Vascular biology of magnesium and its transporters in hypertension

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Abstract. Magnesium may influence blood pressure by modulating vascular tone and structure through its effects on myriad biochemical reactions that control vascular contraction/dilation, growth/apoptosis, differentiation and inflammation. Magnesium acts as a calcium channel antagonist, it stimulates production of vasodilator prostacyclins and nitric oxide and it alters vascular responses to vasoconstrictor agents. Mammalian cells regulate Mg²⁺ concentration through special transport systems that have only recently been characterized. Magnesium efflux occurs via Na⁺-dependent and Na⁺-independent pathways. Mg²⁺ influx is controlled by recently cloned transporters including Mrs2p, SLC41A1, SLC41A2, ACDP2, MagT1, TRPM6 and TRPM7. Alterations in some of these systems may contribute to hypomagnesemia and intracellular Mg²⁺ deficiency in hypertension and other cardiovascular pathologies. In particular, increased Mg²⁺ efflux through dysregulation of the vascular Na⁺/Mg²⁺ exchanger and decreased Mg²⁺ influx due to defective vascular and renal TRPM6/7 expression/activity may be important in altered vasomotor tone and consequently in blood pressure regulation. The present review discusses the role of Mg²⁺ in vascular biology and implications in hypertension and focuses on the putative transport systems that control magnesium homeostasis in the vascular system. Much research is still needed to clarify the exact mechanisms of cardiovascular Mg²⁺ regulation and the implications of aberrant cellular Mg²⁺ transport and altered cation status in the pathogenesis of hypertension and other cardiovascular diseases.

Key words: cations, exchangers, Na⁺/Mg²⁺ exchanger, TRPM7, vascular smooth muscle, blood pressure

An inverse association between body Mg²⁺ levels and blood pressure has been reported in epidemiological studies and hypertensive actions of dietary Mg²⁺ supplementation and hypertensive effects of Mg²⁺ deficiency have been demonstrated in animal models and in patients [1-8]. Molecular processes underlying this relationship between blood pressure and Mg²⁺ are unclear, but Mg²⁺ effects on the vasculature have been suggested to be important.

Magnesium is an essential cation involved in many essential physiological and biochemical processes regulating cardiovascular function, including contraction and dilation, growth and inflammation, production of vasoactive agents and protein and nucleic acid synthesis. Magnesium is critical for many enzymatic reactions, since all phosphate-dependent reactions have an obligatory need for Mg²⁺ [9, 10]. Magnesium also regulates ion channels and is a natural antagonist to Ca²⁺ [11, 12]. Intracellular Mg²⁺ is tightly regulated and since Mg²⁺ is a critical component in multiple biochemical reactions, a small change in [Mg²⁺], could lead to significant effects on signaling pathways that regulate vascular functions [13, 14]. Despite the fact that Mg²⁺ is such an abundant cytosolic cation and that it is so important in biological processes, there is a paucity of information regarding the mechanisms whereby cells regulate Mg²⁺ transport across cell mem-
Transporters and exchangers that have been implicated in transcellular Mg\(^{2+}\) transport include the Na\(^+/\)Mg\(^{2+}\) exchanger, Mg\(^{2+}/\)Ca\(^{2+}\) exchanger and recently identified cation channels, including transient receptor potential melastatin 6 and 7 channels (TRPM6, TRPM7) [15-21]. The present review will discuss the importance of Mg\(^{2+}\) in vascular biology in hypertension and will focus on the transport systems that may play a role in the control of vascular magnesium homeostasis.

**Magnesium, vessels and hypertension**

Hypomagnesemia and decreased tissue Mg\(^{2+}\) levels have been reported in various models of experimental hypertension [22, 23]. Clinical studies examining Mg\(^{2+}\) status in hypertensive patients have shown, for the most part, some form of hypomagnesemia (serum and/or tissue) and an inverse association between blood pressure levels and serum Mg\(^{2+}\) (serum and/or tissue) and an inverse association for the most part, some form of hypomagnesemia Mg\(^{2+}\) status in hypertensive patients have shown, have been reported in various models of experimental hypertension [22, 23]. Clinical studies examining Mg\(^{2+}\) status in hypertensive patients have shown, for the most part, some form of hypomagnesemia [24, 25]. Low serum Mg\(^{2+}\) is a common finding in elderly patients with metabolic syndrome [26]. Some investigations failed to demonstrate hypomagnesemia or decreased cellular Mg\(^{2+}\) in hypertension [27], while others reported elevated levels of erythrocyte [Mg\(^{2+}\)], [28]. Hypertensive patients with low Mg\(^{2+}\) levels require a greater number of antihypertensive medications compared with normomagnesemic patients [29]. A recent meta-analysis of 44 human studies showed that magnesium supplements may enhance the effects of antihypertensive medications in mildly hypertensive patients [30] and those with diabetes [31, 32]. Hypertensive patients treated with 600 mg of magnesium pidolate daily, demonstrated a small but significant reduction in day and night blood pressure as assessed by ambulatory monitoring [33].

