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Neurogenic Inflammation and Cardiac Dysfunction due to Hypomagnesemia

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Abstract

Hypomagnesemia continues to be a significant clinical disorder that is present in patients with diabetes mellitus, alcoholism, and treatment with magnesuric drugs (diuretics, cancer chemotherapy agents, etc.). To determine the role of magnesium in cardiovascular pathophysiology, we have used dietary restriction of this cation in animal models. This review has highlighted some key observations which helped formulate the hypothesis that release of substance P (SP) during experimental dietary Mg deficiency (MgD) may initiate a cascade of deleterious inflammatory, oxidative, and nitrosative events, which ultimately promote cardiomyopathy, *in situ* cardiac dysfunction, and myocardial intolerance to secondary stresses. SP acts primarily through neurokinin-1 (NK-1) receptors of inflammatory and endothelial cells, and may: induce production of reactive oxygen and nitrogen species (superoxide anion, NO•, peroxynitrite, hydroxyl radical), leading to enhanced consumption of tissue antioxidants; stimulate release of inflammatory mediators; promote tissue adhesion molecule expression; and enhance inflammatory cell tissue infiltration and cardiovascular lesion formation. These SP-mediated events may predispose the heart to injury if faced with subsequent oxidative stressors (ischemia/reperfusion, certain drugs) or facilitate development of *in situ* cardiac dysfunction, especially with prolonged dietary Mg-restriction. Significant protection against most of these MgD-mediated events has been observed with interventions that modulate neuronal SP release or its bioactivity, and with several antioxidants (vitamin E, probucol, epicaptopril, d-propranolol). In view of the clinical prevalence of hypomagnesemia, new treatments, beyond magnesium repletion, may be needed to diminish deleterious neurogenic and prooxidative components described in this article.

Keywords

Dietary Mg-deficiency; Hypomagnesemia; Substance P; Oxidative and Nitrosative Stress; Cardiac Inflammation; Cardiac dysfunction; Ischemia/Reperfusion Injury; Neurokinin-1 Receptor Antagonists; NMDA Receptor Blocker; Antioxidants

Clinical and Experimental Hypomagnesemia

Clinical hypomagnesemia has been associated with a higher incidence of arrhythmias,¹ vasospasm,² sudden death with congestive heart failure³ and acute myocardial infarction.^{3,4} In one study,⁵ 45% of patients with acute myocardial infarction had this condition. Several animal models (hamsters,^{6,7} rats,⁸⁻¹⁰ mice,^{11,12} dogs,¹³ primates¹) have been used to simulate clinical hypomagnesemia, and have exhibited histologic, electrical and/or functional abnormalities of the cardiovascular system. Moreover, pre-existing Mg-deficiency (MgD) was shown to amplify myocardial vulnerability to toxic agents¹ and imposed stresses.¹⁴ When the imposed stress was ischemia / reperfusion (I/R), dogs on a Mg-restricted diet were shown to develop larger infarcts than those on a Mg-normal (or Mg sufficient, MgS) diet;¹⁴ and hearts from MgD rats exhibited lower recovery of cardiac function compared to the non-deficient group.¹⁰ Animals placed on Mg-restricted diets also displayed progressive cardiovascular lesion formation, heightened cardiac inflammatory cell infiltration,¹⁵ decreased levels of endogenous antioxidants (glutathione, vitamin E, ascorbate)^{16,17} and higher plasma levels of pro-oxidant metals¹⁸ and lipid peroxidation (LPO) products.^{19,20} Antioxidant treatment attenuated the severity of both cardiovascular inflammation *in vivo*²¹ and postischemic reperfusion injury *in vitro*,²² suggesting that dietary Mg-deficiency progresses into a pro-oxidant condition.

