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Cardioprotective and Antiarrhythmic Effects of Magnesium Pretreatment Against Ischaemia/Reperfusion Injury in Isoprenaline-Induced Hypertrophic Rat Heart

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Abstract The effects of magnesium (Mg^{2+}) on ischaemic complications of pathological cardiac hypertrophy are unclear. In this study, we investigated effects of Mg^{2+} pretreatment on ischaemia/reperfusion (I/R) injury in isoprenaline (ISO)-induced hypertrophic hearts. Wistar rats were treated for seven days with different combinations of ISO (1.25 mg/kg) subcutaneously, $MgSO_4$ (270 mg/kg) intraperitoneally, or vehicle (saline). On eighth day, hearts were either subjected to regional I/R during Langendorff perfusion or histologically stained with haematoxylin and eosin and Masson's trichrome. Hemodynamic- and electrocardiographic parameters were recorded using the PowerLab data-acquisition system. Infarcts were identified by triphenyltetrazolium chloride staining. Plasma Mg^{2+} was measured using photometric assays. Mg^{2+} pretreatment significantly decreased I/R-induced infarct size ($p = 0.001$) and the overall arrhythmia score ($p < 0.001$) of I/R-induced ventricular ectopics, ventricular tachycardia, and ventricular fibrillation in ISO-treated-, but not non-ISO-treated hearts. Mg^{2+} also improved post-I/R left ventricular developed pressure in ISO-treated hearts. However, Mg^{2+} did not reverse the ISO-induced myocyte thickening and interstitial fibrosis or increase in heart weight. Plasma Mg^{2+} was not different among treatment groups. These results suggest that Mg^{2+} pretreatment may protect against I/R-induced injury and malignant arrhythmias in hypertrophic hearts, possibly via mechanisms unrelated to long-lasting changes in plasma Mg^{2+} or prevention of structural changes such as fibrosis.

Keywords Arrhythmia • Cardiac • Hypertrophy • Ischaemia/Reperfusion • Isoprenaline • Magnesium

Introduction

Activation of the sympathetic nervous system is a key compensatory mechanism in heart failure [1]. However, in the long run, the overstimulation of cardiac adrenergic receptors by the sustained release of catecholamines induces pathological cardiac hypertrophy, which worsens heart failure [2]. Pathological cardiac hypertrophy is a maladaptive, structural- and electrical remodelling response of the heart to cardiac stress conditions such as mechanical overload, myocardial injury, and neurohumoral activation [3]. The resultant tissue overgrowth and increased metabolic demands, together with electrical dysfunction, predispose the heart to life-threatening complications. Left ventricular hypertrophy, in particular, more than doubles the risk of cardiac death and is associated with a high risk of myocardial infarction, ventricular dysfunction, and malignant ventricular arrhythmias [4]. However, treatment options aimed at preventing or minimising the ischaemic complications of cardiac hypertrophy have remained limited since modulators of the underlying mechanisms have not yet been clearly identified.

Magnesium (Mg^{2+}) may provide a viable treatment option in cardiac hypertrophy, given that low serum Mg^{2+} concentrations are associated with an increase in ventricular mass [5] and enhance the pro-arrhythmic effects of hypokalaemia in hypertrophied hearts [6]. In non-diseased hearts, Mg^{2+} is involved in a variety of functions, including cellular metabolism, tissue growth and development, vascular reactivity [7], and regulation of ion channels [8]. Clinically, Mg^{2+} is a critical component of cardioplegic solutions used during cardiac surgery, and is effective in treating pre-eclampsia, but the effects in hypertrophic hearts are not fully known.

