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Transient Receptor Potential Melastatin 7 Cation Channel (TRPM7) Kinase: a new player in Ang II-induced hypertension

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Abstract

Transient receptor potential melastatin 7 (TRPM7) is a bi-functional protein comprising a magnesium (Mg²⁺)/cation channel and a kinase domain. We previously demonstrated that vasoactive agents regulate vascular TRPM7. Whether TRPM7 plays a role in the pathophysiology of hypertension and associated cardiovascular dysfunction is unknown. We studied TRPM7kinase-deficient mice (TRPM7 kinase) (heterozygous for TRPM7kinase) and wildtype (WT) mice infused with Ang II (400 ng/kg/min, 4 weeks). TRPM7kinase expression was lower in heart and aorta from TRPM7 kinase versus WT mice, effects that were further reduced by Ang II infusion. Plasma Mg²⁺ was lower in TRPM7 kinase versus WT mice in basal and stimulated conditions. Ang II increased blood pressure in both strains with exaggerated responses in TRPM7 kinase versus WT groups (p<0.05). Acetylcholine-induced vasorelaxation was reduced in Ang II-infused TRPM7 kinase mice, an effect associated with Akt and eNOS downregulation. Vascular VCAM-1 expression was increased in Ang II-infused TRPM7kinase-deficient mice. TRPM7 kinase targets, calpain and annexin-1, were activated by Ang II in WT but not in TRPM7 kinase mice. Echocardiographic and histopathological analysis demonstrated cardiac hypertrophy and LV dysfunction in Ang II-treated groups. In TRPM7kinase-deficient mice, Ang II-induced cardiac functional and structural effects were amplified compared with WT counterparts. Our data demonstrate that in TRPM7 kinase mice, Ang II-induced hypertension is exaggerated, cardiac remodelling and LV dysfunction are amplified and endothelial function is impaired. These processes are associated with hypomagnesemia, blunted TRPM7kinase expression/signaling, eNOS downregulation and pro-inflammatory vascular responses. Our

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findings identify TRPM7kinase as a novel player in Ang II-induced hypertension and associated vascular and target organ damage.

Keywords

Blood pressure; endothelial dysfunction; TRPM; magnesium; signal transduction

Introduction

The transient receptor potential (TRP) melastatin 7 (TRPM7) cation channel is a member of the TRP ion channel superfamily which, together with its homolog TRPM6, has the unique property of possessing a cation channel domain coupled to a cytosolic α -type serine/threonine kinase domain that comprises the carboxy-terminal region of the protein (hence also termed “chanzyme”) (1, 2). While the channel domain regulates transmembrane magnesium (Mg^{2+}) influx, the kinase domain influences downstream signaling targets such as annexin-1, calpain, myosin-IIA heavy chain, Ef2-k and PLC γ 2 (3–6). The kinase domain can also influence TRPM7 channel function (7). TRPM7 regulates many cellular processes including Mg^{2+} homeostasis, cell growth/apoptosis, motility, actin-myosin interaction, differentiation, and mechanosensitivity (8–10). Studies in TRPM7-deficient mice indicate that TRPM7 plays an essential role in embryonic development, kidney morphogenesis, cardiac function and systemic Mg^{2+} homeostasis (11–13). TRPM7 dysfunction has been implicated in numerous pathologies including neurodegenerative disorders, cancer and cardiovascular disease (14–18). Although the physiological and pathological significance of TRPM7 is becoming increasingly apparent, the biology of TRPM7, particularly in the cardiovascular system, still remains elusive.

We previously demonstrated that TRPM7 is expressed in the vasculature and that it is regulated by vasoactive agents, such as bradykinin, aldosterone, endothelin-1, and angiotensin II (Ang II) (19–23). Physical factors such as shear stress and flow stimulate TRPM7 activity implicating TRPM7 as a mechanotransducer in vascular cells (24, 25). TRPM7 plays an important role in modulating vascular smooth muscle cell (VSMC) Mg^{2+} homeostasis (1, 19–22, 26), a major determinant of VSMC function and vascular tone. Experimental evidence indicates that Mg^{2+} deficiency causes endothelial dysfunction, arterial remodeling and hypertension and that normalization of Mg^{2+} improves vascular function and reduces blood pressure (BP) (27).

Mechanisms regulating vascular Mg^{2+} in health and disease remain unclear but TRPM7 could be important. Aberrant TRPM7 expression/activity may contribute to impaired intracellular free Mg^{2+} concentration ($[Mg^{2+}]_i$) and VSMC contraction, proliferation, inflammation, and fibrosis, important determinants of vascular dysfunction and remodelling in hypertension (28). In support of this 1) pro-hypertensive factors, such as Ang II and oxidative stress, regulate TRPM7 expression/activity (22, 29), 2) vascular TRPM7 is dysfunctional in spontaneously hypertensive rats (30), 3) renal TRPM7 is downregulated in aldosterone-infused mice (31), 4) pulsatile atheroprone shear stress stimulates endothelial TRPM7 expression (32), 5) TRPM7 is upregulated after renal ischemia (33), and 6) TRPM7

is implicated in vascular remodeling and inflammation induced by pressure overload (34). Moreover clinical studies showed decreased expression of TRP channels in cerebral vascular tissue from patients after hypertensive intracerebral hemorrhage (35).

The critical importance of TRPM7 is emerging from studies in which TRPM7 gene has been deleted. In cells from TRPM7-knockout mice and in cells in which siRNA is targeted to TRPM7, growth is abnormal, proliferation is arrested and cells undergo apoptosis and death (11, 12). In embryonic cardiomyocytes in which TRPM7 is disrupted, spontaneous Ca^{2+} transient firing is reduced with associated downregulation of voltage-dependent Ca^{2+} channel (36). At the whole animal level, homozygous TRPM7 kinase (kinase) knockout is embryonic lethal, indicating the critical role of TRPM7 kinase for normal development (11,12,37). Heterozygous TRPM7kinase mice are viable, but exhibit signs of hypomagnesaemia.

Considering the importance of TRPM7 in the cardiovascular system, we hypothesised that TRPM7 disruption plays a role in the pathophysiology of hypertension. To address this we induced hypertension by Ang II infusion in mice deficient in TRPM7 kinase (TRPM7 kinase) and investigated BP responses and effects on cardiac, renal, and vascular function. In addition, we explored putative molecular mechanisms underlying TRPM7kinase-dependent cardiovascular effects.

Methods

Please see supplemental text for detailed methods

Animals

Male mice, 3 months of age, mixed background (C57B6 and SV129), were studied including: wildtype (TRPM7^{+/+}) mice and mice heterozygous for the deletion of the TRPM7kinase domain (TRPM7 kinase) (12). Homozygous mice were not studied because of embryonic lethality (12). Mice were infused with Ang II (osmotic minipumps, 4 weeks). Systolic BP was measured by plethysmography.

Echocardiography

Transthoracic echocardiography was performed at the end of the study (Vevo 2100 imaging system with 40-MHz transducer).

Vascular function

Vascular function of small mesenteric resistance arteries was studied by wire myography (38,39). Vessel contractility was assessed by concentration-response curves to norepinephrine (NE). Endothelium-dependent and -independent relaxation was assessed by measuring dilatory responses to acetylcholine (ACh) and diethylamine NONOate (DEA-NO) respectively in pre-contracted vessels.

Vascular structure and mechanics

Vascular structure and mechanics were studied by pressure myography (38).

Western Blotting

Total protein and membrane-enriched fractions were analyzed by immunoblotting.

Reactive Oxygen Species (ROS) Assessment

NADPHoxidase-derived $O_2^{\bullet-}$ was assessed by lucigenin-enhanced chemiluminescence, hydrogen peroxide (H_2O_2) by Amplex Red, and nitric oxide (NO) by the Nitrate/Nitrite Colorimetric Assay Kit.

Statistics

Data are presented as means \pm SEM. Groups were compared using one-way ANOVA with Tukey's correction to compensate for multiple testing. $p < 0.05$ was significant.

Results

Reduced cardiac and vascular TRPM7kinase expression in TRPM7 kinase and Ang II-infused mice

To analyze TRPM7kinase protein expression in mice, we first sought to investigate the specificity of the TRPM7kinase antibody. HEK cells overexpressing full length TRPM7 under the control of tetracycline (tet) and HEK cells deficient in the kinase domain (kinase) were used as we described, (23). Tet-on HEK cells with full length TRPM7 showed significant TRPM7 expression, while in Tet-on kinase cells, protein was undetectable (Fig. 1A). These findings confirmed the specificity of the antibody for TRPM7kinase. Using this antibody, we probed for TRPM7kinase in cardiac and vascular tissue in our experimental groups. TRPM7kinase expression was reduced $\approx 50\%$ in cardiac and vascular tissue in TRPM7 kinase mice compared to TRPM7^{+/+} mice, which reflects the heterozygosity of the TRPM7 kinase mice (Figs 1B, 1C). Ang II reduced TRPM7kinase expression in heart and aorta in both strains.