Mg-deficient rodents also exhibit temporal elevations in circulating inflammatory mediators which preceded significant leukocyte infiltration of cardiac tissue (3 weeks).^{19,23-25} Of further interest was our discovery that rodents experienced a neurogenic response during dietary MgD,^{24,26-28} and this became the underlying basis of our contention²⁶ that neuropeptides may initiate subsequent deleterious events in this model. In this review, experimental findings are described which were instrumental in formulating and refining of our hypothesis that MgD-mediated release of the neuropeptide, substance P, may trigger many of the inflammatory / oxidative / nitrosative events which eventually promote cardiomyopathy, *in situ* dysfunction, and loss of myocardial tolerance to secondary stresses.

Neuropeptide Release during MgD Triggers Inflammation and Oxidative / Nitrosative Stress

Circulating levels of the pro-inflammatory neuropeptide, substance P (SP),^{29,30} were found to be significantly elevated in MgD rodents.^{9,26,31,32} Calcitonin gene-related peptide (CGRP) was also elevated, and probably emanated from sensory-motor neuron fibers which are rich in both neuropeptides.³³ Significant early elevations of SP (dietary day 3) preceded the peak increases (between dietary days 7 and 21),^{9, 26, 29-32} in other inflammatory parameters (circulating interleukin-1 [IL-1], IL-6, tumor necrosis factor alpha [TNF α], histamine, PGE₂, gamma-interferon, circulating white blood cells [WBCs] and cardiac tissue WBC infiltration, CD11b, CD14, ICAM, & VEGF expression), in oxidative indices (LPO products, endogenous antioxidant depletion, iron status changes) and in nitrosative stress markers (nitrosyl-Hgb, plasma NO $\bullet$ oxidation products, cardiac tissue nitrotyrosine, and NOS2 and NOS3 protein expression). Changes in most of these parameters were substantially attenuated by concurrent *in vivo* treatment with specific neurokinin-1 (NK-1, or SP) receptor antagonists or an inhibitor of the N-methyl-D-aspartate (NMDA) receptor/channel during MgD,^{9,10,31,34-37}

Role of The NMDA Receptor In MgD

The NMDA receptor / channel complex enhances neuronal entry of substantial amounts of calcium, which is an important mediator of neuropeptide release (including SP). The NMDA channel is blocked by Mg²⁺ in a voltage-dependent manner.³⁸ Dizocilpine (MK-801), a non-

competitive NMDA receptor/channel blocker, inhibited cardiac inflammation in the Mg-deficient rat. The increases in endothelium adhesion molecule CD54 (iCAM) and of monocyte / macrophage surface protein CD11b in ventricular tissue were attenuated by MK-801. MgD also promoted apoptosis in rat hearts and this was ameliorated by NMDA receptor blockade.^{39,40} NMDA receptor/channel blockade effectively reduced circulating SP levels in MgD rats. Treatment of 3 wk MgD rats with a relatively low dose of MK-801 (0.5 mg/kg/day, s.c.) caused depression in plasma SP levels at all examined time intervals⁴¹ and area integration of the 3 wk time-course revealed an overall 50 % reduction in total SP compared to untreated. The early elevation in circulating SP during MgD may involve SP-rich C-fibers of the dorsal root ganglia or the spinal cord.⁴³ SP immunostaining of dorsal root ganglia showed a time-dependent (1-3 wks) depletion of SP in MgD rats, and this was prevented with MK-801 pretreatment.³⁹

The involvement of neuronal SP in systemic oxidative/nitrosative stress during MgD was also evident from *in vivo* NMDA receptor blockade studies. Plasma NO• product levels were shown to increase 2.5-fold in 2 wk MgD rats, and were reduced 50% by MK-801.⁴² Circulating prostaglandin E₂ (PGE₂, cyclooxygenase [COX]-derived vasoactive mediator) levels were elevated 3.9-fold in 2-wk MgD rats suggesting endothelial activation and were dose-dependently reduced by MK-801 treatment.^{39,42} MK-801 significantly prevented loss of RBC glutathione in 2 and 3 wk MgD rats, resulting in 38 - 56 % higher levels compared to the untreated MgD group.³⁹ Finally, MK-801 treatment substantially reduced (~ 70%) the 5.3-fold elevation in circulating neutrophil basal O₂⁻ production observed in 3 wk MgD rats.⁴² Collectively, these data implicate a central role of the NMDA receptor in modulating tissue and circulating SP levels during MgD.