Catecholamines such as the synthetic β-adrenergic agonist isoprenaline (ISO) have been used to generate cardiac hypertrophy disease models that are associated with myocardial

dysfunction and arrhythmogenesis [9, 10]. Catecholamines are also known to alter cellular Mg^{2+} homeostasis by inducing Mg^{2+} efflux [11, 12], an effect that is enhanced by low extracellular Mg^{2+} concentration [13]. Thus, the ISO-induced cardiac disease model not only results in a hypertrophic myocardium that is prone to ischaemia [14], but also one in which cellular Mg^{2+} homeostasis may be altered. However, it is unclear whether supplementation of extracellular Mg^{2+} may modulate the complications of catecholamine-induced cardiac hypertrophy. In the present study, we explored the effects of Mg^{2+} pretreatment on the sequelae of myocardial ischaemia/reperfusion (I/R) injury in ISO-induced hypertrophic rat heart model.

Materials and Methods

Animals

The study was approved by the Faculty of Health Sciences Animal Research Ethics Committee of the University of Cape Town and was performed in compliance with the *Guide for the Care and Use of Laboratory Animals* (NIH Publication No.85 (23), revised 1996). Adult male Wistar rats (250-300 g) were obtained from the University of Stellenbosch Animal Unit and housed in an animal facility at the University of Cape Town. The rats were kept under standardised laboratory conditions (12-hour light/dark cycle and temperature of ~23 °C) and had free access to food and water.

Treatments and Experimental Protocols

Two similar sets of rats were each divided into four different groups that were treated for 7 consecutive days (Fig. 1a) as described below. Thereafter, one set of rat hearts was used in I/R experiments during Langendorff perfusion, whereas the other set was used for histological analysis, without being subjected to the I/R protocol. The isoprenaline (ISO)-induced cardiac hypertrophy group was treated daily with ISO 1.25 mg/kg subcutaneously (s.c.) [15] and physiological saline intraperitoneally (i.p.). In preliminary experiments, we noticed that higher doses of ISO, such as 5 mg/kg per day [10], resulted in a high mortality rate with time in our rats. The ISO + Mg^{2+} group was injected daily with ISO 1.25 mg/kg s.c. and $MgSO_4$ 270 mg/kg i.p., a Mg^{2+} dose that has been used in neuroprotection studies [16]. The Mg^{2+} group was injected daily with saline s.c. and $MgSO_4$ 270 mg/kg i.p. The control group was

injected daily with two drug-equivalent volumes of saline s.c. and i.p. Physiological saline was used to dissolve ISO or $MgSO_4$. The ISO solutions were prepared on the day of treatment.

Heart Isolation and Langendorff Perfusion

On day 8, rats were anticoagulated with heparin (500 I.U./kg i.p.) and anaesthetised with sodium pentobarbital (60 mg/kg i.p.). Upon the loss of pedal withdrawal reflexes, the heart was extracted and placed in cold (4 °C), filtered (Whatman filter paper, Sigma-Aldrich, South Africa) modified Krebs-Henseleit perfusate containing (in mmol/L): 118.5 NaCl, 4.7 KCl, 25 $NaHCO_3$, 1.2 $MgSO_4$, 1.8 $CaCl_2$, 1.2 KH_2PO_4 and 11 glucose (pH was normalized to 7.4 before adding $CaCl_2$). The aorta was immediately cannulated, ensuring that the tip of the cannula was above the aortic valve. The heart was perfused on a constant-pressure (74 mmHg) Langendorff apparatus, while suspended in a temperature-regulated organ chamber. The perfusate was continually gassed with carbogen (95 %O_2 and 5 %CO_2) and maintained at 37 °C using a pump-driven, warming system. Coronary flow rate was measured by timed collection of effluent from pulmonary trunk drainage. The time taken from excision to cannulation of hearts was limited to 3 min, and hearts that did not attain stable sinus rhythm during the stabilisation period were excluded.

The perfusion protocol consisted of 20 min stabilisation, 30 min regional ischaemia and 60 min reperfusion [17]. Regional ischaemia was induced by occluding the left anterior descending artery using a 5/0-silk suture snare [18], and reperfusion was started by releasing the suture snare. At the end of perfusion, the left anterior descending artery was re-occluded and the aorta was injected with 1 % Evans blue to outline the area-at-risk (AAR). Hearts were blotted, weighed, covered with cling film, and stored at -20 °C for infarct size measurements. Blood collected during cardiac extraction was centrifuged to obtain plasma, which was then

used to measure Mg^{2+} concentrations using automated photometric colour tests (Beckman AU, PathCare, South Africa).

