Mg^{2+} modulates membrane lipids in vascular smooth muscle: a link to atherogenesis

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Abstract Epidemiological studies associate low dietary magnesium intake with an increased incidence of ischemic heart disease and sudden cardiac death. We have used proton-magnetic resonance (^{1}H-NMR) techniques and Mg^{2+}-selective electrodes to monitor changes in lipid extracts of aortic and cerebrovascular smooth muscle as extracellular ionized magnesium ion concentration ($[Mg^{2+}]_o$) is lowered. We have found that, within the pathophysiological range of Mg^{2+} concentrations, fatty acid chain length and double bond content are progressively reduced as $[Mg^{2+}]_o$ is lowered. In contrast, the plasmalogen content is progressively increased. A concomitant decrease in fatty acid chain length and double bonds indicates oxidation of double bonds resulting in truncation of the fatty acids. A decrease in lipid oxidation in the presence of elevated Mg^{2+} could contribute to the apparent protective role of increased Mg^{2+} intake on vascular function in humans.

Key words: Atherogenesis; Lipid; NMR; Smooth muscle; Fatty acid unsaturation; Plasmalogen

1. Introduction

Over the past three decades, an accumulation of epidemiological studies indicates that the low magnesium content in drinking water found in areas with soft water and Mg-poor soil [1] is associated with high incidences of ischemic heart disease (IHD) and sudden cardiac death (SCD) [2]. At present, the average dietary intake of magnesium has declined from about 450–485 mg/day at the turn of the century to about 185–260 mg/day for large segments of the North American population [3]. This shortfall in dietary magnesium may be significant in the etiology of atherogenesis and cardiovascular disease. The coronary arteries of such patients often show deficits of 30–40% in total magnesium content and concomitant elevation in Ca^{2+} content. Magnesium is the only element that has been found to be consistently lowered in cardiac muscle of SCD patients [1].

Both animal and human studies [1–5] have shown a strong relationship between dietary intake of magnesium and atherosclerosis. A number of studies now indicate that in the serum ionized Mg^{2+} is appreciably reduced in the serum of patients with atherosclerosis, IHD, myocardial infarction, stroke, and hypertension, although total magnesium may be normal [6–8]. Magnesium salts have been shown to be effective in the clinical treatment of several cardiovascular diseases (e.g. arrhythmias, hypertension, acute myocardial infarction) and stroke [9–11]. In view of this large body of data on magnesium and the observation that fatty streaks in intimal smooth muscle precede the development of intermediate atherogenic lesions [12], we have investigated the effects of levels of ionized Mg^{2+} found in patients with IHD and stroke [7,8] on the lipids of vascular smooth muscle membranes.

2. Materials and methods

2.1. 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 and immediately placed in normal Krebs-Ringer bicarbonate (NKRB) solution at room temperature. They were cleaned of blood, loose connective tissue and fat and were cut into rings about 2–3 mm long. The endothelium was removed by rubbing with wet filter paper. The composition of the NKRB was (in mM): NaCl, 118; KCl, 4.7; $KHPO_4$, 1.2; $MgSO_4$, 1.2; $CaCl_2$, 2.5; glucose, 10; $NaHCO_3$, 25. The ionic activity of Mg^{2+} in sera and in modified NKRB was monitored by ion selective electrodes (NOVA Biomedical Corp., MA) as described by Altura et al. [7]. $MgSO_4$ was added to Krebs-Ringer bicarbonate (KRB) solutions so as to obtain final concentrations of 0.17, 0.3, 0.6, 1.2 and 4.8 mM Mg^{2+}.

Male mongrel dogs (15 ± 3 kg) were anaesthetized with sodium pentobarbital (40 mg/kg, i.v.) and sacrificed by bleeding from the common femoral arteries. After craniotomy, the brains were removed rapidly and placed in normal NKRB at room temperature. Basilar and middle cerebral arteries were carefully excised and cleaned of arachnoid membranes and blood elements. The vessels were cut into small segments about 3–4 mm in length. For intact tissue preparations, extreme care was taken to avoid damage of endothelial cells. In some rings from canine cerebral arteries, however, the lumen of the vessels were gently rubbed with a small wire to remove endothelial cells.