Immunohistochemical analysis demonstrated that in aorta, TRPM7kinase is expressed in both the vascular media (vascular smooth muscle cells (VSMC)) and in the endothelium (endothelial cells (EC)) (Fig. 1C). In Ang II-infused mice, TRPM7kinase expression in VSMC and ECs was reduced in WT and TRPM7kinase-deficient mice.

Morphological characteristics and biochemical analysis

Body weight was similar between groups (Table 1). Cardiac mass was increased in Ang II-infused TRPM7^{+/+} and TRPM7 kinase mice versus controls. Ang II-induced cardiac hypertrophy was significantly greater in TRPM7 kinase mice versus Ang II-infused TRPM7^{+/+}. Renal mass was similar between vehicle-infused groups, and no kidney hypertrophy was observed in Ang II-infused group.

Biochemical analysis is summarized in Table 2. In vehicle-infused mice, plasma Mg^{2+} levels were lower in TRPM7 kinase than WT mice. Ang II increased plasma Mg^{2+} in TRPM7^{+/+}, but not in TRPM7 kinase mice. Plasma urea levels and urinary albumin-creatinine ratio were increased in Ang II-infused mice, reflecting renal dysfunction. Ang II reduced plasma albumin, calcium and chloride levels only in TRPM7 kinase mice. Urine

nitrate/nitrite, marker of systemic NO production, was reduced in Ang II-infused groups versus control counterparts.

Echocardiography

Echocardiography was performed to determine left ventricle (LV) dimensions and function (Table 3). No differences in echocardiographic measurements were observed between vehicle infused-groups. LV mass and intraventricular septum (IVS) thickness in diastole (d) were increased in Ang II-infused TRPM7^{+/+} mice versus vehicle-infused counterparts. In TRPM7 kinase mice EF and FS were significantly decreased upon Ang II infusion compared to vehicle-infused TRPM7^{+/+}. LV mass, LVIDd, LVID in systole (s) and LVPWd were increased in TRPM7 kinase mice versus vehicle-infused TRPM7^{+/+}. In Ang II-infused TRPM7 kinase mice there was a more pronounced decrease in EF, increase in LV mass and LVIDd than in Ang II-infused controls.

Cardiac histopathology

Cardiac hypertrophy was further analyzed histologically using hematoxylin and eosin (Fig. S1A). Hematoxylin-eosin staining revealed that vehicle-infused TRPM7^{+/+} and TRPM7 kinase mice had similar cardiac morphology with no gross abnormalities. Ang II promoted an increase in heart size in both groups, with a greater effect in Ang II-infused TRPM7 kinase mice, especially in the left ventricle (Fig. S1B).

Cardiac collagen accumulation was assessed by Sirius Red staining (Fig. S2A and table 4) and interstitial fibrosis was assessed by Masson's trichrome staining (Fig. S2B). Hearts from vehicle-infused TRPM7^{+/+} and TRPM7 kinase mice did not exhibit collagen deposition nor fibrosis. In hearts from Ang II-infused mice there were foci of collagen deposition and interstitial fibrosis (Table 4). Perivascular collagen content and fibrosis were increased in Ang II-infused mice versus vehicle-infused controls (Fig. S2A, S2B).

Aortic histopathology

Photomicrographs of Masson's trichrome-stained cross-sections of thoracic aorta showed that vehicle-infused TRPM7^{+/+} and TRPM7 kinase mice have similar media thickness. Ang II infusion led to a significant increase in the media thickness in both groups (Fig. S3A, S3B). The thickening of the aortic media is evidenced by the distance between each concentric elastic lamina intermingled with the smooth muscle cell layers. A pronounced thickening of the perivascular adventitial layer was also observed in Ang II-infused groups.

BP effects of Ang II in TRPM7 kinase mice

Figure 2 shows BP in TRPM7^{+/+} and TRPM7 kinase mice infused with vehicle or Ang II for 4 weeks. No differences in basal BP were observed between vehicle-infused groups. In Ang II-infused TRPM7^{+/+} mice, BP increased gradually over 10 days at which time BP became significantly higher than vehicle-treated controls. BP remained elevated until the end of the experiment. In Ang II-infused TRPM7 kinase mice, the onset of hypertension was rapid, occurring within 6 days of Ang II infusion. The magnitude of BP elevation by Ang II was significantly greater in TRPM7 kinase mice versus vehicle-infused counterparts.

Effect of Ang II on vascular function in TRPM7 kinase mice

As shown in figure 3A, maximal Ach-induced relaxation was significantly reduced in mesenteric resistance arteries from Ang II-infused TRPM7 kinase versus vehicle-infused TRPM7^{+/+} mice. Decreased sensitivity to Ach (pD₂) was observed in arteries from both Ang II-infused groups (Inset Fig. 3A). This impairment was specific for endothelium-dependent relaxation since arteries from Ang II-infused TRPM7^{+/+} and TRPM7 kinase mice showed similar relaxation to DEA-NO (NO donor) (Fig. 3B).

Contractile responses to NE were similar in vehicle-infused TRPM7^{+/+} and TRPM7 kinase mice (Fig. 3C). Ang II-infused mice displayed increased contraction to NE compared to vehicle-infused counterparts.

Ang II effects on vascular structure

Pressurized arteries had comparable lumen diameter at 3 mmHg of intraluminal pressure in all four groups (Fig. S4A). With a progressive increase of intraluminal pressure, the lumen diameter increased to a lesser degree in Ang II-infused versus vehicle-infused TRPM7^{+/+} mice (Fig. 4A). Media cross-sectional area (Fig. S4B) and media to lumen ratio (Fig. S4C) were similar in all groups at intraluminal pressure of 3 mmHg. In response to stepwise increments of intraluminal pressure these parameters were not affected by Ang II.

Vascular mechanics

Mesenteric resistance arteries from all groups had comparable strain (Fig. 4A) at an intraluminal pressure of 3 mmHg. In arteries from vehicle-infused groups, TRPM7 kinase mice showed decreased stress-pressure (Fig. 4B) in response to stepwise increments of intraluminal pressure compared to TRPM7^{+/+}. In both Ang II treated groups, artery deformation and stress-pressure in response to stepwise increments of intraluminal pressure, were lower than in vehicle-infused TRPM7^{+/+} mice. Ang II evoked a shift to the left in the stress-strain curve to an equal extent in both groups, indicating that vessels from both of the Ang II-infused groups are stiffer than vehicle-infused counterparts (Fig. 4C).

Activation of vascular eNOS, Akt and caveolin-1

To investigate putative molecular mechanism underlying endothelial dysfunction in Ang II-infused TRPM7 kinase mice, we examined vascular expression and phosphorylation of eNOS and its upstream regulator Akt in Ang II-infused TRPM7^{+/+} and TRPM7 kinase mice. As demonstrated in figure 5A basal eNOS was similar in TRPM7^{+/+} and TRPM7 kinase mice. However, in Ang II-infused TRPM7kinase-deficient mice, eNOS levels were significantly reduced compared with vehicle- and Ang II-infused TRPM7^{+/+} mice. Phosphorylation of eNOS (serine 1177, activation site) was significantly decreased in Ang II-infused TRPM7 kinase mice versus vehicle-treated mice (Fig. 5B). Akt was phosphorylated in Ang II-infused TRPM7^{+/+} but not in Ang II-infused TRPM7 kinase mice (Fig. 5C). In membrane-enriched fractions, expression of eNOS was lower and that of caveolin-1 was higher in Ang II-infused TRPM7 kinase mice versus control counterparts (Figs. S5A, S5B).

Ang II effects on TRPM7 targets, mitogen-activated protein (MAP) kinases and pro-inflammatory mediators in arteries from TRPM7^{+/+} and TRPM7 kinase mice

Upon activation, calpain and annexin-1, TRPM7 kinase downstream targets, translocate to the plasma membrane. The content of calpain and annexin in membrane-enriched fractions was evaluated as a functional index of TRPM7. As shown in figure 6 Ang II increased calpain and annexin-1 membrane content in arteries from TRPM7^{+/+} mice, but failed to elicit any response in TRPM7 kinase mice.

Expression of the VCAM-1, an adhesion molecule induced by inflammatory mediators, was significantly increased in aorta from TRPM7^{+/-} mice compared to other groups (Fig. 7A).

At the molecular level, MAP kinases play a key role in vascular regulation by Ang II. We evaluated activation of vascular ERK1/2 and JNK in Ang II-infused TRPM7^{+/+} and TRPM7^{+/-} mice. As shown in figure 7B and figure S6 Ang II significantly increased phosphorylation of ERK1/2 and JNK in arteries from TRPM7^{+/+} mice. Similar responses were observed in TRPM7 kinase suggesting that TRPM7 kinase is not critically involved in MAP kinase activation by Ang II.

