Transient Receptor Potential Melastatin 7 (TRPM7) Cation Channels, Magnesium and the Vascular System in Hypertension

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Decreased Mg^{2+} concentration has been implicated in altered vascular reactivity, endothelial dysfunction and structural remodeling, processes important in vascular changes and target organ damage associated with hypertension. Unlike our knowledge of other major cations, mechanisms regulating cellular Mg^{2+} handling are poorly understood. Until recently little was known about protein transporters controlling transmembrane Mg^{2+} influx. However, new research has uncovered a number of genes and proteins identified as transmembrane Mg^{2+} transporters, particularly transient receptor potential melastatin (TRPM) cation channels, TRPM6 and TRPM7. Whereas TRPM6 is found primarily in epithelial cells, TRPM7 is ubiquitously expressed. Vascular TRPM7 has been implicated as a signaling kinase involved in vascular smooth muscle cell growth, apoptosis, adhesion, contraction, cytoskeletal organization and migration, and is modulated by vasoactive agents, pressure, stretch and osmotic changes. Emerging evidence suggests that vascular TRPM7 function might be altered in hypertension. The present review discusses the importance of Mg^{2+} in vascular biology in hypertension and focuses on transport systems, mainly TRPM7, that might play a role in the control of vascular Mg^{2+} homeostasis. Elucidation of the relationship between the complex systems responsible for regulation of Mg^{2+} homeostasis, the role of TRPM7 in vascular signaling, and the cardiovascular impact will be important for understanding the clinical implications of hypomagnesemia in cardiovascular disease. (*Circ J* 2011; **75**: 237–245)

Key Words: Cardiovascular system; Hypertension; Magnesium; TRPM7

Hypertension is the most common chronic disease in developed and developing societies and a major risk factor for morbidity and mortality.^{1,2} The most common form of hypertension is ‘essential hypertension’ the causes of which remain unknown. Among the many factors implicated in the pathophysiology of hypertension are neurohumoral, renal, metabolic, race, genetic, environmental factors and alterations in cellular cations, such as sodium (Na^+), calcium (Ca^{2+}), potassium (K^+) and magnesium (Mg^{2+}) have been shown to be related to the increase in systolic blood pressure.^{3–7}

Mg^{2+} , an abundant intracellular divalent cation, functions as an allosteric modulator of several proteins, controls nucleotide and protein synthesis, regulates Na^+ , K^+ , and Ca^{2+} channels and is critical for myriad enzymatic reactions, particularly those involving kinases.^{8,9} Mg^{2+} is stored primarily in bone and the intracellular compartments of muscle and soft tissues, with less than 1% of total body Mg^{2+} circulating in the blood.^{9,10} Mg^{2+} homeostasis depends primarily on the balance between intestinal uptake and renal excretion. Mg^{2+} deficiency results from reduced dietary intake, intestinal malabsorption

or renal loss. Mammalian cells tightly control magnesium levels within a narrow range (0.70–1.1 mmol/L) by specific regulatory mechanisms operating at the level of intraorganellar compartmentalization, intracellular magnesium buffering and at regulating magnesium entry and efflux across the cell membrane.

Epidemiological studies indicate that low Mg^{2+} status is associated with many diseases including diabetes, metabolic syndrome, dyslipidemia and hypertension.^{11–15} In the vascular system Mg^{2+} regulates vascular tone, cardiac rhythm and platelet-activated thrombosis.^{16,17} Increased intracellular Mg^{2+} concentration ($[Mg^{2+}]_i$) causes vasodilation and attenuates agonist-induced vasoconstriction, whereas reduced $[Mg^{2+}]_i$ has opposite effects, leading to hypercontractility and impaired vasorelaxation.^{18–21} Low $[Mg^{2+}]_i$ also results in abnormal vascular cell growth, inflammation and increased membrane permeability to Na^+ and Ca^{2+} accumulation in vascular smooth muscle cells (VSMC), which might be important in modulating vascular tone and function in hypertension.^{9,22}