NK-1 Receptor Blockade *in vivo* Attenuates SP Bioactivity and Associated Inflammation

Additional support for a critical involvement of SP in the pathology of experimental MgD comes from *in vivo* NK-1 (SP) receptor blockade. The actions of SP on inflammatory cells *in vivo* are mediated through NK-1 receptors⁴⁴ and this interaction can: induce degranulation of tissue mast cells and histamine release;⁴⁵ induce T-cell proliferation⁴⁶ and leukocyte adhesion;⁴⁵ activate macrophages;⁴⁷ prime neutrophils for oxidative metabolism (superoxide anion production);⁴⁸ and induce production of nitric oxide by endothelial cells.⁴⁹ *In vivo* treatment with the SP receptor antagonist, L-703,606, significantly attenuated the enhanced neutrophil production of O₂⁻ observed in 3 week MgD rats,^{35,50} suggesting a substantial role for SP in neutrophil activation. Three weeks of MgD also elicited a 13-fold elevation in circulating PGI₂ levels (COX-derived vasoactive mediator), and this was dramatically reduced (80%) by L-703,606.³⁵ *In vivo* treatment with another SP receptor antagonist, CP-96,345, also prevented most of the MgD-mediated loss of RBC glutathione, the rise in plasma levels of lipid peroxidation products (TBAR-reactive materials), and the formation of myocardial inflammatory lesions in this model.^{10,31,36} Changes in these oxidative stress markers resulting from SP receptor blockade are consistent with our observation that NMDA receptor blockade (MK-801) also attenuated these oxidative stress parameters.

MgD also induced NO• production and NOS up-regulation in the rat model. A moderate (1.7-fold) increase of plasma NO• oxidation products (nitrate + nitrite) was evident at day 7 of MgD and levels became 2 to 2.5-fold higher during weeks 2 and 3, indicating activation of NO• synthesis. MgD was shown to significantly induce iNOS (NOS2) protein expression in rat ventricular tissue after only 7 days.^{51,52} SP receptor blockade (L-703,606) *in vivo* inhibited the rise in plasma nitrate + nitrite by 75 %, ³⁵ raising the possibility that excessive NO• could mediate some of the deleterious effects of SP. Collectively, these findings

strongly suggest that SP promotes neutrophil activation and systemic oxidative/nitrosative stress during MgD and that interventions which reduce circulating SP levels or block its bioactivity at the NK-1 receptor are effective against MgD-induced injury.

Increased Intestinal Permeability and Endotoxemia During MgD

Since MgD has a systemic impact in our rodent model, we considered whether this condition predisposed animals to changes in intestinal permeability, endotoxemia and resultant cardiac inflammation. The gut is rich in neuropeptides⁵³ and may contribute to the early rise in plasma SP during MgD. During week 1 of MgD, intestinal inflammation was evident and pronounced PMN infiltration occurred by week 3, when significant decreases in mucosal barrier function were observed. Gastrointestinal mast cells (MCs) express receptors for SP and secrete tryptase II, the major mast cell protease (MCP-II) in rats.⁵⁴ MgD increased intestinal MCP-II expression in rats.⁵⁵ Moreover, eNOS (NOS3) expression (mRNA by real time reverse-transcription PCR) in small intestinal tissue of 3 week MgD rats was shown to significantly decrease (65 %) compared to control and may be associated with increased vascular permeability.⁵⁵ This is consistent with our findings that intestinal permeability (Evans blue dye) increased significantly (19-fold > control) in 3 week MgD rats and suggests the potential for endotoxin (LPS) translocation. LPS may induce systemic increases in TNF α , IL-1 β and IL-6 levels,⁵⁶ and can stimulate secretion of TNF α from adult rat cardiac myocytes. This process was dependent on the presence of LPS receptors (CD14).⁵⁷ In this light, we have observed enhanced CD14 receptor expression in MgD rat cardiac and intestinal tissues. These findings, as illustrated in Figure 1, implicate a potential role for SP in the development of enhanced intestinal permeability, endotoxemia and cardiac dysfunction during prolonged MgD.