Electrocardiographic and Hemodynamic Measurements

Electrocardiographic- and hemodynamic parameters were recorded using the PowerLab data-acquisition system and LabChart 7 software (ADInstruments, Australia). An apex-to-base electrogram (equivalent to Lead II on an electrocardiogram) was recorded via an Animal Bio Amplifier (ML136, ADInstruments, Australia). Electrocardiographic data were analysed using LabChart 7 Pro ECG Analysis Module (ADInstruments, Australia). Arrhythmias such as ventricular premature beats (VPB), ventricular tachycardia (VT), and ventricular fibrillation (VF) were classified according to the Lambeth Conventions [19]. The episodes of VT and VF induced under these experimental conditions were relatively brief and therefore the arrhythmias were allowed to recover spontaneously without manipulation. Arrhythmias were quantified using the following scoring equation [20]:

$$\text{Arrhythmia score} = (\log_{10} \text{VPB}) + (\log_{10} \text{episodes VT}) + 2[(\log_{10} \text{episodes of VF}) + (\log_{10} \text{total duration of VF})] \quad \text{(Equation 1)}$$

Left ventricular pressure was measured using a water-filled, intraventricular balloon inserted through the left atrial appendage and mitral valve and mounted at the tip of a catheter connected to a blood pressure transducer (MLT1199) and amplifier (Bridge Amp ML221, ADInstruments, Australia). At steady-state during stabilisation, the balloon was inflated to a left ventricular end-diastolic pressure (LVEDP) of 5-10 mmHg and the balloon volume was not altered thereafter. Hemodynamic parameters were measured before and after I/R. The left ventricular developed pressure (LVDP) was obtained as the difference between peak systolic

pressure and LVEDP. Hemodynamic data were analysed using the LabChart 7 Pro blood pressure module (ADInstruments, Australia) on which additional parameters such as maximal rates of LV pressure increase ($+dP/dt_{max}$) and of LV pressure decline ($-dP/dt_{max}$) were quantified.

Infarct Size Measurement

Ventricles of frozen hearts were cut transversely into a series of 2-mm slices from the apex to base and thawed for 2,3,5-triphenyltetrazolium chloride (TTC) staining [21]. The slices were incubated in a solution of 1 % TTC in phosphate buffer (pH 7.4) at 37 °C for 20 min and agitated periodically while protected from light. The slices were then washed with the buffer and fixed with 10 % formalin to enhance contrast, stored in the dark at room temperature for 24 h, and scanned using a flatbed scanner. Infarct size was measured as TTC-negative area on the slices from each heart using ImageJ software (Version 1.44p, NIH, USA) and was expressed as a percentage of the Evans blue-free AAR.

Histological Studies

Hearts were fixed with 10 % buffered formalin and processed to paraffin wax. Slices of ventricular sections (4-µm thick) were cut with a rotary microtome (Leica RM2125RT, South Africa) and stained with routine haematoxylin and eosin stain (H&E) or with Masson's trichrome connective tissue stain. Images were taken with a camera (Zeiss AxioCam, Germany) attached to an optical microscope (Zeiss AxioSkop, Germany). The histological changes were semi-quantified by grading the degree of myocyte enlargement (H&E) or

interstitial fibrosis (Masson's trichrome) as follows: (-), none; (+), mild; (++), moderate; (+++), severe [22].

Chemicals and Reagents

Sodium pentobarbitone was purchased from Kyron Laboratories (Johannesburg, South Africa). All other drugs and chemicals were obtained from Sigma (Sigma-Aldrich, South Africa).