Although our understanding of renal and intestinal Mg^{2+} homeostasis has been greatly enhanced over the past few

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years there is still a paucity of information regarding the molecular mechanisms whereby cells regulate intracellular Mg^{2+} homeostasis and transport across membranes. Transporters and exchangers that have been implicated in transmembrane Mg^{2+} transport include, among others, the Na^+/Mg^{2+} exchanger, Mg^{2+}/Ca^{2+} exchanger and the recently identified including transient receptor potential melastatin 6 and 7 channels (TRPM6, TRPM7).^{23–27} The present review will discuss the importance of Mg^{2+} in vascular biology in hypertension and will focus on the transport systems, mainly TRPM6/7, that might play a role in the control of vascular magnesium homeostasis.

Cellular Mg Homeostasis

Unlike our knowledge of other major cations, mechanisms regulating cellular Mg^{2+} handling are poorly understood. More than 95% of Mg^{2+} is sequestered by chelators or bound to other biomolecules, including phospholipids, ribosomes and phosphonucleotides (ATP, ADP).^{9,26} Intracellular Mg^{2+} is maintained below the concentration predicted from the transmembrane electrochemical potential. This control is achieved through a balance of Mg^{2+} uptake, intracellular storage, and Mg^{2+} efflux mediated through specific Mg^{2+} transporters.

Mg^{2+} efflux occurs against the electrochemical gradient, therefore an energy-coupled mechanism for its extrusion must be present. Mg^{2+} efflux appears to be regulated by at least 2 pathways: the Na^+-Mg^{2+} exchange driven by the Na^+ gradient, and the Na^+ -independent 'passive' Mg^{2+} transport via Mg^{2+} -permeable channels.^{28,29} Na^+ -dependent Mg^{2+} transport occurs mainly via the Na^+/Mg^{2+} exchanger and has been demonstrated in many cell types, including VSMC and cardiomyocytes.^{30–32} However, Na^+ -independent transport, demonstrated mainly in erythrocytes and hepatic cells, involves Ca^{2+} (Ca^{2+}/Mg^{2+} exchanger), Mn^{2+} (Mn^{2+}/Mg^{2+} antiporter) and Cl^- (Cl^-/Mg^{2+} co-transporter)-dependent mechanisms.^{26,33–35} Regulation of these exchangers remain unclear, although Ang II, aldosterone, and other vasoactive agents have been shown to influence these transporters.

Until recently little was known about protein transporters controlling transmembrane magnesium influx. A few Mg^{2+} transporters had been demonstrated, 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 mitochondrial RNA splicing2 (Mrs2), responsible for mitochondrial Mg^{2+} uptake.^{36,37} 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).^{38–41} Recently it has been shown by microarray analysis that the NIPA1 and 2 genes, named for 'non-imprinted 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.^{42–44}

Analysis of different forms of human disorders characterized by low serum Mg^{2+} concentrations 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.⁴⁵ 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 dis-

ease. Genetic analyses of patients with primary hypomagnesemia and secondary hypocalcemia (HSH), another Mg^{2+} -wasting disorder, identified TRPM6 (and its homologue TRPM7) as a key component of epithelial Mg^{2+} reabsorption.^{46,47} 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.^{48,49}

TRPM Channels

TRPM proteins, members of the transient receptor potential superfamily, possess 6 predicted transmembrane domains and display the structural hallmark of long intracellular NH2 and COOH termini.^{50–52} TRPM have been implicated in cardiovascular pathologies.^{52,53} The founding member, TRPM1 (melastatin), was detected in malignant melanoma cells.⁵⁴ Members of the TRPM family are divided into 4 groups:⁵¹ TRPM1/3, TRPM2/8, TRPM4/5 and TRPM6/7. TRPM channels exhibit highly varying cation permeability, from Ca^{2+} impermeable (TRPM4/5) to highly Ca^{2+} and Mg^{2+} permeable (TRPM6 and TRPM7).