2.2. Lipid extraction and ^{1}H-NMR

Total lipid was extracted by a modification of the method of Bligh and Dyer [13]. Tissue samples were homogenized in 4 volumes of $CHCl_3$:methanol (1:2 v/v). The suspensions were transferred to 15 ml glass-stoppered centrifuge tubes, using successive 0.3 ml aliquots of water and $CHCl_3$ to wash the homogenizer. The homogenate and wash volumes were combined, vortexed for 1 min, and centrifuged at $2000 \times g$ for 5 min. The upper aqueous and lower lipid-containing phases were separated, the interfacial material was washed twice with 0.35 ml volumes of $CHCl_3$ and the upper and lower phases pooled with the first fractions. The lower $CHCl_3$ phase was washed twice with fresh upper phase and taken to dryness under N_2. Lipids were stored in $CHCl_3$ in the dark at −35°C under N_2.

One-pulse spectra were taken on a Varian VXR-500 spectrometer operating at a proton frequency of 500 MHZ. All spectra were taken at 25°C using a 60° pulse, a spectral width of 5000 Hz, a repetition time of 10 s and 8K data points. 256 acquisitions were required to attain adequate signal/noise ratios. Resonance intensities were measured as peak areas in fully relaxed spectra. The average number of double bonds or vinyl ether linkage per acyl chain and the average carbon chain length were determined as indicated in Fig. 1, below.

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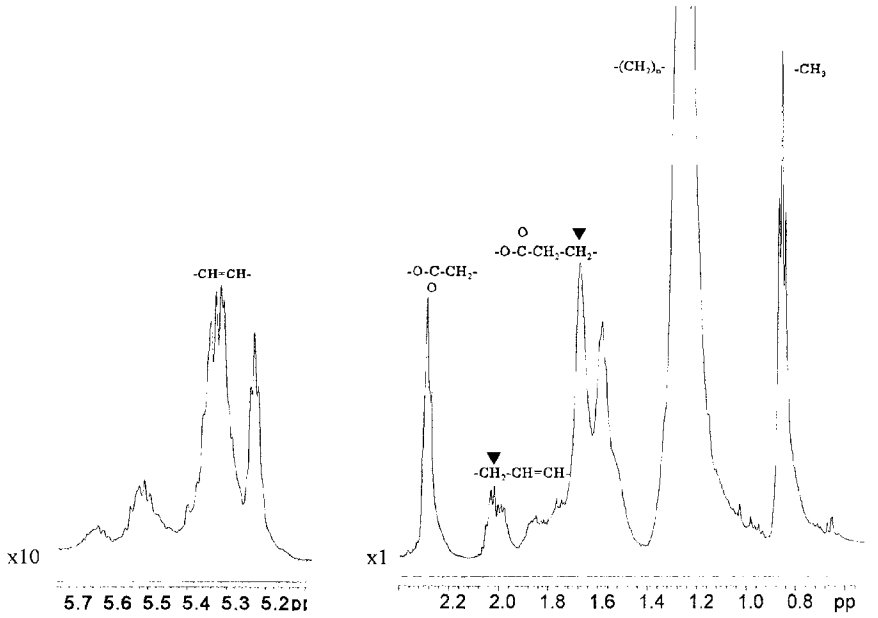
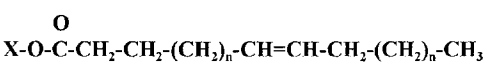
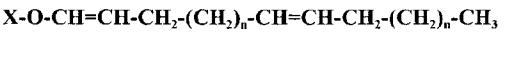


Fatty Acid



Plasmalogen



X = Sn1 and/or Sn2 position of glycerol lipid

Fig. 1. Typical 500 MHZ ^{1}H-NMR spectra of an extract of total lipids from a 35 mg sample of rat aorta free of endothelial cells. Upper left: expanded spectrum of the 5.7 to 5.2 ppm region demonstrating the proton peaks associated with fatty acid double bonds. Upper right: expanded spectrum of the 2.3 to 0.7 ppm region with assignment of the peaks corresponding to the protons of the fatty acid backbone. The 5.7 to 5.2 ppm region was magnified 10 times compared to the 2.3 to 0.7 ppm region. Representative fatty acid and plasmalogen structures are illustrated in the lower portion.