Hearts from MgD Rats are Predisposed to Ischemia/Reperfusion Injury and are Protected by In Vivo Treatment with Antioxidants and SP Receptor Blockers

Our prior studies^{22,24,34} showed that 3 weeks of severe MgD₉ (9% recommended daily allowance [RDA] for Mg) causes reduced myocardial tolerance to *in vitro* postischemic stress: greater mechanical dysfunction, enhanced tissue injury, and greatly elevated lipid peroxidation (LPO-derived lipid hydroperoxide) and free radical production. There were no significant differences in pre-ischemic baseline functional / hemodynamic properties of 3 week MgD₉ and MgS hearts prior to I/R.^{22,34} Thus, *in vivo* events during the 3 weeks of MgD₉ predisposed hearts to I/R injury.

Treatment *in vivo* with antioxidants prevented much of the MgD₉-induced loss of myocardial tolerance to I/R stress.^{22,24,32,34} Vitamin E improved recovery of function, and reduced total lipid alkoxyl radical levels (electron spin resonance spin trapping of LO $\bullet$), tissue LDH release, and tissue protein oxidation in 40 min I/R hearts.²² However, antioxidant protection may be secondary to the *in vivo* actions of SP during MgD. I/R hearts from L-703,606 (NK-1 receptor antagonist)-treated 3 week MgD₉ rats exhibited a significant improvement in cardiac work recovery, whereas similarly-stressed MgS hearts did not significantly benefit.³⁴ L-703,606-treatment of MgD₉ rats led to significant reductions in postischemic tissue LDH loss, lipid hydroperoxide production, and alkoxyl radical formation.³⁴ MgD rat hearts also produced substantially more (2.4 x) NO $\bullet$ during reperfusion and *in vivo* SP receptor blockade significantly reduced this heightened NO $\bullet$ production.³⁵ This further increase in NO $\bullet$ production from I/R-stressed MgD hearts is likely to involve prior SP-mediated enhancement of iNOS (NOS2) activity during MgD. Similar treatment *in vivo* did not provide additional protection to I/R MgS hearts with

respect to the above parameters, and acute L-703,606 treatment *in vitro* proved ineffective.³⁴ Thus, SP receptor blockade must inhibit *in vivo* SP-triggered inflammatory responses observed during MgD, and these responses must influence myocardial vulnerability to I/R injury.

Severity & Duration of Dietary Mg-Restriction Influences Myocardial I/R Tolerance

Varying dietary Mg-intake in rats directly influenced plasma SP levels,⁵⁸ the associated severity of systemic oxidative/nitrosative stress,^{59,60} and loss of myocardial tolerance to I/R stress.⁵⁸ We demonstrated that SP release also occurred in rats fed moderate MgD diets (MgD₂₀ = 20 % RDA; MgD₄₀ = 40% RDA)⁵⁸ and the literature suggests that many of the same pathological characteristics observed in the severe MgD₉ animal also occurred in these animals.^{59,60} MgD₉, MgD₄₀, and Mg₁₀₀ (100% RDA=Mg-Sufficient or MgS) rats⁵⁸ displayed proportional changes in their plasma Mg concentrations (% of Mg₁₀₀: MgD₉ = 31; MgD₂₀ = 50; MgD₄₀ = 75). When placed on increasingly restricted Mg diets for 3 weeks, they exhibited proportionately greater SP levels: total SP levels for MgD₄₀ and MgD₉ were 2.43- and 5.14-fold higher than Mg₁₀₀, respectively. This confirms that the magnitude of the SP elevation can be modulated by the extent of dietary Mg-restriction, and that neurogenic inflammation can occur at clinically-relevant levels of hypomagnesemia.^{12,58}

Changes in RBC glutathione and plasma malondialdehyde, were also monitored in these 3 week Mg-restricted rats.⁵⁸ An inverse relationship for plasma malondialdehyde formation versus Mg-dietary content was observed: levels from MgD₄₀, MgD₂₀, and MgD₉ rats were elevated 20%, 60%, and 148% compared to Mg₁₀₀ rats. Moreover, the decline in RBC glutathione was also directly proportional to the extent of Mg-restriction: levels from MgD₄₀, MgD₂₀, and MgD₉ rats fell 20.6%, 29.4%, and 50% compared to the Mg₁₀₀ group.