TRPM3, abundantly expressed in the kidney, are Ca^{2+} -permeable ion channels activated by cell swelling.^{55,56} TRPM4 and TRPM5 are permeable to monovalent cations but impermeable to Ca^{2+} and display voltage-dependent modulation and rapid activation and deactivation kinetics on receptor stimulation and depolarization. TRPM4 exhibits the highest expression in heart, pancreas and placenta, whereas TRPM5 is enriched in taste receptor cells.^{57–59} A different role in sensory physiology has been ascribed to TRPM8, which is expressed in sensory neurons and prostate carcinoma cells and activated by menthol, icillin, and cool temperatures. TRPM8 acts as a plasmalemmal Ca^{2+} channel and as an intracellular Ca^{2+} release channel.^{51,57,59}

Three members of the TRPM family, TRPM2, TRPM6, and TRPM7, are set apart from other known ion channels because they harbor enzyme domains in their respective COOH termini and thus represent prototypes of an intriguing new protein family of enzyme-coupled ion channels termed 'chanzyme' (channel plus enzyme).^{25,50,51,60} TRPM2 is expressed mainly in the brain and is a non-selective cation channel permeable to Na^+ , Ca^{2+} , K^+ and Cs^+ . TRPM2 is COOH terminally fused to an ADP-pyrophosphatase and is activated by one of the products of NAD hydrolysis, ADP-ribose.⁶¹ TRPM2 is highly sensitivity to H_2O_2 and other reactive oxygen species.⁶²

TRPM6 and TRPM7 are now well accepted as important regulators of Mg^{2+} homeostasis. These channels also transport other cations such as Ca^{2+} , Zn^{2+} , Mn^{2+} , and Co^{2+} and in the absence of Ca^{2+} and Mg^{2+} they can mediate Na^+ flux.^{27,63,64} TRPM6 and TRPM7 share ~50% sequence homology, are constitutively active and contain protein kinase domains in their COOH termini, which bear sequence similarity to elongation factor 2 (eEF-2) serine/threonine kinases.⁴⁹

TRPM6 mRNA shows a restricted expression pattern, with the highest levels along the intestine and the distal convoluted tubule (DCT) of the kidney. TRPM6 co-localizes with cytosolic proteins that buffer intracellular Mg^{2+} such as Na^+/Cl^- cotransporter NCCT, parvalbumin and calbindin-D28K.^{46,47,65–67} The critical role of TRPM6 for epithelial Mg^{2+} transport became evident when positional cloning showed that HSH, an autosomal-recessive disorder and characterized by low serum Mg^{2+} concentrations due to diminished intestinal Mg^{2+} absorption and decreased renal Mg^{2+} reabsorption, is

caused by mutations in the TRPM6 gene.^{46,47,66} Factors implicated in TRPM6 regulation remain unclear, but 17β -estradiol and Mg^{2+} are important,⁶⁸ TRPM6 currents are inhibited by millimolar concentrations of intracellular Mg^{2+} .^{64,65,67} Recent studies identified epidermal growth factor (EGF) as an autocrine/paracrine magnesiotropic hormone. EGF binding to its EGFR at the basolateral membrane of the DCT and stimulates endomembrane trafficking and increased number of TRPM6 channels at the plasma membrane.^{69,70} The signaling pathway downstream of EGFR activation, which stimulates TRPM6-mediated Mg^{2+} influx is dependent on Src, ERK/MEK, PI3K and AP-1.^{70,71} The role of EGFR signaling in Mg^{2+} homeostasis is further supported by the observation that patients treated with cetuximab, a mAb directed against the EGFR, develop hypomagnesemia.^{72,73}

To date little is known about the TRPM6 α -kinase domain. Receptor for activated C-kinase 1 and repressor of estrogen receptor activity interact with the α -kinase domain and inhibit

channel activity in a phosphorylation-dependent manner.⁷⁴ Intracellular ATP modulation of TRPM6 channel activity requires the ATP-binding motif in the α -kinase domain. Also, the α -kinase of TRPM6 is capable of phosphorylating TRPM7 but not vice versa, underlying the functional non-redundancy of these 2 channels.²⁵