3. Results and discussion

Proton nuclear magnetic resonance (^{1}H-NMR) spectroscopy was used to quantitate both fatty acid unsaturation and the vinyl ether linkage of plasmalogen and to estimate average fatty acid chain length in lipid extracts of vascular smooth muscle cells incubated in media containing a range of extracellular magnesium ion concentrations ($[Mg^{2+}]_o$). ^{1}H-NMR is a rapid and comprehensive technique for monitoring lipid composition of cells, body fluids and tissues (e.g. [14,15]). Fig. 1 illustrates two regions of the ^{1}H-NMR spectra of the total lipid extracted from a 35 mg sample of rat aorta stripped of endothelial cells. The peaks corresponding to the protons of the fatty acid double bonds appear around 5.3 ppm (Fig. 1, upper left). The protons associated with the fatty acid backbone are shown in the 2.5–0.7 ppm region (Fig. 1, upper right). The resonances associated with the protons of the vinyl ether (α,β-unsaturated ether) moiety of plasmalogen are measurable at 5.90 ppm (region not shown) in extracts of as little as 10 mg of dog cerebrovascular smooth muscle cells (see ref. [16]).

Representative fatty acid and plasmalogen structures, the assignments of peaks corresponding to fatty acid double bonds (5.34 ppm), methyl group protons (0.85 ppm), $-(CH_2)_n-$ protons (1.25 ppm) and protons associated with other carbons of the fatty acid chain, are indicated in the lower portion of Fig. 1. Assignments are based on those published by Sze and Jardetzky [14] and confirmed in our laboratory. Fatty acid unsaturation and vinyl ether (plasmalogen) content were determined using integrated peak intensities of resonances unique to those moieties. Average carbon chain length was determined by dividing the sum of appropriately normalized fatty acid proton intensities of the carbons indicated in Fig. 1 by the normalized proton intensities of the methyl carbon.

Total serum Mg in humans normally varies between 0.70 and 0.96 mM, but the free Mg^{2+}, which is of direct biochemical significance in this study, varies between 0.54 and 0.65 mM, with an average value of 0.6 mM [6–8]. Pathophysiological values (from clinical studies) can approach 0.3 mM [7,8]. The lowest reported blood free Mg^{2+} in humans is about 0.24 mM [7,8,17]. Intravenous infusion with saline solutions containing 4.8 mM Mg^{2+} is used for certain types of electrolyte and anticonvulsive therapy and approaches the total serum level of Mg found in women after Mg^{2+} therapy for eclampsia. For comparison, ^{31}P-NMR spectroscopy and Mag-FURA-2 measurements indicate intracellular free Mg^{2+} in

several cell types to also be about 0.5–0.6 mM [18–20]. Clinical work-ups almost invariably measure total serum Mg, but free Mg^{2+} can be determined by using either an ion-selective electrode or ^{31}P-NMR. In the present study, free ionized Mg^{2+} levels in the culture medium were measured using an ion selective electrode (NOVA Biomedical Corp., MA) [7].

We found that in vascular smooth muscle cells, both the level of fatty acid unsaturation (double bonds) and the average fatty acid chain length decrease as extracellular Mg^{2+} is lowered from the physiological into the pathophysiological range. Fig. 2 shows the correlation between extracellular Mg^{2+} concentration, fatty acid unsaturation and chain length of rat aorta smooth muscle lipids. Aortic segments, with endothelial cells removed, were incubated for 18 h in Krebs-Ringer bicarbonate solution (pH 7.4) containing 0.17, 0.3, 0.6, 1.2 or 4.8 mM Mg^{2+}, as measured with an ion-selective electrode [7]. Within the pathophysiological range found in vivo in humans (0.3 to 0.5 mM) there was nearly a two-fold decrease in fatty acid unsaturation and 2–3 carbon decrease in average chain length. Similar studies were carried out using aortic smooth muscle cell cultures as well as segments of dog cerebrovascular blood vessels with endothelial cells removed. Fig. 3 illustrates the changes in fatty acid unsaturation and average fatty acid chain length in cerebrovascular vessels as a function of extracellular magnesium ion concentration in the range of 0.17 to 1.2 mM. As Mg^{2+} increased, there was a 2–3 fold increase in unsaturation and an increase in average chain length of about 3 carbons. Thus, both aortic and cerebrovascular smooth muscle cells respond in the same manner as in vitro changes in extracellular Mg^{2+} ion concentration. Similar results were obtained with aortic smooth muscle cell cultures (data not shown).