When dietary duration was extended to 7 weeks, hearts from MgD₂₀ and MgD₄₀ rats displayed normal baseline (control) hemodynamic properties (Fig. 2),⁵⁸ yet were more susceptible than Mg₁₀₀ (MgD₂₀ > MgD₄₀ > Mg₁₀₀) to *in vitro* I/R stress (40- minute global I/30-minute R). This was shown by the greater oxidative (alkoxyl radical production) and tissue (myocardial LDH loss) injury. Significant (p<0.05) reductions in cardiac output, and LV developed pressure, along with increased mean aortic diastolic pressure, were responsible for further decreases in I/R cardiac work observed with MgD₂₀ and MgD₄₀ hearts.⁵⁸

When exposure to the moderate MgD₂₀ diet was extended to 8 weeks, perfused hearts from these rats displayed significant (p<0.05) declines (18%-21%) in baseline cardiac output and cardiac pressure-volume work (Fig. 2, top); such significant changes were not observed at 7 weeks (Fig 2, top).⁵⁸ Hearts from 8 week MgD₂₀ rats also displayed greater I/R contractile dysfunction (13 % lower cardiac work recovery) and enhanced oxidative injury (15% more alkoxyl radical production) (Fig. 2, bottom) compared to the I/R-stressed 7 week group. This demonstrates that duration of moderate dietary Mg-restriction is also an important determinant of myocardial susceptibility to imposed stresses.

SP-Mediated Oxidative Stress Persists with Extended Exposure to Severe MgD

We previously showed that elevations in circulating SP during 3 weeks of MgD was transient.⁵⁸ After an initial rise in SP levels during week 1 of MgD, levels declined during week 2, and this was followed by substantially greater elevations during week 3.^{9,31,34-36}

Changes in systemic oxidative stress markers occurred with or subsequent to the initial increase in SP and were largely attenuated by SP receptor blockade *in vivo*. The effects of extending severe dietary Mg-restriction to 5 weeks on circulating SP levels and indices of oxidative stress were determined in the rat (Fig. 3). Rats exposed to 5 weeks of MgD exhibited a 6.5-fold increase in circulating SP levels. This was associated with enhanced oxidative stress, as indicated by the 52% decline in RBC glutathione and elevations in plasma 8-isoprostane levels (LPO), which were 80 % higher at week 3, but 203 % higher at 5 week. Circulating PMNs isolated from 5 week MgD rats displayed a nearly 4-fold higher basal superoxide-generating activity compared to the MgS group, indicative of endogenous basal PMN activation. These findings suggest that *in vivo* oxidative stress was actively occurring even during later periods of MgD.

Echocardiographic Evidence that Extended Dietary MgD Induces Cardiac Dysfunction

Extending the duration of severe dietary MgD to 5 weeks also had a negative impact on *in situ* rat heart function, as shown by noninvasive echocardiography.³⁵ Three weeks of MgD did not significantly alter LV systolic function, but 5 weeks of MgD (Table 1) led to a significant ($p<0.05$) depression in LV systolic function (LV ejection fraction fell by 6.1%; % fractional shortening by 16.5%; maximum aortic pressure [Pmax] by 24.4 %, and maximum aortic velocity [Vmax] by 13.5 %). Interventricular septum diameter in systole decreased by 18.6%, possibly indicative of dilated cardiomyopathy, and the mitral valve E/A wave ratio fell by 27.7%, suggestive of LV diastolic dysfunction. These findings may represent the first echocardiographic demonstration of cardiac dysfunction in a rodent model of MgD. We anticipate that future studies with SP receptor antagonists may confirm a causal role for SP in the development of oxidative injury and cardiac dysfunction during long-term MgD.