TRPM6 and TRPM7 can form hetero-oligomeric channel complexes. TRPM7 but not other members of the TRPM family seems to be necessary for TRPM6 trafficking to the plasma membrane.^{23,25,27,66} TRPM7 is functional on its own, but homo-oligomerization might be needed for correct positioning of TRPM7 to the plasma membrane.^{23,25} Single-channel conductance and biophysical properties studies suggest that TRPM6, TRPM7 and the complex TRPM6/7 are distinct ion channels that exhibit different divalent cation permeability, pH sensitivity and unique single channel conductance.⁷⁵ Also, TRPM6 and TRPM7 have distinct properties with respect to the pharmacological agent 2-aminoethoxydiphenyl

borate (2-APB). Whereas micromolar levels of 2-APB maximally increase TRPM6 activity, they inhibit activity of TRPM7 channels.⁷⁵ The exact role of TRPM6/TRPM7 complex in Mg^{2+} homeostasis remains to be elucidated.

TRPM7

TRPM7 is ubiquitously expressed.^{48,49} In physiological conditions TRPM7 preferentially transports Mg^{2+} and to a lesser extent Ca^{2+} and other divalent cations. Because Mg^{2+} plays such an important role in cell function, it seems unlikely that TRPM7 is the only transporter involved in maintaining intracellular Mg^{2+} concentration. In support of this, a recent study using primary mammalian cells found that deletion of TRPM7 did not alter cellular Mg^{2+} concentration.⁷⁶ Several other studies demonstrated that targeted disruption of TRPM7 gene in cell lines is lethal, underpinning a salient and non-redundant role of this channel in cell physiology and cell survival.^{23,48}

TRPM7 is constitutively active and like TRPM6 is negatively regulated by changes in cytosolic Mg^{2+} or Mg^{2+} -ATP.^{23,25,48,49,63,77} Several studies have demonstrated that ATP acts as an intracellular Mg^{2+} chelator, decreasing the concentration of intracellular free Mg^{2+} , leading to increased TRPM7-mediated conductance.

The functional α -kinase domain at the carboxyl terminus of TRPM7 has been structurally characterized by X-ray crystallography and displays the unique features of a zinc finger domain as well as a region involved in the catalysis of ATP-triggered reactions.⁷⁸ 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.^{48,49} The role of TRPM7 kinase domain for the channel function is still controversial. While some studies reported that phosphotransferase-deficient TRPM7 mutants displayed reduced sensitivity for intracellular Mg^{2+} and reduced current amplitude upon G protein-coupled receptor stimulation or were even not functional, others found no changes in Mg^{2+} sensitivity of TRPM7 channels lacking the α -kinase domain when compared to wild-type channel.^{23,48,49,80} Since the α -kinase is adjacent to the conducting pore of TRPM7, changes in free Mg^{2+} concentrations might activate the associated kinase domain. The kinase in turn would signal via other phosphorylated substrates and/or feedback to modulate TRPM7 channel gating. Thus TRPM7 could function as a ' Mg^{2+} sensor'.^{25-27,48,49,79,80} It is also possible that the kinase domain might play a role in channel assembly or subcellular localization to the plasma membrane (Figure 1).

To date, 3 known TRPM7 kinase substrates have been identified, annexin-1, myosin IIA heavy chain and calpain.⁸¹⁻⁸³ Annexin-1 is an endogenous mediator of the anti-inflammatory actions of glucocorticoids, and is implicated in cell growth and apoptosis.⁸¹ Considering that both annexin-1 and TRPM7 have been linked to processes of cell survival and cell growth it is possible that the regulation of cell death and proliferation by TRPM7 involves phosphorylation of annexin-1 by TRPM7 kinase. Myosin II heavy chain is involved in cell migration, growth, apoptosis and cytoskeletal organization. Identification of myosin IIA heavy chain as another substrate for TRPM7 α -kinase domain further supported the role of this channel in cell growth and differentiation.⁸² TRPM7 also activates calpain and initiates the disassembly or turnover of peripheral adhesion complexes, which is involved in cell adhesion, motility, metastasis, apoptosis, and ischemic cell death. In fact, after neural injury, TRPM7 might accelerate the anoxia-induced production of reactive oxygen species

and exacerbate cell death.⁸⁴ Other possible functions of the kinase domain include a role in phospholipase signaling.^{49,85} The biological significance of TRPM7-regulated downstream targets still remains unclear, because studies on these substrates were performed mainly in cell lines.