Since the enzymes that form double bonds in fatty acids (desaturases) also introduce the double bond in the vinyl ether linkage of plasmalogen [21], Mg^{2+}-dependent changes in plasmalogens were compared with changes in fatty acid unsaturation. Plasmalogens constitute a unique class of N-containing phospholipids in which the sn-1 position (outermost carbon of

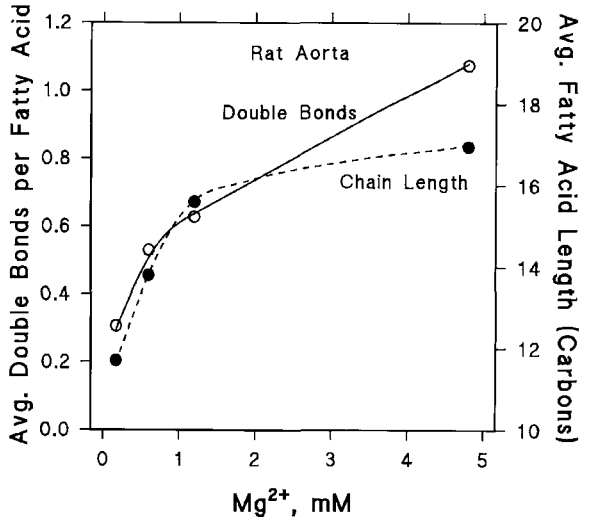


Fig. 2. Changes in fatty acid unsaturation (– ○ –) and average fatty acid chain length (– ● –) in rat aorta smooth muscle cell lipids as a function of extracellular Mg^{2+} concentration. Aortic segments free of endothelial cells were incubated in Kreb's Ringers bicarbonate medium containing the ionized $[Mg^{2+}]_o$ indicated for 18 h as described in text. Points represent pooled tissue from 6 animals.

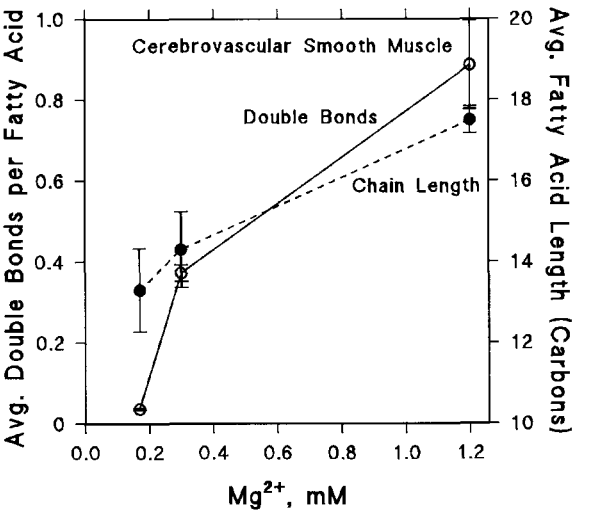


Fig. 3. Changes in fatty acid unsaturation (– ○ –) and average fatty acid chain length (– ● –) in dog cerebrovascular smooth muscle cell lipids as a function of extracellular Mg^{2+} concentration. Cerebrovascular segments free of endothelial cells were incubated in Kreb's Ringers bicarbonate medium containing the $[Mg^{2+}]_o$ indicated for 18 h as described in text. Points represent means ± SD for individual tissue samples ($N = 3$).

the glycerol backbone) is occupied by a vinyl ether-linked fatty alcohol. Fig. 4 compares the per cent change in vinyl ether bonds per acyl side chain and the vinyl ether:double bond ratio in dog cerebrovascular smooth muscle lipids as a function of Mg^{2+}. In contrast to the Mg-dependent increase in classical double bonds noted above, the vinyl ether content decreases with increasing $[Mg^{2+}]_o$. The ratio of fatty acid double bonds to vinyl ether double bonds in cerebrovascular cells increased about 5-fold as extracellular Mg^{2+} was increased from the pathophysiological to the physiological range (0.3–0.8 mM). The data suggest that different desaturases must be involved in the synthesis of plasmalogens and unsaturated fatty acids, although one cannot rule out the possibility that Mg^{2+} has different effects on the same enzyme acting on different substrates. Since plasmalogens are high in excitable and contractile tissue (reviewed in [22]), the increased contractility of vascular smooth muscle in low Mg^{2+} in vivo [1,8,23] may be facilitated by an increased plasmalogen content.