Data Analysis

Data are expressed as mean and standard error of mean (SEM), with *n* indicating the number of rats studied under each condition. Statistical analysis was conducted using Prism 5 (GraphPad, USA). Differences among multiple groups for normally-distributed data (Kolmogorov-Smirnov and Shapiro-Wilk normality tests) were evaluated using one-way analysis of variance (ANOVA), followed by the Tukey's *post-hoc* test. Hemodynamic parameters were compared using repeated measures ANOVA. For non-parametric data, a Kruskal-Wallis test was conducted followed by the Dunn's *post-hoc* test. A two-tailed $p \leq 0.05$ was considered statistically significant.

Results

General Parameters

The initial body weight was not significantly different among the treatment groups (Fig. 1b), hence the heart weight-to-initial body weight ratio was used as an index of cardiac hypertrophy [10]. By day 8, ISO treatment caused a significant increase in heart weight-to-initial body weight ratio compared with control ($p < 0.001$, ISO *vs*. control; Fig. 1c). Co-treatment with Mg^{2+} did not prevent ISO-induced increase in heart weight-to-initial body weight ratio ($p = 0.97$, ISO + Mg^{2+} *vs*. ISO). Mg^{2+} treatment, on its own, did not alter heart weight-to-initial body weight ratio compared to control ($p = 0.52$).

On day 8, the actual body weight was also not significantly different among the groups. However, ISO decreased the percentage gain in body weight (change of -0.3 ± 1.6 % for ISO *vs*. +4.0 ± 0.7 % for control, $p < 0.05$; $n \geq 5$ rats per group), and the effect was not reversed by co-treatment with Mg^{2+} (-0.6 ± 1.4 % for ISO + Mg^{2+}; $p > 0.05$ *vs.* ISO; $n \geq 5$ rats per group). Pretreatment with Mg^{2+} alone did not significantly affect gain in body weight (+1.6 ± 0.8 % for Mg^{2+}; $p > 0.05$ *vs*. control; $n \geq 5$ rats per group). The day 8 plasma Mg^{2+} concentrations, measured 24 h after the final $MgSO_4$ or equivalent saline injection, were not significantly different among the treatment groups (plasma Mg^{2+} in mmol/L: 0.91 ± 0.05 for control, 0.90 ± 0.03 for ISO, 0.91 ± 0.02 for ISO + Mg^{2+}, and 0.92 ± 0.01 for Mg^{2+}; $p > 0.05$; $n \geq 5$ rats per group).

Infarct Size

Representative images of TTC-stained mid-ventricular slices are shown in Fig. 2a. The sizes of AAR relative to the total ventricular area were not significantly different among the treatment groups ($p = 0.52$; Fig. 2b). ISO-treatment significantly increased I/R-induced infarct size compared to control ($p = 0.002$, ISO *vs.* control; Fig. 2c), which was returned to control level by co-treatment with Mg^{2+} ($p = 0.001$, ISO + Mg^{2+} *vs*. ISO; Fig. 2c). However, pretreatment with Mg^{2+} alone had no significant effect on infarct size compared to control hearts ($p = 0.87$, Mg^{2+} *vs*. control), suggesting that Mg^{2+} did not protect non-hypertrophied hearts against I/R injury.

In preliminary experiments to demonstrate that the perfusion-protocol conditions and duration were not by themselves detrimental to hearts in the absence of I/R, a separate subgroup of saline-treated hearts was perfused without undergoing coronary occlusion. The coronary ligation suture was left in place and used to demarcate AAR at the end of perfusion. The size of the baseline TTC-negative non-viable myocardium attributable to tissue-handling artefacts in this subgroup of hearts was significantly smaller (5.9 ± 0.7 %; $n = 5$ rats) than the size of real infarcts in I/R protocol hearts ($p < 0.05$), yet the AAR (31 ± 3.4 %) was not significantly different ($p > 0.05$).