Ang II effects on systemic and vascular ROS in TRPM7^{+/+} and TRPM7 kinase mice

The role of ROS generation by Ang II was analyzed in TRPM7^{+/+} and TRPM7 kinase mice. Plasma H₂O₂ concentration was similar between vehicle-infused TRPM7^{+/+} and TRPM7 kinase mice. Ang II had no significant effect on plasma H₂O₂ in either group (Figs. S7A, S7B). NADPH-dependent superoxide anion generation was measured in aortic homogenates by lucigenin-enhanced chemiluminescence and showed similar responses in TRPM7^{+/+} and TRPM7 kinase mice (Fig. S7C). Translocation of the Nox cytosolic subunit p47phox from the cytosol to membrane was evaluated as an index of oxidase activation. Expression of p47phox in membrane-enriched fractions was similar between groups (Fig. S7D).

Discussion

This study demonstrates a distinct cardiovascular phenotype in Ang II-infused TRPM7 kinase mice characterised by severe hypertension, exaggerated cardiac hypertrophy, worsening of left ventricular dysfunction and persistent hypomagnesemia compared with wildtype counterparts. Associated with these changes is impaired endothelial function, downregulation of eNOS and blunted signaling through TRPM7kinase, without effect on MAP kinases or redox status. Our findings identify TRPM7kinase as a novel player in the regulation of BP and cardiovascular function and indicate that TRPM7kinase perturbations may be important in the pathophysiology of Ang II-induced hypertension and associated vascular and target organ damage (Fig. 8). Cardiac and vascular expression of TRPM7kinase was significantly downregulated by Ang II in WT and TRPM7 kinase mice indicating an important negative interaction between these systems.

Phenotypically TRPM7 kinase mice in basal conditions were similar to wildtype controls, except for significant hypomagnesemia, likely due to reduced intestinal Mg²⁺ absorption and increased renal Mg²⁺ wasting, since TRPM7 and its homologue TRPM6 are major

Mg²⁺ transporters in epithelial cells in the gastrointestinal tract and kidneys as we reported (1–3, 12, 13). Similar to other knockout models used to interrogate the pathophysiology of hypertension, a pathological phenotype was only unmasked when TRPM7 kinase mice were challenged with Ang II. In mice deficient in TRPM7kinase, Ang II-induced hypertension was exaggerated and target organ damage was amplified. These phenomena were associated with pronounced endothelial dysfunction and blunted TRPM7kinase signaling through Akt, calpain and annexin-1. The relationship between reduced TRPM7 expression/activity, hypomagnesemia and BP has previously been suggested in experimental models of hypertension (16, 27, 40–42). Here we advance this concept and suggest that TRPM7kinase fine-tunes Ang II actions, because when it is downregulated, Ang II effects were augmented. This is supported by our previous findings in another mouse model with hereditary hypomagnesemia and associated downregulation of TRPM7 (42). While exact molecular mechanisms underlying the regulatory role of TRPM7 are still unclear, dampened TRPM7 signaling, as demonstrated here, may be important. However, we cannot exclude the possibility that some of the observed effects in our study may also relate to altered Mg²⁺ homeostasis and hypomagnesemia, especially since our previous studies demonstrated that alterations in TRPM7 kinase mice were ameliorated by Mg²⁺ supplementation (12).

TRPM7 plays an important role in the regulation and function of endothelial cells as demonstrated in human umbilical vein endothelial cells, where TRPM7 deficiency leads to altered growth and migration (43). In our *in vivo* study here, endothelial function was not significantly altered by Ang II in wildtype mice but was markedly impaired in Ang II-infused TRPM7 kinase mice as evidenced by reduced endothelium-dependent vasodilation. Reasons why Ang II did not influence endothelial function, or ROS production, in wildtype mice, as expected, are unclear but may relate, in part, to the fact that the mice studied here were of mixed genetic background, where responses to vasoactive agents are variable.

Decreased acetylcholine-induced vasorelaxation in Ang II-infused TRPM7 kinase mice was associated with decreased eNOS phosphorylation and Akt activation and increased membrane-associated caveolin-1. Since eNOS is negatively modulated by caveolin-1, upregulation of caveolin-1 may reduce eNOS bioactivity, as previously reported (44,45). In addition blunted effects of Ang II on Akt, an upstream regulator of eNOS, may play a role in reduced eNOS activation in TRPM7 kinase mice. These molecular processes likely contribute to dampened eNOS phosphorylation and NO generation with consequent endothelial dysfunction and reduced vasorelaxation, important determinants of BP elevation. It is also possible that hypomagnesemia in TRPM7 kinase mice may influence endothelial function, since decreased [Mg²⁺]_i enhances agonist-stimulated vasoconstriction and impairs vasodilation, while increased [Mg²⁺]_i is associated with vasorelaxation. These effects may occur through Mg²⁺ interactions with Ca²⁺, or through eNOS modulation by Mg²⁺ (46).

One of the earliest indicators of endothelial inflammation is expression of adhesion molecules, such as VCAM-1, which promotes leukocyte adhesion to the endothelium and migration of macrophages through the vascular media. Increased VCAM expression has been demonstrated in atherosclerosis and hypertension and is closely associated with vascular inflammation through processes that involve activation of MAP kinases and oxidative stress. In TRPM7kinase-deficient mice, Ang II induced a significant increase in

VCAM-1 expression. Molecular mechanisms underlying the inflammatory response are unclear, but may relate to decreased activation of TRPM7kinase targets, annexin-1 and calpain, which have anti-inflammatory and pro-migratory functions respectively (3, 47). Reduced annexin-1 expression and increased inflammation have been demonstrated in aortas from hypomagnesemic mice (40). We recently demonstrated that the TRPM7kinase domain, but not the TRPM7 channel domain, regulates annexin-1 and calpain signalling (23). The inflammatory and endothelial responses observed in our study appear to be independent of changes in oxidative stress or redox-sensitive MAP kinase signaling, because TRPM7kinase-deficiency had little impact on Ang II-induced phosphorylation of ERK1/2 and JNK.

The hypertensive vascular phenotype is characterised not only by endothelial dysfunction, but also by vascular remodeling (28). Previous studies demonstrated a role for TRPM7 and Mg^{2+} in vascular hypertrophy in hypertension (28,41). Here we show that in Ang II-treated mice, lumen diameter was reduced and the stress-strain relationship had a leftward shift compared with controls. Effects were similar in TRPM7^{+/+} and TRPM7 kinase mice indicating that remodeling and altered mechanics (increased stiffness) induced by Ang II are independent of TRPM7 kinase. These findings are in contrast to those for endothelial dysfunction, which had a significant TRPM7 component, suggesting that TRPM7 kinase may be more important in endothelial than vascular smooth muscle cell regulation, at least in 3 month-old mice. Whether vascular structural changes become more apparent with aging in TRPM7 kinase mice awaits confirmation.

TRPM7 has also been implicated in cardiac regulation and growth, since disturbed TRPM7 activity leads to abnormal cardiogenesis and impaired ventricular function (36, 37). In rat cardiomyocytes increased TRPM7 activity was involved in Ang II-induced cardiac fibrosis (48). In mouse heart and aorta, upregulation of TRPM7 channels by Ang II was associated with a proliferative phenotype of ascending aortic smooth muscle cells, an effect that was mediated through TRPM7 suppression of Pyk2-ERK1/2-Elk-1 signaling (49). Moreover cardiac arrhythmias have been linked to both up- and down-regulation of TRPM7 (35, 36). In our study, decreased TRPM7kinase activity was associated with exaggerated cardiac hypertrophy and pronounced left ventricular dysfunction in Ang II-infused mice. While this might be an adaptive response secondary to severe hypertension, it is also possible that decreased myocardial TRPM7kinase activity itself may contribute to structural and functional cardiac changes.

In conclusion, we show that in heterozygous TRPM7 kinase mice Ang II induces an exaggerated hypertensive response, pronounced cardiac hypertrophy, and worsening of left ventricular function. Associated with these changes is endothelial dysfunction and inflammation, processes linked to blunted TRPM7kinase signaling and eNOS downregulation. Vascular remodeling and vascular MAPK activation, typically associated with Ang II-induced hypertension, were not influenced by TRPM7kinase deficiency. These observations indicate that TRPM7kinase differentially regulates vascular function in hypertension, with a primary target being the endothelium. Our findings identify TRPM7kinase as a modulator of BP regulation, which when downregulated promotes severe hypertension and worsening of cardiovascular function. Moreover, we show that Ang II is a

negative regulator of TRPM7. Taken together this study defines a novel TRPM7kinase-sensitive mechanism involved in Ang II-induced hypertension and target organ damage.