Conclusion

Key findings are presented which support our proposal that neurogenic inflammation (SP) during early stages of dietary MgD in rodents is the principle trigger of inflammatory, oxidative, and nitrosative events, which promote the subsequent development of *in situ* cardiac dysfunction, cardiomyopathy, and the loss of myocardial tolerance to imposed stresses (I/R). This view is largely supported by observations that *in vivo* treatments which either alter neuronal SP release / bioavailability (NMDA receptor/channel blockade, varying dietary Mg content), reduced SP bioactivity (NK-1 receptor blockade), or limited oxidative/ nitrosative stress injury (antioxidants, nitric oxide synthase inhibitors), afforded significant protection against most of the pathology associated with MgD. Moderate Mg-restricted diets (20% and 40% RDA) in rodents produced neurogenic inflammation, as well as levels of hypomagnesemia that are comparable¹² to those found in various clinical situations (cardiovascular disease, diabetes, alcoholism, AIDS/HIV, cancer, drugs with Mg wasting properties).^{1-3,61} Thus, the use of additional agents (antioxidants, NK-1 receptor blockers), which lessen the potential cardiac pathology caused by this mineral deficiency, might be worthy of future clinical applications in addition to Mg-replacement therapy.

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Figure 1. Proposed events occurring during MgD, secondary to increased circulating SP. Increased oxidative stress in multiple cells; SP enhanced intestinal permeability; and subsequent endotoxemia contributing to cardiac injury and dysfunction.

Figure 2.

Extending moderate MgD (20% RDA) exposure from 7 to 8 weeks enhanced preischemic dysfunction (**top**) and I/R myocardial injury (**bottom**) compared to Mg₁₀₀ (MgS). Hearts were perfused in working mode for 20 min (pre-ischemic basal function), and then were exposed to 40 min global I/R. I/R values were normalized to basal levels. LVSP and LVDP = left ventricular systolic and developed pressures; A-DP = aortic diastolic pressure; CO = cardiac output; and CW = cardiac pressure-volume work. Means $\pm$ SE of 5-8. **Top:** * p < 0.05 vs Mg₁₀₀; **bottom:** **p < 0.02 vs Mg₁₀₀.

Figure 3.

Effects of extending dietary MgD exposure to 5 weeks on rat plasma SP levels (EIA kit from R&D Systems; time-course data were integrated over the specified 7 day period); plasma 8-isoprostane levels (immunoassay kit from Cayman Chemical); RBC glutathione (GSH) loss; and neutrophil (PMN) basal superoxide generating activity (SOD-inhibitable cytochrome c reduction assay). Values are means $\pm$ SE from 3-5 rats. *p<0.05, **p<0.01 vs MgS.

Table 1

Echocardiographic Parameters for Control and MgD Rats after 5 Weeks.

Parameters	5 wk Control	5 wk MgD % change ↑↓	Suggestive of:
LVEF	0.87 ± 0.02	↓ 6.1 ± 0.02 *	systolic dysfunction
%FS	52.0 ± 3.60	↓ 16.5 ± 0.07 *	systolic dysfunction
E/A	1.56 ± 0.17	↓ 27.7 ± 1.47 *	diastolic dysfunction
Pmax, mmHg	6.55 ± 0.45	↓ 24.4 ± 2.21 *	reduced aortic pressure
Vmax, m/s	1.283 ± 0.08	↓ 13.5 ± 0.55	Reduced aortic velocity
IVSs, mm	3.23 ± 0.23	↓ 18.6 ± 0.71 *	dilated cardiomyopathy

Echo parameters were measured using a GE VingMed System Five with a 10 MHz pediatric probe. **IVSs** = thickness of the interventricular septum in systole; **Pmax** = the pressure gradient of the velocity of the aortic outflow; **Vmax** = velocity of aortic outflow; **LVEF** = left ventricular ejection fraction; % **FS** = % fractional shortening; **E/A** = Mitral valve E/A ratio. Means ± SE of 4-5 rats, and values for treatment group are % changes (increased ↑ or decreased ↓) vs control.

*
p<0.05 vs Control.