Large population-based studies have also demonstrated variable associations between serum magnesium and blood pressure. Data from the Framingham Heart Study offspring cohort, failed to demonstrate that low serum magnesium is a risk factor for developing hypertension or CVD in 3,531 middle-aged adult participants [34]. On the other hand hypomagnesemia was found to be one of the strongest predictors of gain in left ventricular mass following 5 years in subjects (n = 1,348) enrolled in the “Study of Health in Pomerania” [35]. Moreover data from the WHO-coordinated CARDIAC (Cardiovascular Diseases and Alimentary Comparison) Study (3960 individuals from 41 WHO-CARDIAC study populations) demonstrated that among 5 diet-related factors, namely total cholesterol, body mass index, sodium, magnesium and taurine to creatinine (Cr) ratio in 24-hour urine, both Taurine/Cr and Mg\(^{2+}/\)Cr were inversely related to coronary heart disease mortalities in males and females, suggesting that higher intakes of taurine and Mg\(^{2+}\) are associated with significantly lower all cardiovascular risks [36]. Similar trends were observed for stroke in the Atherosclerosis Risk in Communities Study cohort (14,221 men and women aged 45-64 years). Higher serum magnesium levels were associated with lower prevalence of hypertension and diabetes mellitus at baseline, while the 15-year follow-up, revealed 577 ischemic strokes, with serum magnesium being inversely associated with ischemic stroke incidence [37].

Low magnesium intake and associated risk for hypertension may be particularly important in females, both during childhood as well as in postmenopausal women. Data from the National Heart, Lung, and Blood Institute Growth and Health Study that enrolled 2,368 girls (49% Caucasian, 51% African-American) aged 9 or 10 years, showed that a lower intake of magnesium, fiber, potassium and calcium, and higher intake of caffeine and calories were each associated with increased incidence of hypertension [38]. In the Women’s Health Initiative Observational Study, 3,713 postmenopausal women aged 50-79 years were studied with respect to circulating markers of inflammation (hs-CRP, IL-6, TNF-α, ICAM-1, VCAM-1, and e-Selectin), and daily Mg\(^{2+}\) intake [39]. High magnesium intake was associated with lower concentrations of markers of systemic inflammation and endothelial dysfunction, suggesting an inverse association between body magnesium and inflammation and an endothelial protective effect of magnesium in postmenopausal women.

Magnesium influences blood pressure, in part, by regulating vascular tone and reactivity. When infused intravenously in stroke patients, magnesium induces a significant vasodilatory response with an associated decrease in blood pressure [40]. Magnesium pidolate (368 mg/day of Mg ion) administered for 1 month to elderly diabetic patients, was associated with a significant improvement of the post-ischemic endothelial-dependent flow-mediated dilation, indicating improved vascular function [40]. In experimental animals, increased levels of extracellular magnesium caused vasorelaxation, decreased vascular resistance and attenuated agonist-induced vasoconstriction [41-43], whereas decreased concentrations caused contraction, potentiated agonist-induced vasoreactivity and increased vascular tone and blood pressure [44, 45]. Magnesium
also influences vascular tone by preventing oxidative stress and by regulating cell growth and apoptosis [46-52]. It attenuates the generation of reactive oxygen species and pro-inflammatory mediators [48, 49] and it regulates MAP kinases in vascular cells [50, 51]. In intact arteries and arterial endothelial and vascular smooth muscle cells, Mg2+ stimulates production of prostacyclins and nitric oxide (NO), which induce vasodilation [46, 47]. In venous endothelial cells, TRPM7 downregulation and associated decreased cellular magnesium are associated with increased NOS activity and increased NO production, which may promote vasorelaxation [53]. Increased NOS/NO in this context of reduced TRPM7-mediated Mg2+ transport, may relate more to TRPM7 kinase deficiency than to changes in magnesium status. For the most part, magnesium is considered to induce vasodilation, as further evidenced by findings that magnesium lithospermate B, which activates eNOS and ameliorates endothelial dysfunction in diabetes by enhancing vasodilation, additionally reduces oxidative stress [54].

Because of the importance of Mg2+ in modulating vascular function, cellular levels need to be tightly regulated. Specific transporters controlling Mg2+ efflux and influx in cardiovascular and renal cells have recently been identified [21, 55-59] (table 1), some of which may be altered in hypertension [60].