Perspectives

Alterations in electrolyte status and ion transporters have been implicated in the pathophysiology of hypertension. While there is extensive data on calcium, sodium and potassium in hypertension, there is a paucity of information about magnesium and its transporters. Here we identify TRPM7, a magnesium/cation channel with kinase activity, as a regulator of magnesium homeostasis and modulator of BP regulation. Altered activity of TRPM7, particularly the kinase domain, is associated with hypomagnesemia, endothelial dysfunction, vascular inflammation, cardiac dysfunction and exaggerated pressor responses to Ang II. Our findings identify TRPM7kinase and its downstream signaling pathways as a novel paradigm in Ang II-induced hypertension. Perturbed activation of TRPM7kinase may be an important mechanism linking hypomagnesemia and cardiovascular injury in hypertension,

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Novelty and Significance

What Is New?

- TRPM7, a unique Mg^{2+} transporter with kinase activity, influences vascular function and blood pressure in Ang II-induced hypertension.
- TRPM7 kinase deficiency leads to exaggerated hypertension with associated eNOS downregulation and amplified endothelial dysfunction.
- TRPM7 kinase/eNOS is a novel system through which Ang II signals.

What Is Relevant?

- Clinical hypertension is associated with perturbed Mg^{2+} homeostasis and vascular dysfunction, possibly through TRPM7 dysfunction.
- Targeting TRPM7 kinase may have potential as a novel therapeutic target in hypertension.

Summary

TRPM7 kinase regulates vascular function in hypertension, with a primary target being the endothelium. TRPM7 kinase may have vasoprotective functions, which when dysregulated contributes to endothelial dysfunction, vascular inflammation and hypertension. We identify a novel TRPM7 kinase-sensitive mechanism in Ang II-induced hypertension.

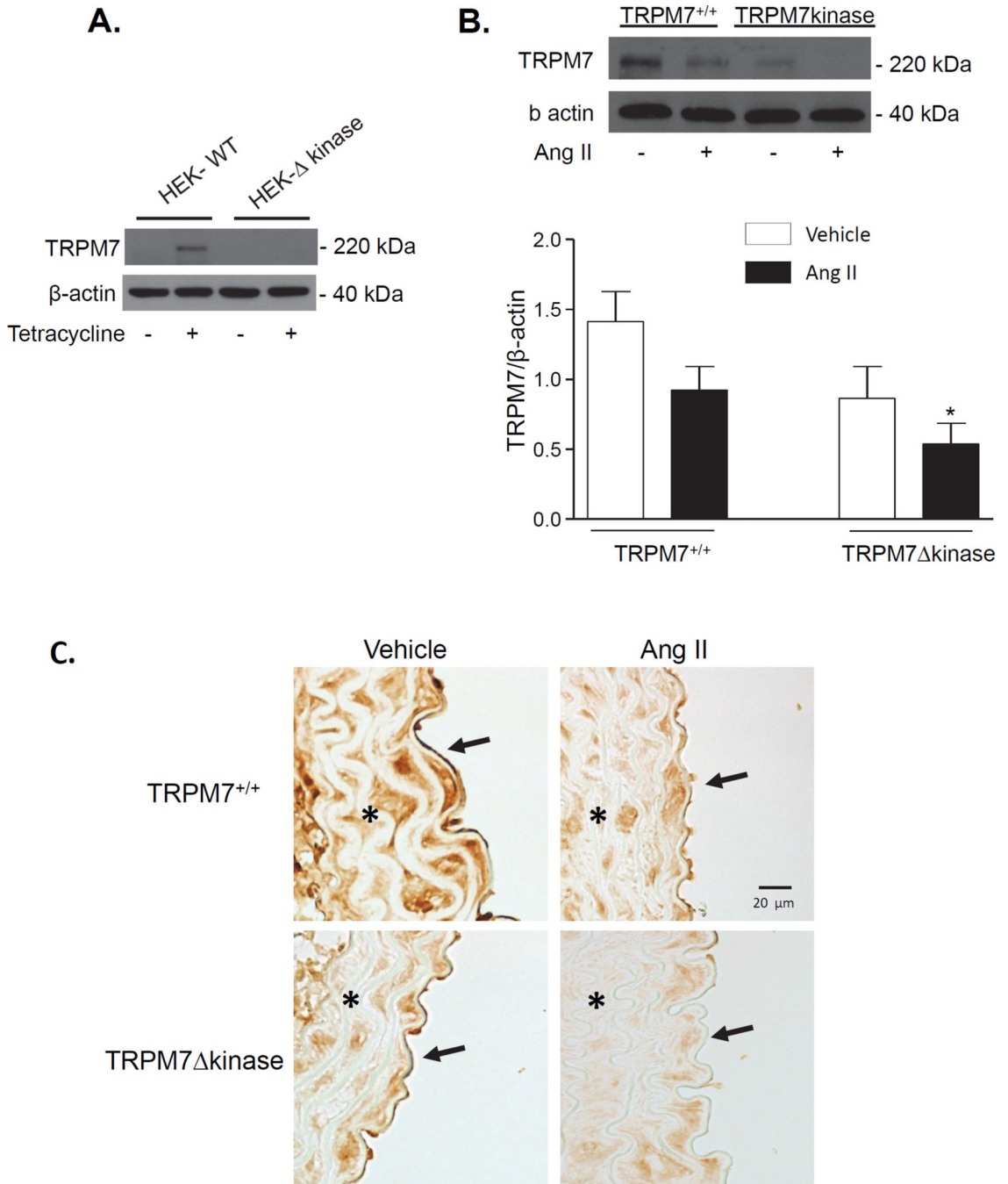


Figure 1. TRPM7kinase expression in heart and aorta of TRPM7 $^{+/+}$ and TRPM7 kinase mice
 A. Validation of TRPM7kinase antibody (Epitomics; # 3828-1). TRPM7 kinase expression in HEK cells expressing TRPM7 full length protein (WT) and TRPM7 lacking the kinase domain (Δ kinase) under the control of tetracycline. Cells deficient in TRPM7kinase failed to express the protein. B. Hearts were isolated from TRPM7 $^{+/+}$ and TRPM7 kinase mice infused with vehicle or Ang II. Protein expression of TRPM7kinase was analyzed as a ratio to β -actin. Upper panels, representative immunoblots of TRPM7 and β -actin. Bar graphs represent mean \pm SEM of 9–11 mice per group. * p <0.05 vs vehicle-infused TRPM7 $^{+/+}$. C.

TRPM7kinase expression was analyzed in aortas from vehicle and Ang II-infused mice. Panels are representative photomicrographs of paraffin-embedded aortic sections processed for immunohistochemistry of TRPM7kinase, with original magnification of $\times 100$. Data were obtained from 5 mice/group. Stars (*) and arrows (\uparrow) indicate the presence of TRPM7kinase in vascular media and endothelium respectively.

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- TRPM7^{+/+} vehicle
- TRPM7^{+/+} Ang II
- TRPM7 Δ kinase vehicle
- TRPM7 Δ kinase Ang II