The Mg^{2+}-dependent changes in fatty acid unsaturation and average chain length occurred in medium free of exogenous fatty acids and/or lipids. Thus, the changes seen here are due to altered turnover and/or modification of endogenous membrane lipids. Recently, low Mg^{2+} environments have been shown to lead to the formation of different types of free radicals with peroxidative capabilities in cultured endothelial cells [24], heart [25], and intact rat skeletal muscle and lipoproteins [26]. The low Mg^{2+}-induced decrease in both fatty acid chain length and in number of double bonds most probably reflects free radical oxidation of the double bonds and chain shortening in the sn-2 position (middle carbon) of the glycerol backbone. This oxidative modification of the polyunsaturated fatty acyl residues of membrane phospholipids could generate platelet-activating factor (PAF)-like lipids [27], believed to be associated with leucocyte diapedesis [27]. Heery et al. [28] have found that exposure of smooth muscle cells to oxidized low density lipoprotein (LDL), which

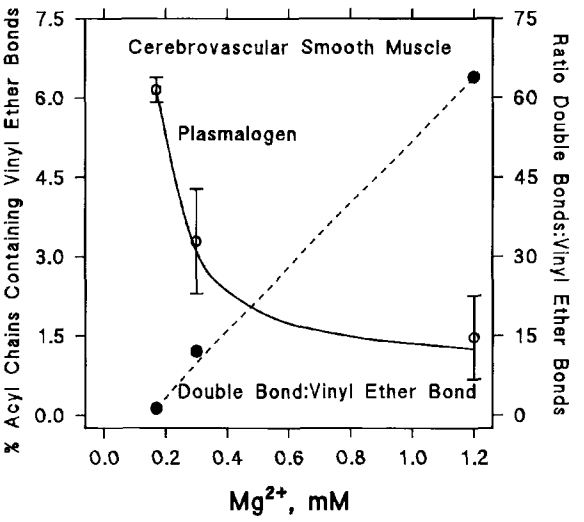


Fig. 4. Changes in vinyl ether (plasmalogen) content (– ○ –) and the ratio of double bonds:vinyl ether bonds (– ● –) as a function of extracellular Mg^{2+} concentration. Points represent means ± SD ($N = 3$). See experiment described in Fig. 3.

produces PAF-like lipids, was mitogenic; this is significant since atherogenesis requires mitosis of intimal smooth muscle cells. The mitogenic activity reported by Heery et al. was blocked by PAF-receptor antagonists [28]. On the other hand, Zoeller et al. [29] have shown that an increase in membrane plasmalogen protects photosensitized Chinese hamster ovary cells from UV; thus, the increase in plasmalogen seen in low $[Mg^{2+}]_o$ (Fig. 4) may represent a possible compensatory mechanism against lipid oxidation.

The role of Mg^{2+} in maintaining normal contractility has been attributed to its ability to maintain Ca^{2+} homeostasis. Experiments with $^{45}Ca^{2+}$, molecular fluorescent probes and with patch clamps demonstrate that in vascular smooth muscle Mg^{2+} competitively inhibits Ca^{2+} transport, acting as a Ca^{2+} channel blocker [1]. Much of the protective effect of magnesium against cardiovascular disease, stroke and myocardial infarction has been attributed to the ability of this divalent cation to act as a vasodilator and a natural calcium antagonist [1]. A decrease in extracellular Mg^{2+} concentration results in an increased contractility of vascular smooth muscle [1,8,23]. The loss of fatty acid double bonds and decrease in fatty acid chain length described here occurs in the same low concentrations of $[Mg^{2+}]_o$, found recently in the sera of patients with IHD and stroke [7,8,17], associated with the increased Ca^{2+}, lipoprotein and macrophage permeability observed in atherosclerotic blood vessels (cit. [12]). These Mg^{2+}-dependent changes in membrane lipids may link various cardiovascular disease risk factors (e.g. hypercholesterolemia, hypertension, diabetes mellitus, immune injury, end-stage renal disease, prolonged stress), and atherogenesis [12,30,31].

In summary, the studies outlined here suggest a novel mechanism for Mg^{2+} action in blood vessels. Fatty acid unsaturation (double bonds) and average fatty acid chain length decrease in vascular smooth muscle cells from both aortic and cerebrovascular tissue with decreasing concentrations of extracellular ionized Mg^{2+}. The sharpest rate of change occurs within the range bracketing the normal to pathophysiological Mg^{2+} concentrations. Since increased dietary intake of polyunsaturated fatty acids is thought to be beneficial to the cardiovascular system [31], and since epidemiological studies indicate a beneficial function for increased dietary Mg [2–4], Mg^{2+} may function to protect the polyunsaturated fatty acids from oxidation and/or to facilitate their incorporation into membrane phospholipids. These Mg^{2+}-dependent changes in membrane lipids could account in part for the experimentally and clinically observed beneficial effects of Mg^{2+} on the maintenance of cardiovascular function [9–11].