TRPM7, Mg^{2+} and Hypertension

Epidemiological studies have linked the prevalence of cardiovascular diseases, including hypertension, and the quality of drinking water or diet. Among the many factors studied in drinking water, the highest inverse correlation has been observed between Mg^{2+} and cardiovascular mortality and morbidity,⁸⁶ and later by other epidemiological and clinical investigations that supported the hypothesis that increased Mg^{2+} intake contributes to prevention of hypertension and cardiovascular disease.⁸⁷⁻⁸⁹

Magnesium supplementation has been shown to lower blood pressure in some experimental models of hypertension.^{90,91} Clinical studies have shown increased, decreased and no effect of Mg^{2+} depletion or supplementation in hypertension. Despite these inconsistencies there are some groups of hypertensive patients who consistently demonstrate altered Mg^{2+} metabolism. These include individuals of African descent, obese participants, patients with severe or malignant forms of hypertension, elderly patients and those with metabolic syndrome.^{92,93} Such hypertensive patients might be Mg^{2+} -sensitive and potentially could benefit from Mg^{2+} supplementation.

Blood pressure is regulated by a variety of Mg^{2+} -sensitive factors that influence systemic vascular tone and resistance.^{94,95} Ion channels, which are essential for the control of membrane potential and thus vascular tone are modulated by changes in intracellular Mg^{2+} . Increased $[Mg^{2+}]_i$ suppresses voltage-dependent Ca^{2+} channels (VDCC), directly blocks inward rectifier K^{+} channels, and stimulates large conductance Ca^{2+} -activated K^{+} (BKCa) channels.⁹⁶ Ca^{2+} , a major determinant of cell contraction, proliferation, migration and secretion, is negatively modulated by Mg^{2+} . Magnesium inhibits Ca^{2+} depolarizing effects, Ca^{2+} -related excitation-contraction coupling, and also acts as a weak Ca^{2+} channel blocker promoting vasorelaxation.⁹⁷ The endothelium has also been shown to play a functional role in the regulation of vasomotor tone by generating and releasing endothelium-derived relaxing factors. Mg^{2+} promotes vasodilation through nitric oxide-dependent pathways and by cyclo-oxygenase-dependent mechanisms, via production of prostacyclin (PGI₂) in isolated aorta from rats.^{98,99} Magnesium-mediated endothelial release of PGI₂ from human umbilical arteries and cultured umbilical vein endothelial cells might be particularly relevant in $MgSO_4$ treatment of eclampsia and pre-eclampsia.^{99,100}

Magnesium also alters responses to vasoconstrictor and vasodilator agents. Absence of Mg^{2+} significantly potentiates contractile responses of vessels to bradykinin, Ang II, serotonin and prostaglandin F_{2 α} . These effects might be related to altered binding of agonists to their specific cell membrane receptors and/or to production of vasoactive agents.^{99,101} Magnesium infusion also inhibits endothelin-1-induced vasoconstriction and high extracellular Mg^{2+} concentration reduces the affinity of endothelin-1 for its specific receptors.¹⁰¹ Furthermore, in magnesium-deficient rats, plasma ET-1 levels are elevated, whereas in magnesium-supplemented rats, plasma ET-1 levels are reduced.^{94,101}

Another possible mechanism whereby Mg^{2+} could have an impact on vascular function is via its antioxidant, anti-