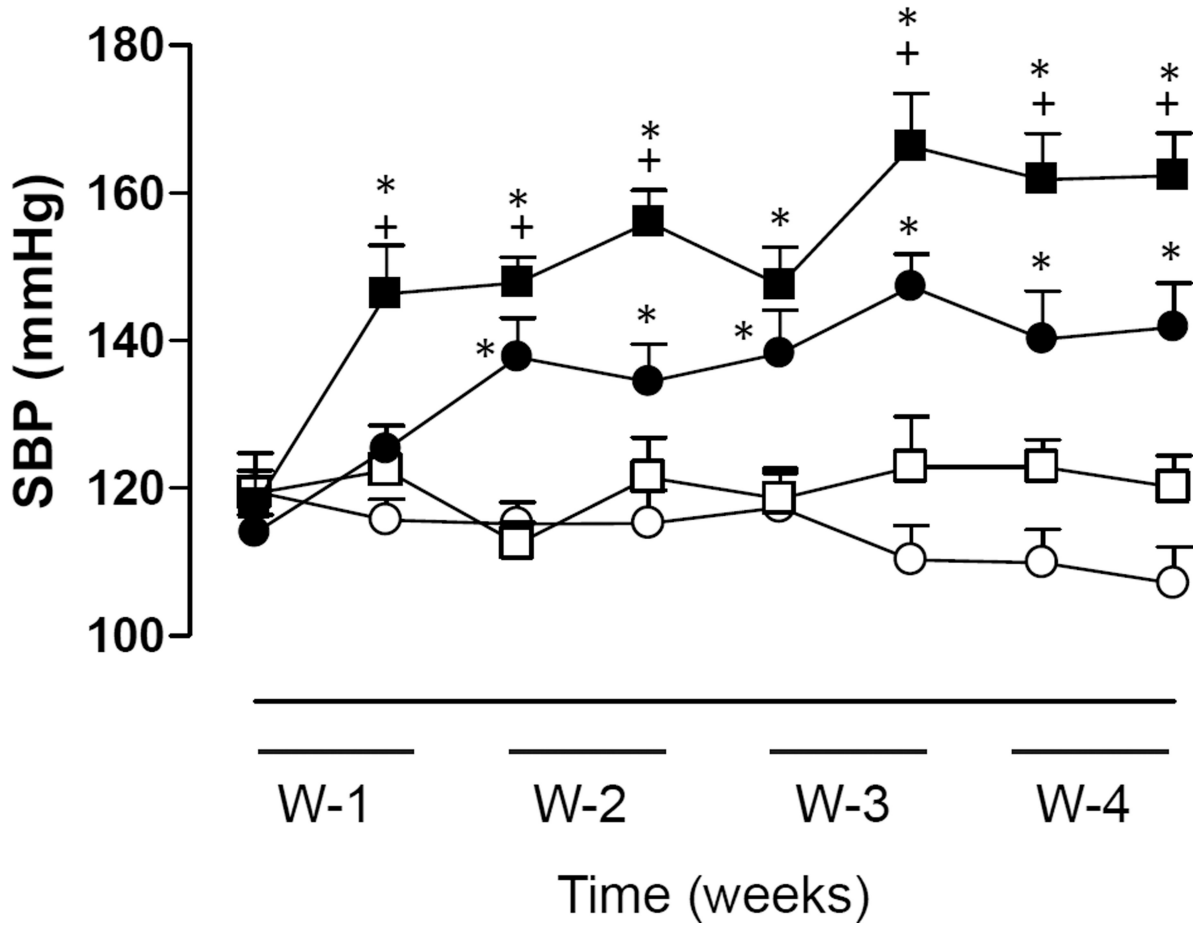
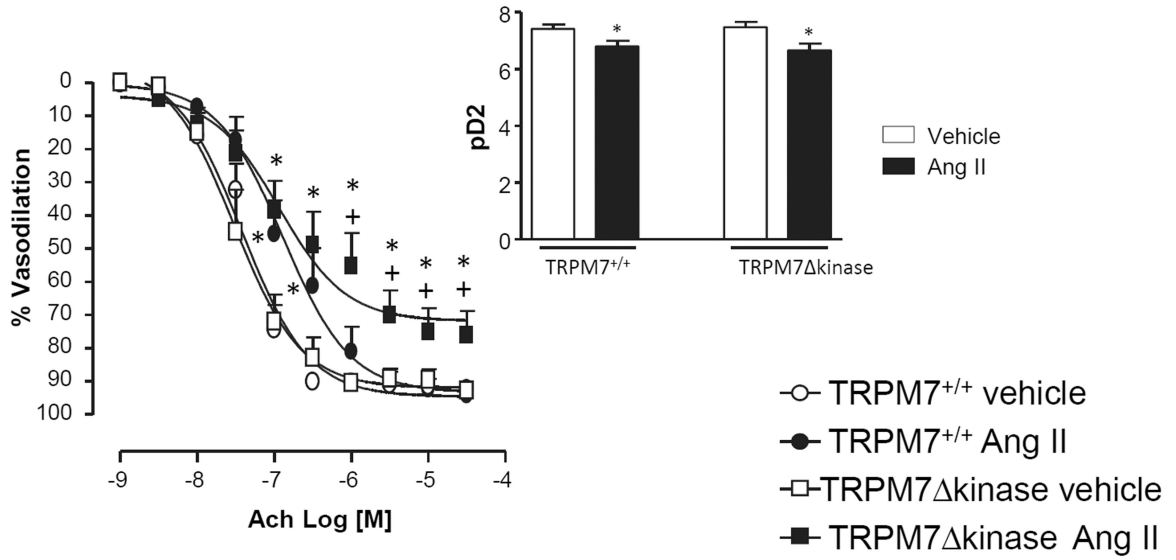
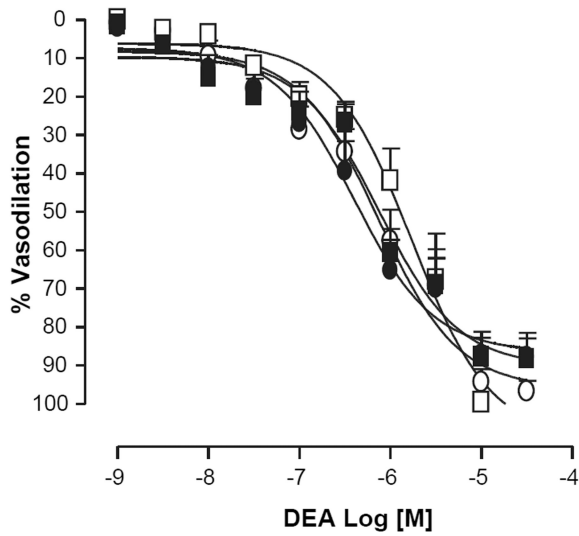


Figure 2. Systolic blood pressure (BP) in TRPM7^{+/+} and TRPM7^{+/-} mice infused with Ang II TRPM7^{+/+} and TRPM7^{+/-} mice were infused with vehicle or Ang II (400 ng/Kg/min., osmotic minipumps; 4 weeks). BP was measured weekly for 4 weeks (W) by tail-cuff plethysmography. Line graphs represent mean \pm SEM of 10–19 mice per group. *p<0.05 vs vehicle-infused counterparts; +p<0.05 vs Ang II-infused TRPM7^{+/+}.

A.



B.



C.

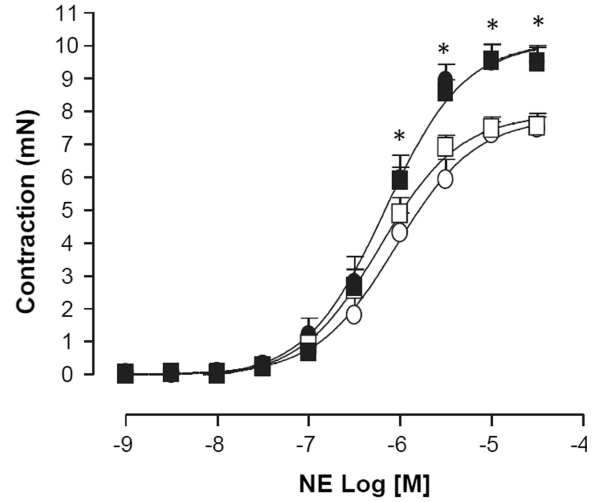
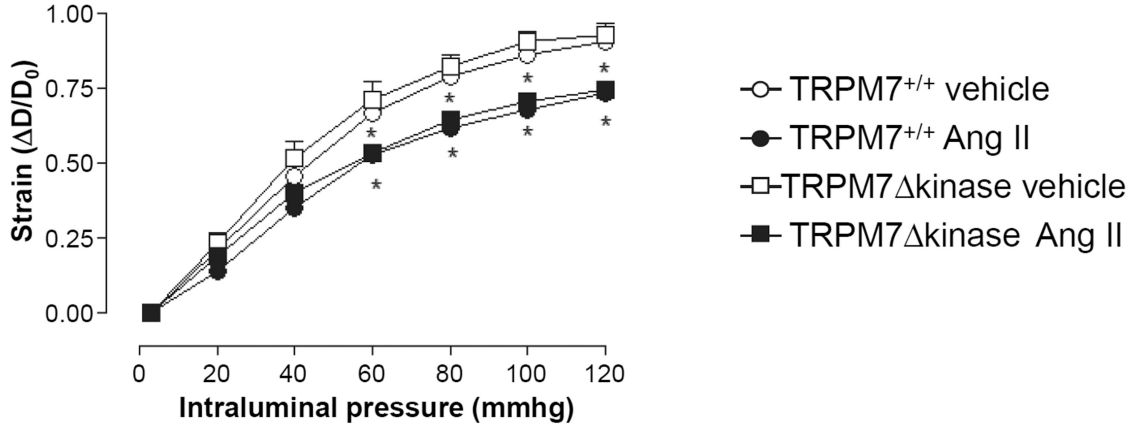


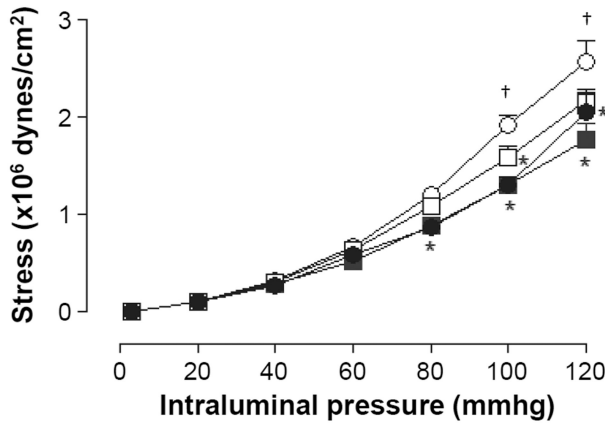
Figure 3. Functional responses in mesenteric arteries from Ang II-infused TRPM7^{+/+} and TRPM7^{+/-} mice

Mesenteric arteries were isolated from TRPM7^{+/+} and TRPM7^{+/-} mice infused with vehicle or Ang II. Concentration-response curves to cumulative concentrations of acetylcholine (Ach, 10⁻⁹- 3 × 10⁻⁵ M) (A), diethylamine NONOate (DEA-NO, 10⁻⁹- 3 × 10⁻⁵ M) (B), and norepinephrine (NE, 10⁻⁹- 3 × 10⁻⁵ M) (C) were analyzed in vessels mounted on wire myographs. Line graphs represent mean ± SEM of 7- 17 mice per group. Inset (A) shows the sensitivity to Ach expressed by pD₂ values. *p<0.05 vs vehicle-infused counterparts; +p<0.05 vs Ang II-infused TRPM7^{+/+}.