Arrhythmias

ISO treatment significantly increased the total number of I/R-induced VPB ($p = 0.01$), VT ($p = 0.005$), and VF ($p = 0.005$) compared to control hearts (Fig. 3a-3c). Co-treatment with Mg^{2+} significantly decreased the episodes of these arrhythmias in ISO-treated hearts ($p < 0.01$, ISO + Mg^{2+} *vs*. ISO for VPB, VT, or VF; Fig. 3a-3c). Mg^{2+} treatment alone did not significantly alter the episodes of VPB, VT or VF compared to control ($p > 0.05$, Mg^{2+} *vs*. control; Fig. 3a-3c). Taken together (as a score derived from Equation 1), Mg^{2+} reversed ISO-

induced increase in the overall arrhythmia score ($p = 0.0002$, ISO + Mg^{2+} *vs*. ISO; Fig. 3d). The arrhythmia score in Mg^{2+} only-treated hearts was not statistically-different from that in control ($p = 0.22$, Mg^{2+} *vs*. control).

Hemodynamic Parameters

Fig. 4 shows the trends of hemodynamic parameters among the treatment groups. In ISO-treated hearts, the pre-I/R baseline LVDP was significantly higher than that in control ($p < 0.05$, ISO *vs*. control; Fig. 4a), but decreased post-I/R to a level similar to that in control. In contrast, in ISO + Mg^{2+} hearts, although the baseline LVDP was also higher than that in control, the post-I/R value remained significantly higher than that in control or in ISO-only treated hearts ($p < 0.05$, ISO + Mg^{2+} *vs*. control or ISO). As part of promoting LVDP, Mg^{2+} also attenuated I/R-induced increase in LVEDP in ISO-treated hearts (Fig. 4b). Mg^{2+} alone did not alter LVDP or LVEDP compared to control. The post-I/R $+dP/dt_{max}$ (Fig. 4c) and $-dP/dt_{max}$ (Fig. 4d) were not significantly different among the treatment groups ($p > 0.05$). However, the pre-I/R $+dP/dt_{max}$ and $-dP/dt_{max}$ were slightly higher in ISO-treated or ISO + Mg^{2+} hearts compared to control. The pre- and post-I/R coronary flow rates (normalised to heart weight) were not significantly different among the groups (pre- and post-I/R coronary flow rates in ml/min/g: 6.2 ± 0.6 and 3.1 ± 1.1 for control, 6.8 ± 1.0 and 3.4 ± 0.7 for ISO, 6.9 ± 1.0 and 4.1 ± 0.3 for ISO + Mg^{2+}, 7.0 ± 1.1 and 3.0 ± 1.1 for Mg^{2+}; $n \geq 5$ rats per group).

Histological Analyses

Given the relatively low dose of ISO used in this study in order to minimise toxicity, we examined the nature of histological changes induced by ISO, and evaluated the effects of Mg^{2+} pretreatment. Examples of images of H&E-stained ventricular slices (Fig. 5a) show the presence of larger myocytes in the ISO-treated heart compared to control heart. Images of Masson's trichrome-stained ventricular slices (Fig. 5b) show more prominent collagen-rich, connective tissue (suggestive of fibrosis) in the interstitium of the ISO-treated heart compared to control. Qualitatively, Mg^{2+} pretreatment did not reverse any of these ISO-induced histological features (Fig. 5c). Mg^{2+} alone did not alter the normal histological appearance of the heart as detected by either H&E- or Masson's trichrome stain.

Discussion

In this study, we showed that Mg^{2+} pretreatment minimised the degree of I/R-induced infarction and attenuated I/R-induced arrhythmogenesis in catecholamine-stimulated hypertrophic hearts, but not in controls. Mg^{2+} also improved post-I/R left ventricular function, but did not reverse catecholamine-induced cardiac histological changes or the increase in heart weight.