Figure 2. TRPM7 have been implicated in a number of vascular pathologies, including hypertension. Ang II, bradikinin (BK) and ET-1 regulate vascular transient receptor potential melastatin 7 (TRPM7) acutely by inducing phosphorylation and chronically by increasing expression at the mRNA and protein levels. Fluid flow and shear stress stimulate TRPM7 cytosol-to-membrane translocation in vascular smooth muscle cell (VSMC) and the amplitude of a native TRPM7-like current. These observations suggest that TRPM7 might also act as a mechanotransducer. Altered cellular Mg^{2+} homeostasis and abnormal VSMC function in hypertension might be related to defective TRPM7 expression/activity, which could contribute to proliferation, inflammation, fibrosis and contraction, important in vascular remodeling associated with high blood pressure.

inflammatory and growth-regulatory properties.^{102,103} Mg^{2+} has antioxidant/anti-inflammatory properties scavenging oxygen radicals possibly by the spontaneous dismutation of superoxide anions ($\cdot O_2^-$). Magnesium is also an essential requirement for the synthesis of some important natural antioxidants that could attenuate damaging actions of oxidative stress and

inflammation in the vasculature.^{102,103} In fact, Mg^{2+} deficiency enhanced oxidative damage, increased plasma concentrations of lipoproteins and induced oxidative damage to erythrocytes, neuronal and vascular cells.^{102–104} Increased production of $\cdot O_2^-$ and hydrogen peroxide (H_2O_2) provoked by reduced Mg^{2+} concentrations, is associated not only with contraction,

hypertrophy and hyperplasia of VSMC but also with inflammatory responses and subsequent tissue injury.^{102–106}

Underlying mechanisms for altered magnesium homeostasis in hypertension are unclear, but inborn errors of Mg^{2+} handling, decreased membrane permeability, altered Na^+/Mg^{2+} exchange, defective membrane binding and impaired cellular responsiveness have been implicated.¹⁰⁷ We and others showed that the Na^+/Mg^{2+} antiport function, which plays a major role in Mg^{2+} extrusion in cardiac, renal and VSMC, is altered in hypertension.^{108–111} Furthermore, amiloride and quinidine-mediated inhibition of Na^+/Mg^{2+} antiport increased vascular $[Mg^{2+}]_i$ and attenuated the development of hypertension in SHR.^{104–111} Also, in Ang II-induced hypertension in rats, inhibition of the Na^+/Mg^{2+} antiport resulted in reduced blood pressure, normalization of vascular and renal MAP kinase activity and improved vascular structure.^{104–111}

TRPM6 and TRPM7 have been implicated in a number of vascular pathologies, including endothelial dysfunction and hypertension.^{112,113} We and others demonstrated that VSMC from mouse, rat and human resistance arteries possess TRPM6 and TRPM7 cation channels and that TRPM7 is critically involved in regulating Mg^{2+} influx, cell viability, proliferation and contraction/dilation.^{47,67,114,115} Fluid flow and fluid-induced shear stress stimulates cytosol-to-membrane translocation of TRPM7 in VSMC and the amplitude of a native TRPM7-like current.¹¹⁶ These observations suggest that TRPM7 might act as a mechanotransducer, which could be important in pathological responses to vessel wall injury, particularly in the context of endothelial damage. Ang II and aldosterone, important vasoactive agents, regulate vascular TRPM7 acutely by inducing phosphorylation and chronically by increasing expression at the mRNA and protein levels.^{114,115} We recently identified a novel signaling pathway whereby bradykinin, through TRPM7, also influence proinflammatory responses in VSMC.¹¹⁷ Also, downregulation of vascular TRPM7 by siRNA reduced basal $[Mg^{2+}]_i$ and Ang II-stimulated $[Mg^{2+}]_i$ transients and attenuated Ang II-mediated VSMC growth. These findings confirmed that TRPM7 is a key regulator of magnesium homeostasis and that it plays a major role in vascular cell function (Figure 2).