A.



B.



C.

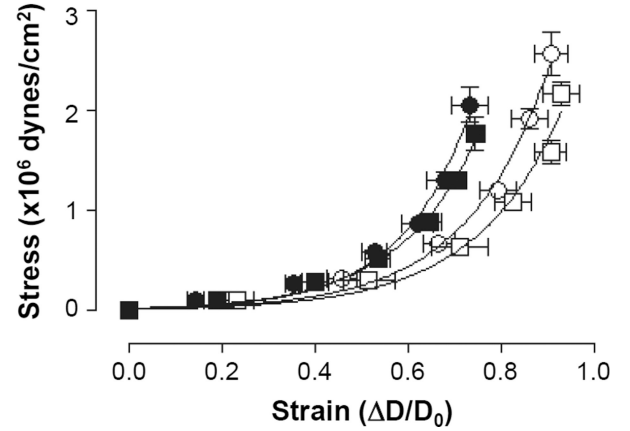


Figure 4. Mechanical properties in mesenteric resistance arteries from Ang II-infused mice
 Mesenteric resistance arteries were isolated from TRPM7^{+/+} and TRPM7^{+/-} mice infused with vehicle or Ang II. Strain (A), Stress (B), and Stress-strain relationship curves (C) were analyzed by pressure myography. Mesenteric arteries were incubated in 0 Ca²⁺-physiological salt solution containing 10 mmol/L EGTA and were subjected to increasing levels of intraluminal pressure. Line graphs represent mean \pm SEM of 5 mice per group. *p<0.05 vs vehicle-infused counterparts; †p<0.05 vehicle-infused TRPM7^{+/-}

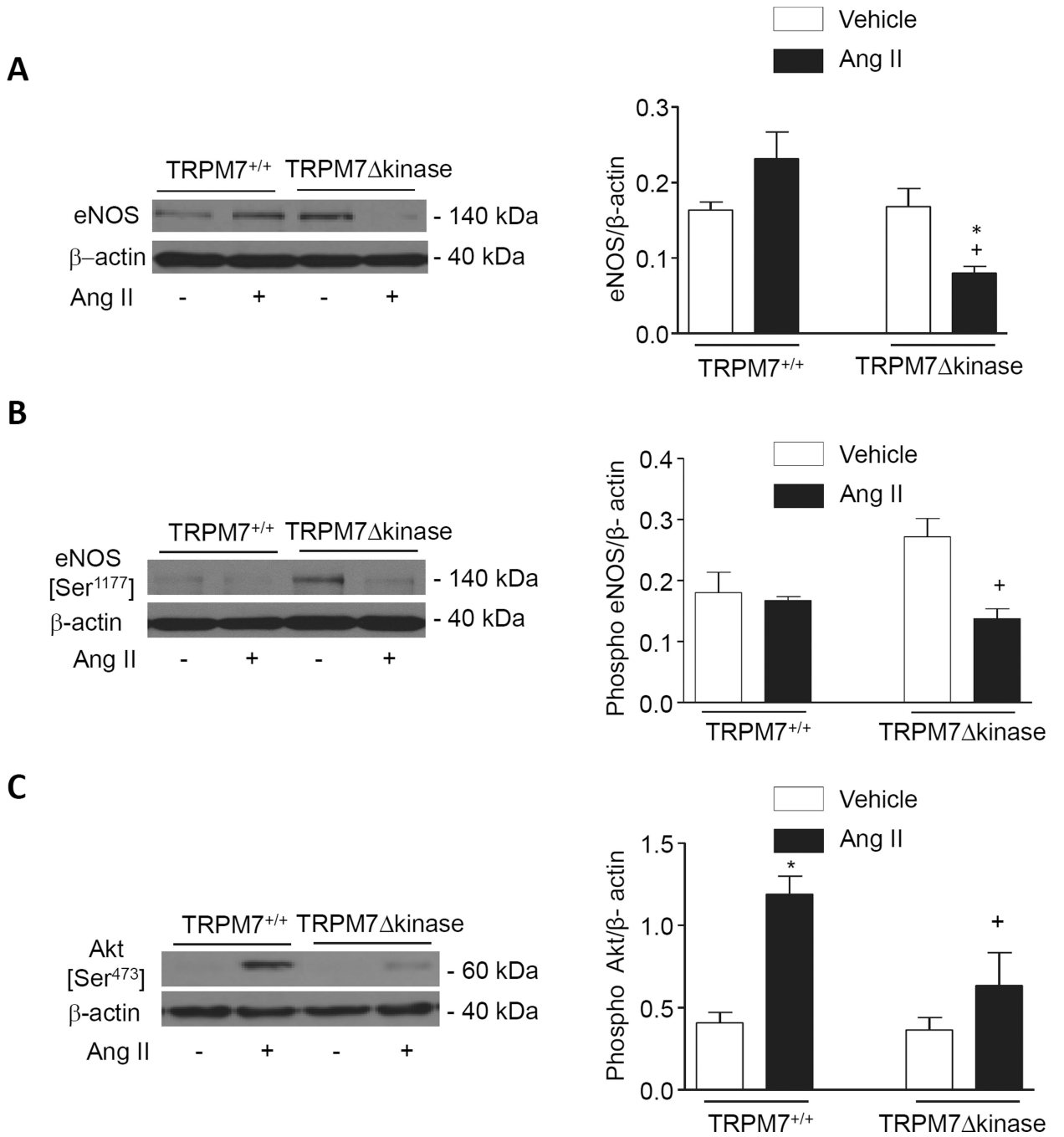
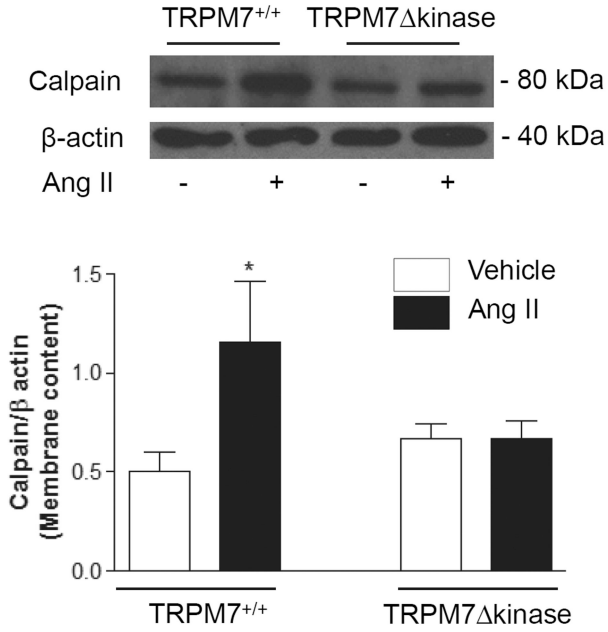


Figure 5. Ang II effects on expression and phosphorylation of eNOS and Akt in aorta from TRPM7^{+/+} and TRPM7^{+/-} mice

Representative immunoblots and bar graphs of eNOS content (A), eNOS (B) and Akt (C) phosphorylation in vessels from vehicle- and Ang II-infused TRPM7^{+/+} and TRPM7^{+/-} mice. Levels of total eNOS, phospho eNOS and phospho Akt are expressed relative to expression of β -actin. Results are presented as mean \pm SEM of 8–9 mice per group. *p<0.05 vs vehicle-treated groups; + p<0.05 vs Ang II-infused TRPM7^{+/+} mice.