The infarct size reduction by Mg^{2+} observed in our study suggests a myocardial protective effect specifically in hypertrophy, a unique feature of Mg^{2+}. However, the mechanism underlying this protective effect of Mg^{2+} in hypertrophy remains unclear. The presence of larger infarcts in ISO-treated hearts compared to control hearts observed in our study is consistent with greater susceptibility of hypertrophic hearts to infarction than non-hypertrophic hearts [4]. Mg^{2+} may therefore act by attenuating this susceptibility, given the lack of Mg^{2+} protection in non-hypertrophic hearts. Such a protective action of Mg^{2+} may involve the modulation of specific molecules involved in myocardial injury that are altered in hypertrophy. These molecules may include an inflammation-mediating protein, P-selectin, which is up-regulated in hypertrophy [23], and for which the expression is attenuated by magnesium [24]. Furthermore, the known pro-survival actions of Mg^{2+} such as anti-apoptosis [25] and anti-oxidant effects [26], reported in other cardiac conditions unrelated to catecholamines, could also be involved in counteracting ISO-induced stress.

In any case, the mechanism underlying the protective effects of Mg^{2+} is likely to be independent of structural modulation in hypertrophy since Mg^{2+} did not reverse the ISO-induced histological changes or the increase in heart weight. Furthermore, the effects of Mg^{2+} could not be accounted for by a correction of plasma Mg^{2+} deficit, given that there was no detectable hypomagnesemia in hypertrophic rats. The lack of differences in plasma Mg^{2+}

between non-diseased Mg^{2+}-treated rats and controls, at 24 h after the last injections, was also not surprising since, at the dose administered, Mg^{2+} is expected to revert to baseline level after approximately 3.5 h [16]. Therefore, the Mg^{2+} effects in this study were likely to be due to long-lasting down-stream cellular effects induced by the transient supramaximal Mg^{2+} levels achieved at the time of treatment [16], since other cardioprotective effects of Mg^{2+} were shown to be present even after the tissue Mg^{2+} levels had returned to baseline levels [27]. However, the possibility of a correction of an intracellular Mg^{2+} deficit in hypertrophic hearts cannot be excluded since tissue Mg^{2+} was not directly measured.

The reason why sometimes Mg^{2+} fails to protect against infarction in non-diseased hearts, as was observed in the present- or other previous studies [21, 28, 29], is not clear. The timing of the administration of Mg^{2+} at reperfusion has been proposed as a key factor in inducing cardioprotection [30, 31]. In addition, the presence of Mg^{2+} protection only in hypertrophied hearts observed in the present study may suggest that the health status of the myocardium may determine sensitivity to Mg^{2+} protective effects.

The suppression of VPB, VT, and VF by Mg^{2+} pretreatment in hypertrophied hearts in our study is also consistent with infarct size reduction, since arrhythmogenesis is known to depend on the magnitude of myocardial damage [32]. As was the case with infarcts, the anti-arrhythmic effects of Mg^{2+} (as determined by the overall arrhythmia score) were minimal in non-hypertrophied hearts. Similarly, Evans et al., [6] showed that the enhancement of pro-arrhythmic effects of hypokalaemia by low Mg^{2+} concentrations in spontaneously hypertensive rats was observed only in hypertrophied, but not in normal hearts. The evidence in our study suggests that the anti-arrhythmic mode of action of Mg^{2+} in hypertrophy was not related to modulation of fibrosis, which is known to be pro-arrhythmogenic [33], or to sustained alterations in plasma Mg^{2+} since these parameters were not different among the treatment groups. However, Mg^{2+} has membrane-stabilising effects that are proposed to play

a role in the treatment of cardiac rhythm disorders such as *torsades de pointes* [34]. In addition, the modulation of cellular Mg^{2+} extrusion has been implicated in mediating anti-arrhythmic effects of drugs in catecholamine-induced stress [12], but details of this mechanism are still unclear.

