang, A., Cheng, T.P.-O. and Altura, B.M. (1992) Biochim. Biophys. Acta 1134, 25–29.
[21] Bocckino, S.B., Blackmore, P.F. and Exton, J.H. (1985) J. Biol. Chem. 260, 14201–14207.
[22] Touchstone, J.C., Alvarez, J.G., Levin, S.S. and Storey, B.T. (1985) Lipids 20, 869–875.
[23] Skidmore, W.D. and Entenman, C. (1962) J. Lipid Res. 3, 471–475.
[24] Sze, D.Y. and Jardetzky, O. (1990) Biochim. Biophys. Acta 1054, 198–206.
[25] Devlin, T.M. (1992) Textbook of Biochemistry with Clinical Correlations, 3rd Edn., Wiley-Liss, New York, NY.
[26] Kolesnick, R. and Golde, D.W. (1994) Cell 77, 325–328.
[27] Hannun, Y.A. (1996) Science 274, 1855–1859.
[28] Kostellow, A.B., Altura, B.M., Altura, B.T., Zhang, A., Gupta, R.K. and Morril, G.A. (1996) FASEB J. 10, 60.
[29] Altura, B.M. and Altura, B.T. (1995) Cell. Mol. Biol. Res. 41, 347–359.
[30] Turlapaty, P.D.M.V. and Altura, B.M. (1978) Eur. J. Pharmacol. 52, 421–423.