A



B

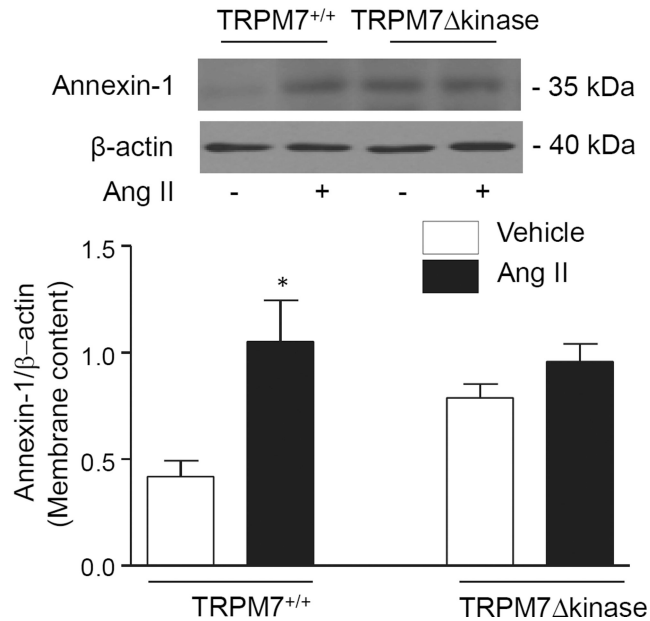


Figure 6. Expression of TRPM7 kinase targets calpain and annexin-1 in membrane-enriched fractions of mesenteric arteries from Ang II-infused TRPM7^{+/+} and TRPM7 kinase mice Mesenteric arteries were isolated from TRPM7^{+/+} and TRPM7 kinase mice infused with vehicle or Ang II. Protein content of calpain (A) and annexin-1 (B) was measured in membrane-enriched fractions. Data are presented as the expression of calpain or annexin-1 relative to the expression of β -actin. Upper panels, representative immunoblots of calpain, annexin-1 and α actin. Results are presented as mean \pm SEM of 6–9 mice per group. * p <0.05 vs vehicle-infused TRPM7^{+/+}.

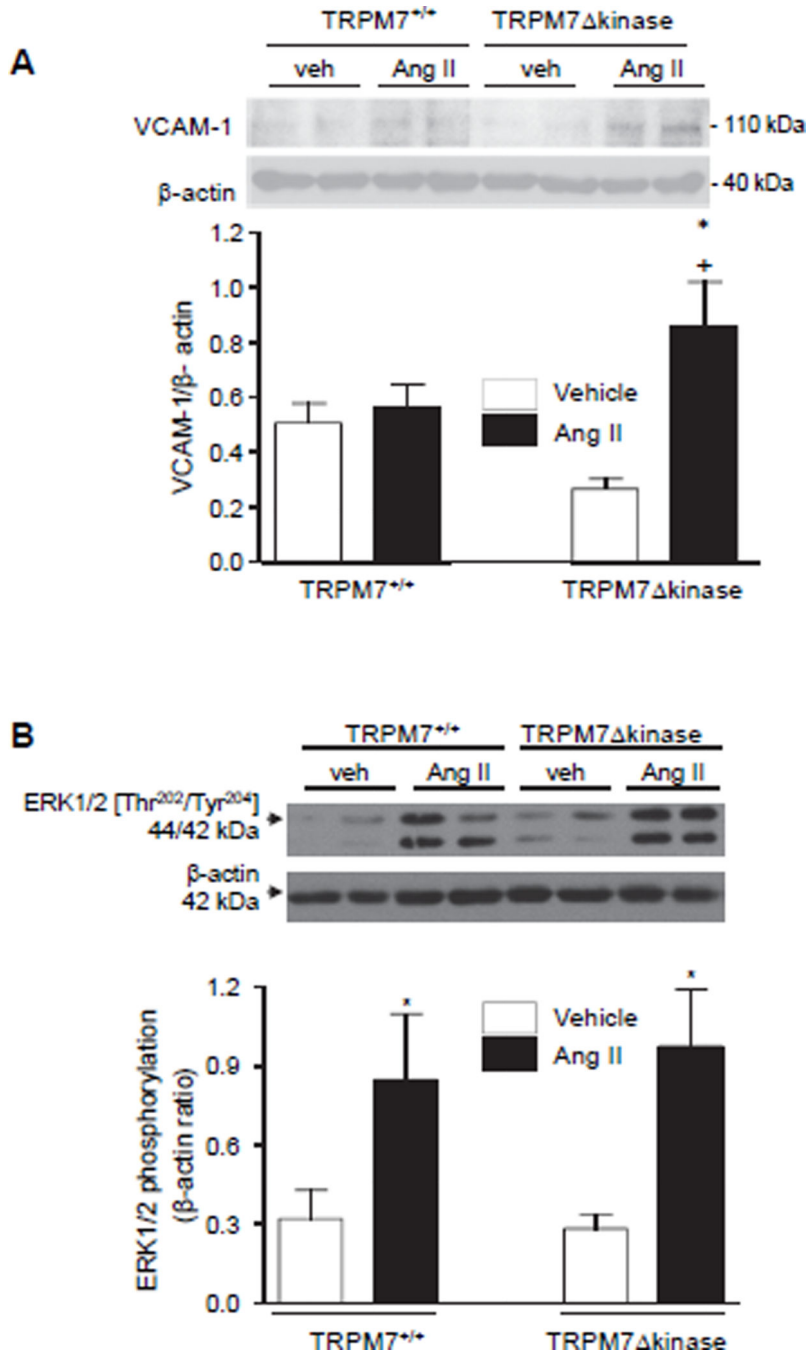


Figure 7. Expression of VCAM-1 and ERK1/2 in vessels from Ang II-infused TRPM7^{+/+} and TRPM7 kinase mice
 Aortas were isolated from TRPM7^{+/+} and TRPM7 kinase mice infused with vehicle or Ang II. (A) Upper panels, representative immunoblots of VCAM-1 and β -actin. Data are presented as the expression of VCAM-1 relative to the expression of β -actin. (B) Phosphorylation levels of ERK1/2 are presented relative to expression of β -actin. Upper panels, representative immunoblots of phospho-ERK1/2 and β -actin. Results are presented as mean \pm SEM of 5–9 mice per group. * p <0.05 vs vehicle infused counterparts; + p <0.05 vs Ang II-infused TRPM7^{+/+}.