**Cellular Mg homeostasis**

Unlike our knowledge of other major cations, the mechanisms regulating cellular Mg2+ handling are poorly understood. More than 95% of Mg2+ is sequestered by chelators or bound to other biomolecules, including phospholipids, ribosomes and phosphonucleotides (ATP, ADP) [9, 13]. Intracellular Mg2+ is maintained below the concentration predicted from the transmembrane electrochemical potential. This control is achieved through a balance of Mg2+ uptake, intracellular storage, and Mg2+ efflux mediated through specific Mg2+ transporters.

Mg2+ efflux occurs against the electrochemical gradient, therefore an energy-coupled mechanism for its extrusion must be present. Mg2+ efflux appears to be regulated by at least two pathways: the Na+-Mg2+ exchange driven by the Na+ gradient, and the Na+-independent "passive" Mg2+ transport via Mg2+-permeable channels [14]. Na+-dependent Mg2+ transport occurs mainly via the Na+/Mg2+ exchanger and has been demonstrated in many cell types, including vascular smooth muscle cells (VSMC) and cardiomyocytes [18, 21]. On the other hand, Na+-independent transport, demonstrated mainly in erythocytes and hepatic cells, involves Ca2+ (Ca2+/Mg2+ exchanger), Mn2+ (Mn2+/Mg2+ antiporter) and Cl− (Cl-/Mg2+ co-transporter)-dependent mechanisms (18,21). Regulation of these exchangers remains unclear, although angiotensin II (Ang II), aldosterone, and other vasoactive agents have been shown to influence these transporters [7, 17].

Until recently little was known about protein transporters controlling transmembrane magnesium influx. A few Mg2+ transporters had been demon-

**Table 1.** Magnesium transporters, functional role and distribution ion the cardiovascular and renal systems.

<table>
<thead>
<tr>
<th>Transporter</th>
<th>Protein</th>
<th>Function</th>
<th>Cardiovascular-renal distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mitochondrial RNA splicing 2 protein</td>
<td>Mrs2p</td>
<td>Mitochondrial Mg2+ influx</td>
<td>Inner mitochondria</td>
</tr>
<tr>
<td>Solute carrier family 41, member 1</td>
<td>SLC41A1</td>
<td>General transporter for divalent cations</td>
<td>Heart, kidney</td>
</tr>
<tr>
<td>Solute carrier family 41, member 2</td>
<td>SLC41A2</td>
<td>General transporter for divalent cations, but not Ca2+</td>
<td>Heart</td>
</tr>
<tr>
<td>Magnesium Transporter 1</td>
<td>MagT1</td>
<td>Mg2+-specific transporter convoluted tubules</td>
<td>Distal</td>
</tr>
<tr>
<td>Ancient Conserved Domain Protein 2</td>
<td>ACDP2</td>
<td>General transporter for divalent cations, but not Ca2+</td>
<td>Kidney cortex</td>
</tr>
<tr>
<td>Transient receptor potential melastatin 6</td>
<td>TRPM6</td>
<td>Renal and gastrointestinal Mg2+ absorption</td>
<td>Kidney tubules, Vessels</td>
</tr>
<tr>
<td>Transient receptor potential melastatin 7</td>
<td>TRPM7</td>
<td>Cell viability, Cellular Mg2+ homeostasis</td>
<td>Kidney, heart, Vessels</td>
</tr>
<tr>
<td>Paracellin-1</td>
<td></td>
<td>Paracellular Mg2+ and Ca2+ reabsorption in the thick ascending limb of loop of Henle</td>
<td>Kidney</td>
</tr>
</tbody>
</table>
strated, but only at the biophysical and functional levels. Recent advances in the field have now identified specific transmembrane Mg\(^{2+}\) transporters. The first mammalian Mg\(^{2+}\) transporter to be identified at the molecular level was Mrs2 (mitochondrial RNA splicing2), responsible for mitochondrial Mg\(^{2+}\) uptake [21]. Other proteins shown to regulate Mg\(^{2+}\) homeostasis include Mg\(^{2+}\) transporter subtype 1 (MagT1), the solute carrier (SLC) family 41 subtype 1 and 2 (SLC41A1, SLAC41A2, respectively) and the Ancient Conserved Domain Protein 2 (ACDP2). Microarray analysis showed that the NIPA1 and 2 genes, named for “nonimprinted in Prader-Willi/Angelman”, membrane Mg\(^{2+}\) transporters 1 and 2 (MMgT1 and 2 respectively) and Huntington interacting protein genes, HIP14 and HIP14L also encode a Mg\(^{2+}\) transporter (reviewed in 21). The exact physiological role of these novel Mg\(^{2+}\) transporters with respect to Mg\(^{2+}\) homeostasis still awaits clarification, but many of the identified mammalian Mg\(^{2+}\) transporters have been associated with congenital disorders encompassing a wide range of tissues, including intestine, kidney, brain, nervous system, and skin.