In the context of high blood pressure, we showed that reduced Mg^{2+} influx in VSMC in SHR was associated with downregulation of vascular TRPM7, but not of TRPM6.¹¹⁵ Also, Ang II-mediated TRPM7, but not TRPM6, expression and activity were attenuated in hypertensive rats suggesting that altered regulation of Mg^{2+} in VSMC involve TRPM7-dependent mechanisms.¹¹⁵ In SHR, aldosterone-infused mice and other experimental models of hypertension, TRPM7 is downregulated and this is associated with altered VSMC Mg^{2+} transport, decreased $[Mg^{2+}]_i$ and impaired vasodilation.^{114–120} These data suggest that altered cellular Mg^{2+} homeostasis and abnormal VSMC function in hypertension might be related to defective TRPM7 expression/activity, which could contribute to proliferation, inflammation, fibrosis and contraction, important in processes involved in vascular remodeling associated with high blood pressure (Figure 2).

To further explore the pathophysiological significance of TRPM channels and vascular damage, we used a model of inherited hypomagnesemia to investigate effects of chronic Mg^{2+} deficiency on the cardiovascular system. Using bidirectional selective breeding, mice from a heterogeneous population mice were selected for low (MgL) and normal-high (MgH) levels of erythrocyte magnesium. MgL mice demonstrate inherited hypomagnesemia, with significant reductions in plasma, bone, and kidney magnesium concentrations.¹²¹ In-

bred mice with intracellular Mg^{2+} deficiency exhibit increased systolic blood pressure, impaired endothelial function, outward hypertrophic remodeling of arteries and vascular inflammation. We also reported altered vascular TRPM7 and annexin-I expression, thus, identifying a vascular phenotype associated with magnesium deficiency and highlighting an important role for magnesium and TRPM7 in the maintenance of vascular integrity and function.¹¹³

Clinically, there are a number of well-defined conditions in which high blood pressure is associated with hypomagnesemia, including hyperaldosteronism, use of diuretics in the management of hypertension and congestive cardiac failure and treatment with calcineurin inhibitors as immunosuppressive drugs.^{122,123} Hyperaldosteronism is associated not only with increased systolic blood pressure but also with electrolyte imbalances, including hypomagnesemia,^{124,125} possibly through modulation of TRPM6/TRPM7.^{115,116} In patients with congestive cardiac failure and hyperaldosteronism, spironolactone, a mineralocorticoid receptor blocker, improved arrhythmias and prevented intraerythrocyte Mg^{2+} depletion,¹²⁶ possibly through effects on cellular Mg^{2+} transport systems as we showed in experimental models.^{115,116}

Conclusions

Inadequate magnesium intake and hypomagnesemia might contribute to the pathophysiology of chronic diseases, such as hypertension. Magnesium normally regulates vascular tone and reactivity by modulating Na^+ , K^+ and Ca^{2+} and by influencing activity of multiple enzymes. Several studies have demonstrated that magnesium plays an important role in the maintenance of vascular integrity, where decreased Mg^{2+} content is associated with endothelial dysfunction, increased reactivity, enhanced contractility, vascular remodeling and inflammation and elevated blood pressure. However, increased vascular Mg^{2+} concentrations are associated with vasodilation, anti-inflammatory responses and reduced blood pressure. Unlike our knowledge of other major cations, cellular Mg^{2+} handling is poorly understood and until recently little was known about pathways regulating Mg^{2+} transport. To date, a number of Mg^{2+} efflux pathways, including the Na^+/Mg^{2+} antiporter and the Ca^{2+}/Mg^{2+} exchanger, have been demonstrated at a functional level. Recently TRPM6 and TRPM7 have been identified as unique Mg^{2+} transporters regulating Mg^{2+} influx. Vascular TRPM7 expression/activity appears to be altered in experimental models of hypertension. Such aberrations might contribute to Mg^{2+} dysregulation and vascular dysfunction associated with high blood pressure. There is still much to be discovered regarding the function and regulation of TRPM6, TRPM7 and TRPM6/7 and of the importance of these channels in cardiovascular biology in health and disease.

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