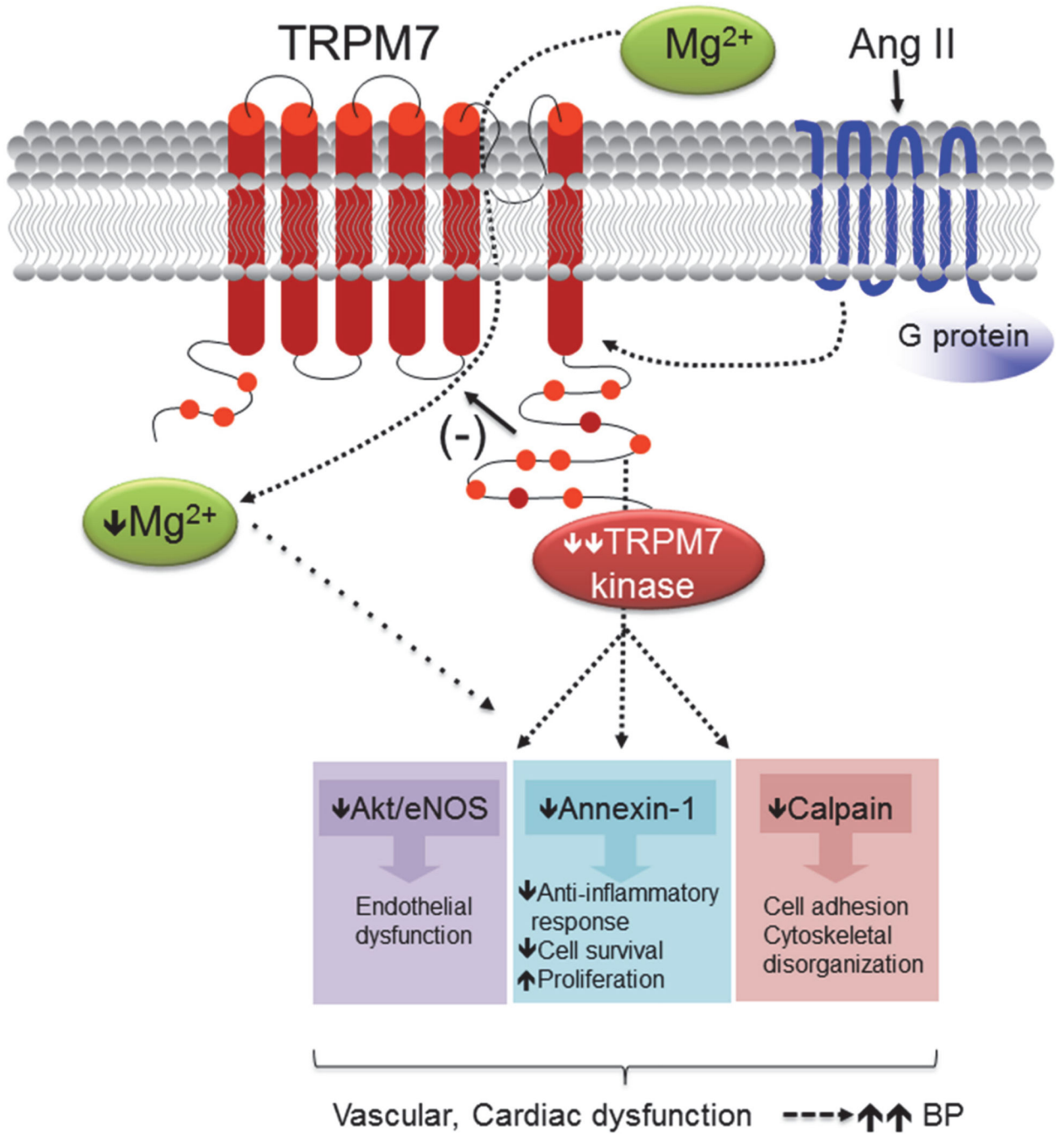


Figure 8. Possible molecular mechanisms whereby TRPM7 kinase deficiency amplifies Ang II cardiovascular and blood pressure (BP) effects

Blunted TRPM7 kinase signaling by Ang II through its G protein-coupled receptor is associated with reduced activation of TRPM7 kinase downstream targets, including Akt, calpain and annexin-1, leading to vascular and cardiac dysfunction and amplified increase in BP. These processes may also be influenced by reduced intracellular Mg^{2+} , possibly due to decreased transmembrane Mg^{2+} transport as a consequence of TRPM7 kinase

downregulation (as we previously demonstrated (1)). (–) denotes inhibitory effect. Dashed lines are possible pathways.

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Table 1

Body weight, kidney and heart mass of vehicle and Ang II-infused TRPM7^{+/+} and TRPM7 kinase mice.

	TRPM7 ^{+/+} vehicle	TRPM7 ^{+/+} Ang II	TRPM7 kinase vehicle	TRPM7 kinase Ang II
Morphological Parameter				
BW (g)	24.8 ± 0.7	25.5 ± 1.0	23.4 ± 0.7	24.2 ± 1.0
HM (mg)/BW(g)	4.4 ± 0.1	5.4 ± 0.1 *	4.5 ± 0.1	5.8 ± 0.1 * [†]
KM (mg)/BW(g)	6.1 ± 0.2	5.8 ± 0.1	6.2 ± 0.1	6.2 ± 0.1

TRPM7^{+/+} and TRPM7 kinase mice were infused with vehicle or Ang II (400 ng/Kg/min, 4 weeks). Results are mean ± SEM. n=6–16 mice per group. Body weight (BW), heart mass (HM), kidney mass (KM).

* p<0.05 versus vehicle counterparts;

[†] p<0.05 versus Ang II-infused TRPM7^{+/+}.

Table 2Plasma and urine profile in vehicle- and Ang II-infused TRPM7^{+/+} and TRPM7 kinase mice.

	TRPM7 ^{+/+} vehicle	TRPM7 ^{+/+} Ang II	TRPM7 kinase vehicle	TRPM7 kinase Ang II
Plasma				
Total protein	44.1 ± 0.7	41.4 ± 0.9 *	43.2 ± 0.9	39.8 ± 1.2 *
Albumin	6.0 ± 0.4	5.2 ± 0.5	6.2 ± 0.7	4.4 ± 0.3 *
Cholesterol	2.3 ± 0.07	2.4 ± 0.09	2.2 ± 0.06	2.2 ± 0.07
Triglycerides	1.5 ± 0.1	1.2 ± 0.1	1.3 ± 0.1	1.9 ± 0.4
Glucose	11.2 ± 0.5	11.2 ± 0.4	10.5 ± 0.5	10.5 ± 0.6
Urea	7.9 ± 0.2	11.2 ± 0.7 *	7.9 ± 0.4	10.6 ± 0.5 *
Creatinine	14.9 ± 0.9	14.0 ± 0.8	13.0 ± 1.3	12.3 ± 1.0
Calcium	1.51 ± 0.04	1.55 ± 0.06	1.46 ± 0.06	1.28 ± 0.08 *
Chloride	96.8 ± 1.2	92.8 ± 1.9	95.0 ± 1.2	88.7 ± 2.4 *
Phosphorus	1.2 ± 0.04	1.2 ± 0.07	1.1 ± 0.07	1.2 ± 0.06
Bicarbonate	19.1 ± 0.4	26.5 ± 0.5	20.0 ± 1.1	20.9 ± 1.7
Magnesium	0.65 ± 0.02	0.74 ± 0.04 *	0.60 ± 0.01 [‡]	0.64 ± 0.02 [‡]
Urine				
Albumin:creatinine ratio (µg/mg)	25 ± 6	394 ± 99 *	47 ± 11	449 ± 118 *
Nitric oxide (µM)	3.7 ± 0.2	3.0 ± 0.2 *	3.9 ± 0.2	3.2 ± 0.1 *

TRPM7^{+/+} and TRPM7 kinase mice were infused with vehicle or Ang II (400 ng/Kg/min, 4 weeks). Results are mean ± SEM. n=6–16 mice per group.

* p<0.05 versus vehicle-treated counterparts.

[‡] p<0.05 versus Ang II-infused TRPM7^{+/+}.

[‡] p<0.05 versus vehicle-treated TRPM7^{+/+}

Table 3

Echocardiographic measurements from M-mode images of vehicle- and Ang II-infused TRPM7^{+/+} and TRPM7 kinase mice.

Echocardiographic Parameter	TRPM7 ^{+/+} vehicle	TRPM7 ^{+/+} Ang II	TRPM7 kinase vehicle	TRPM7 kinase Ang II
EF (%)	49.9 ± 5.5	43.6 ± 7.2	42.8 ± 3.1	22.9 ± 3.7 * [†]
FS (%)	25.1 ± 3.3	19.8 ± 4.3	21.6 ± 1.7	10.5 ± 1.9 *
LV mass (mg)	89.9 ± 3.4	127.1 ± 10.2 *	105.7 ± 5.9	160.9 ± 8.3 * [†]
IVS; d (mm)	0.77 ± 0.06	1.03 ± 0.03 *	0.89 ± 0.04	0.94 ± 0.04
LVID; d (mm)	3.6 ± 0.07	3.6 ± 0.18	3.6 ± 0.10	4.13 ± 0.15 * [†]
LVID; s (mm)	2.7 ± 0.1	3.1 ± 0.2	2.9 ± 0.1	3.7 ± 0.2 *
LVPW; d (mm)	0.76 ± 0.04	0.9 ± 0.03	0.79 ± 0.05	0.96 ± 0.05 *
Heart rate (bpm)	501 ± 20	472 ± 15	455 ± 13	474 ± 23

TRPM7^{+/+} and TRPM7 kinase mice were infused with vehicle or Ang II (400 ng/Kg/min., osmotic minipumps, 4 weeks). EF = Ejection fraction, FS = Fractional shortening, LV = Left ventricle, IVS = Interventricular septum, LVID = Left ventricle internal diameter, LVPW = Left ventricle posterior wall, d = in diastole, s = in systole, bpm = beats per minute. Results are mean ± SEM. n = 5–10 mice per group.

* p < 0.05 vs vehicle-treated counterparts;

[†] p < 0.05 vs Ang II-infused TRPM7^{+/+}.

Scores for collagen accumulation and fibrosis of vehicle- and Ang II-infused TRPM7^{+/+} and TRPM7 kinase mice.

Table 4

Experimental Group	Sirius Red					Masson's Trichrome						
	-	+	++	+++	-	+	++	+++	-	+	++	+++
TRPM7 ^{+/+} Vehicle	8	0	0	0	8	0	0	0	0	0	0	0
TRPM7 ^{+/+} Ang II	0	2	4	1	0	1	4	2				
TRPM7 kinase Vehicle	9	0	0	0	9	0	0	0				
TRPM7 kinase Ang II	0	2	3	2	0	1	5	2				

Hearts were isolated from TRPM7^{+/+} and TRPM7 kinase mice infused with vehicle or Ang II (400 ng/Kg/min., osmotic minipumps, 4 weeks). Paraffin-embedded of heart sections were processed for Sirius Red (collagen) and Masson's trichrome (fibrosis). Staining for both collagen content and fibrosis were scored according to the following: - indicates intact; +, mild, ++, moderate, +++ severe. Values are numbers of mice displaying collagen deposition or fibrosis. n= 8-9 mice per group.