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References

[1] B.M. Altura, B.T. Altura, in: J.H. Laragh, B.M. Brenner (eds.), Hypertension: Pathophysiology, Diagnosis, and Management, 2nd ed., Raven Press, New York, 1995, p. 1213.
[2] M.J. Eisenberg, Am. Heart J. 124 (1992) 544–549.
[3] M.S. Seelig, Magnesium Deficiency in the Pathogenesis of Disease, Plenum, New York, 1980.
[4] M.S. Seelig, Am. J. Cardiol. 68 (1991) 1221–1222.
[5] B.T. Altura, M. Brust, S. Bloom, R.L. Barbour, J.G. Sternpak, B.M. Altura, Proc. Natl. Acad. Sci. USA 87 (1990) 1840–1844.
[6] M.S. Markell, B.T. Altura, R.L. Barbour, B.M. Altura, Clinical Sci. 85 (1993) 315–318.
[7] B.T. Altura, F. Bertschat, A. Jeremias, H. Ising, B.M. Altura, Scand. J. Clin Lab. Invest. 54 (Suppl. 217) (1994) 77–82.
[8] B.M. Altura, B.T. Altura, Sci, Am. Sci. Med. 2 (1995) 28–37.
[9] H.S. Rasmussen, P. McNair, P. Norregard, V. Backer, O. Lindeneg, S. Balslev, Lancet 1 (1986) 234–236.
[10] M. Schechter, H. Hod, N. Marks, S. Behar, E. Kaplinsky, B. Rabinowitz, Am. J. Cardiol. 66 (1990) 271–2774.
[11] K.W. Muir, K.R. Lees, Stroke 26 (1995) 1183–1188.
[12] R. Ross, Nature 362 (1993) 801–809.
[13] E. Bligh, W. Dyer, Can J. Biochem. Physiol. 37 (1959) 911–917.
[14] D.Y. Sze, O. Jardetzky, Biochim. Biophys. Acta 1054 (1990) 198–206.
[15] G.T. Choi, M. Casu, W.A. Gibbons, Biochem. J. 290 (1993) 717–721.
[16] A.B. Kostellow, G.A. Morrill, G.-Y. Ma, A. Zhang, B.T. Altura, B.M. Altura, R.K. Gupta, Proc. Soc. Mag. Res. 3 (1995) 1622.
[17] M. Maj-Zurawska, Scan. J. Clin. Lab. Invest. 217 (1994) 69–76.
[18] R.K. Gupta, P. Gupta, R.D. Moore, Annu. Rev. Biophys. Bioeng. 13 (1984) 221–246.
[19] A. Zhang, T.P. Cheng, B.T. Altura, B.M. Altura, Pflugers Arch. – Eur. J. Physiol. 421 (1992) 391–393.
[20] B.M. Altura, R.L. Barbour, T.L. Dowd, B.T. Altura, R.K. Gupta, Biochim. Biophys. Acta 1182 (1993) 329–332.
[21] R.L. Wykle, M.L. Blank, B. Malone, F. Snyder, J. Biol. Chem. 247 (1972) 5442–5447.
[22] L.A. Horrocks, M. Sharma, in: New Comprehensive Biochemistry, vol. 4, Elsevier, Amsterdam, 1982, pp. 51–93.
[23] B.M. Altura, B.T. Altura, Blood Vessels 15 (1978) 5–16.
[24] B.F. Dickens, W.B. Weglicki, S. Li, I.T. Mak, FEBS Lett. 311 (1992) 187–191.
[25] F. Wu, B.T. Altura, J. Gao, R. Barbour L, B.M. Altura, Biochim. Biophys. Acta 1225 (1994) 158–164.
[26] Y. Rayssiguier, E. Gyeux, L. Bussiere, J. Durlach, A. Mazur, J. Am. Coll. Nutrit. 12 (1993) 133–137.
[27] G.A. Zimmerman, S.M. Prescott, T.M. McIntyre, J. Nutrition 125 (Suppl. 6) (1995) 1661S–1665S.
[28] J.M. Heery, M. Kozak, D.M. Stafforini, D.A. Jones, G.A. Zimmerman, T.M. McIntyre, S.M. Prescott, J. Clin. Invest. 96 (1995) 2322–2330.
[29] R.A. Zoeller, O.H. Morand, C.R. Raetz, J. Biol. Chem. 263 (1988) 11590–11596.
[30] J.H. Campbell, G.R. Campbell, J. Hypertension 12 (Suppl. 10) (1994) S129–S132.
[31] S.M. Schwartz, D. deBois, E.R. O'Brien, Circ. Res. 77 (1995) 445–465.