Analysis of different forms of human disorders characterized by low serum Mg\(^{2+}\) levels due to defective intestinal absorption and/or renal Mg\(^{2+}\) wasting led to the identification of a paracellular (between cells) magnesium transporter, paracellin-1 (claudin 16), a member of the claudin family of tight-junction proteins [55]. Paracellin-1 mutations are associated with a hereditary disease, hypomagnesemia with hypercalciuria and nephrocalcinosis (FHHNC), characterized by massive renal Mg\(^{2+}\) and Ca\(^{2+}\) wasting leading to end-stage renal disease. Genetic analyses of patients with primary hypomagnesemia and secondary hypocalcemia (HSII), another Mg\(^{2+}\)-wasting disorder, identified TRPM6 (and its homologue TRPM7) as a key component of epithelial Mg\(^{2+}\) reabsorption [56, 57]. The ubiquitously expressed TRPM7 was characterized functionally as a constitutively active ion channel permeable for divalent cations including Mg\(^{2+}\) and regulated by intracellular concentrations of Mg\(^{2+}\), magnesium-nucleotide complexes, and humoral factors [58, 59].

**TRPM cation channels and magnesium**

Whereas TRPM6 is expressed primarily in intestinal epithelia and kidney tubules, TRPM7 is ubiquitous, and is expressed in blood vessels, the heart, brain, lungs, liver, spleen and intestine [60-64]. We demonstrated that VSMC from rat and human resistance arteries possess functionally active TRPM7 cation channels [65]. TRPM7 has also been identified in vascular cells from pulmonary arteries [65].

TRPM7 is permeable to the dominant physiological divalent cations Ca\(^{2+}\) and Mg\(^{2+}\) and also to essential trace metal (Zn\(^{2+}\), Cu\(^{2+}\), Fe\(^{2+}\), Mn\(^{2+}\), Co\(^{2+}\), Ni\(^{2+}\)) and toxic metal ions (Cd\(^{2+}\), Ba\(^{2+}\), Sr\(^{2+}\)) [66-69]. Of the divalent cations, TRPM7 appears to be most selective for Mg\(^{2+}\) and is essential for cellular function [58, 59, 69, 70]. TRPM7 knockout cells, which undergo growth arrest and compromised viability, can be rescued by Mg\(^{2+}\) supplementation [70-73], although in HUVECs, TRPM7 downregulation by siRNA was associated with MAP kinase activation and cell growth [53]. TRPM6 and TRPM7 have the unique feature of possessing a transmembrane domain linked to an α-kinase domain at the C-terminus [75, 76]. Despite the high homology of TRPM6 and TRPM7, and although they are both implicated in Mg\(^{2+}\) distribution in the body, they have physiologically and pharmacologically distinct roles in Mg\(^{2+}\) homeostasis [76, 77]. Whereas TRPM6 appears to be involved mainly in regulating total body Mg\(^{2+}\) levels through the kidney and gastrointestinal tract [78, 79], TRPM7 may be more important in regulating intracellular Mg\(^{2+}\) homeostasis and [Mg\(^{2+}\)]\(_i\) [76, 79].

TRPM7, like TRPM6, is regulated by changes in cytosolic Mg\(^{2+}\) or Mg\(^{2+}\)-ATP [80-82]. This is the reason why TRPM7 and TRPM6 were previously called MIC (magnesium inhibiting channel) or MagNuM (magnesium-nucleotide-regulated metal ion channel) [82]. Intracellular Mg\(^{2+}\) inhibits TRPM7, which may serve as a negative feedback mechanism to reduce Mg\(^{2+}\) uptake when the cell has sufficient Mg\(^{2+}\). Under conditions of low intracellular Mg\(^{2+}\), recovery from this inhibition may open the channel to normalize cytosolic Mg\(^{2+}\) levels. The modulatory effect of Mg\(^{2+}\) and Mg\(^{2+}\)-ATP may be related to the protein kinase domain of the channel [82-84]. The dual ability of TRPM7 to act as a channel and at the same time as a kinase, suggests that this protein is involved in regulating both cellular Mg\(^{2+}\) status and intracellular signaling pathways. To date, three known TRPM7 kinase domain substrates have been identified, annexin-1, myosin IIA heavy chain and calpain [85-87], all important in controlling cell functions such as cell adhesion, cytokkeletal organization, cell migration, cell death and cell growth. Deletion of TRPM7 in mice is embryonic lethal and TRPM7-deficient mice (heterozygotes) reveal a defect in intestinal Mg\(^{2+}\) absorption, supporting
the critical function of TRPM7 in normal magnesium homeostasis and in fundamental cellular processes [74].

Factors influencing TRPM7 expression and activity are still under investigation. Other than Mg\(^{2+}\) and Mg-ATP, the role of second messengers in TRPM7 regulation is unclear. PIP2 may inhibit TRPM7 channel activity [80, 88]. Some studies suggested that cAMP influences TRPM7 channel activity [89], whereas others failed to demonstrate that cAMP/cGMP signaling influences TRPM7 activity (90). TRPM7 binds directly to several PLC isoforms, including PLC and PLC [90], which may be important for G protein-coupled receptor activation of TRPM7. Humoral factors, including bradykinin, Ang II, aldosterone and estrogen [65, 90-92], and mechanical factors, such as shear stress and stretch [87, 92-95], which are involved in the regulation of vascular tone and structure, also modulate TRPM7. Bradykinin increases TRPM7 channel activity in N1E-115 cells [90]. In VSMC, Ang II and aldosterone modulate TRPM6 and TRPM7 expression and influence TRPM7-dependent Mg\(^{2+}\) transport [65]. Much research, especially in physiologically relevant systems and not in cell lines, is still needed to understand exactly how TRPM7 is regulated, what stimuli activate or inhibit TRPM7 activity, how intracellular signaling molecules regulate the channel and kinase domains, what the downstream signaling targets of TRPM7 kinase are and how TRPM7 influences cellular functional responses.

\textbf{Mg}^{2+} \textit{transporters in hypertension}

Studies from our laboratory and those of others demonstrated that decreased [Mg\(^{2+}\)]\textsubscript{i} in hypertension is associated with alterations in both Mg\(^{2+}\) efflux and influx. The Na\(^+/\text{Mg}^{2+}\) antiport plays a major role in Mg\(^{2+}\) extrusion in cardiac, renal and VSMC and in hypertension, the Na\(^+/\text{Mg}^{2+}\) antiporter function is altered [96-98]. This exchanger is inhibited by amiloride, quinine, imipramine and manganese [99]. In spontaneously hypertensive rats (SHR), amiloride and quinidine administration was associated with an increase in vascular [Mg\(^{2+}\)]\textsubscript{i} and attenuation of the development of hypertension. In Ang II-induced hypertension in rats, inhibition of the Na\(^+/\text{Mg}^{2+}\) antiporter resulted in reduced blood pressure, normalization of vascular and renal MAP kinase activity and improved vascular structure [100]. Other studies have also demonstrated alterations in Na\(^+/\text{Mg}^{2+}\) exchanger activity in hypertension [96, 98, 101].

Altered Mg\(^{2+}\) influx in VSMC in SHR was associated with downregulation of vascular TRPM7, but not TRPM6 [102]. The mechanisms underlying this are unclear. Similarly to what was reported in cell lines, we also found that TRPM7 and Mg\(^{2+}\) are critical for vascular cell viability, because TRPM7 knockdown with siRNA in VSMC resulted in reduced cell growth, which was restored upon Mg\(^{2+}\) supplementation [65]. Hence, aberrations in vascular TRPM7-regulated Mg\(^{2+}\) homeostasis in hypertension may contribute to altered vascular growth and contraction, important in vascular remodeling in hypertension [92, 103, 104].

\textbf{Conclusion}

At the vascular level, increased [Mg\(^{2+}\)]\textsubscript{i} is associated with vasodilation, anti-inflammatory responses and reduced blood pressure. On the other hand, decreased [Mg\(^{2+}\)]\textsubscript{i} is associated with endothelial dysfunction, increased reactivity, enhanced contractility, vascular remodeling and inflammation and elevated blood pressure. Vascular Na\(^+/\text{Mg}^{2+}\) exchanger activity and TRPM7 expression/activity appear to be altered in experimental models of hypertension and may contribute to magnesium dysregulation and altered vascular function in hypertension. Since the recent identification and characterization of Mg\(^{2+}\)-selective transporters, there has been enormous interest in the field. However, there is a paucity of information and much research is needed to clarify the exact mechanisms of magnesium homeostasis in the cardiovascular system and the implications of aberrant cellular magnesium transport in the pathogenesis of hypertension and other vascular diseases. The role for magnesium in the management of hypertension still awaits clarification.

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