The improvement of post-I/R LVDP by Mg^{2+} in our study was consistent with the reduction in ventricular infarct size. Given that the calculation of LVDP includes LVEDP, the Mg^{2+} effect on LVDP in ISO-treated hearts was partly due to the attenuation of I/R-induced increase in LVEDP, and suggested that Mg^{2+} minimised ischaemic contractures. The Mg^{2+} effect was however unrelated to changes in the myocardial perfusion status (as indicated by an unaltered coronary flow rate) or to modulation of contractility indices (as shown by lack of differences in $+dP/dt_{max}$ and $-dP/dt_{min}$). The higher pre-I/R values of parameters (LVDP, $+dP/dt_{max}$, and $-dP/dt_{min}$) in ISO-only or ISO + Mg^{2+} hearts compared to control were probably due to residual positive inotropic- and lusitropic effects of catecholamine (ISO)-mediated β-adrenergic receptor stimulation [35]. Our findings are in agreement with the Mg^{2+}-mediated attenuation of ISO-induced ventricular dysfunction in dogs *in vivo* [36], but that study was not done in an MI disease model. In humans, post-MI elevation of extracellular Mg^{2+} was shown to be correlated with preservation of LV function [37]; however that study was done outside the context of catecholamine stimulation or hypertrophy. Mg^{2+} and K^{+} supplementations in blood cardioplegic solutions were also shown to improve post-operative cardiac function recovery in patients with cardiac hypertrophy [38].

Limitations of this study include the presence of systemic effects of ISO like failure to gain body weight, which may confound morphological- and functional changes in the heart or other organs. Loss of body weight is known to occur with ISO treatment [39], but in our study an effort was made to minimise such effects (~5 % body weight difference) by using a

lower optimal dose (1.25 mg/kg) of ISO [15] compared to the commonly-used 5 mg/kg [10]. Another limitation is that, while the dose of Mg^{2+} used in our study is expected to achieve therapeutic plasma levels [16], the intermittent injections could have made the attainment of steady-state conditions less predictable. Steady-state conditions can be achieved by using automated devices like implantable infusion pumps, but such devices usually require invasive methods of insertion performed under anaesthesia. Finally, though this study demonstrated the cardiac conditions under which Mg^{2+} might work in isolated hearts, the transferability of the effect to hearts *in vivo*, where other systemic factors participate, remains unestablished. The *in vivo* tests therefore need to be a key part of follow-up studies.

Results of this study showed a potentially clinically-relevant cardioprotective- and anti-arrhythmic role of Mg^{2+} pretreatment in catecholamine-stimulated cardiac hypertrophy. The findings thereby advance knowledge of the cardiac conditions under which Mg^{2+} supplementation therapy may be beneficial. Mg^{2+} may act by attenuating pro-ischaemic changes in cardiac hypertrophy that are unrelated to histological changes such as fibrosis or to long-lasting changes in plasma Mg^{2+}, but further studies are required to clarify the exact mechanisms underlying the protection in hypertrophy.

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Conflict of Interests The authors declare no conflict of interest.

References

1. Triposkiadis, F., Karayannis, G., Giamouzis, G., Skoularigis, J., Louridas, G. & Butler, J. (2009). The sympathetic nervous system in heart failure physiology, pathophysiology, and clinical implications. *Journal of the American College of Cardiology, 54*, 1747-62.
2. Jaffre, F., Callebert, J., Sarre, A., Etienne, N., Nebigil, C. G., Launay, J. M., et al. (2004). Involvement of the serotonin 5-HT2B receptor in cardiac hypertrophy linked to sympathetic stimulation: control of interleukin-6, interleukin-1beta, and tumor necrosis factor-alpha cytokine production by ventricular fibroblasts. *Circulation, 110*, 969-74.
3. Armoundas, A. A., Wu, R., Juang, G., Marban, E. & Tomaselli, G. F. (2001). Electrical and structural remodeling of the failing ventricle. *Pharmacology and Therapeutics, 92*, 213-30.
4. Levy, D., Garrison, R. J., Savage, D. D., Kannel, W. B. & Castelli, W. P. (1990). Prognostic implications of echocardiographically determined left ventricular mass in the Framingham Heart Study. *New England Journal of Medicine, 322*, 1561-6.
5. Reffelmann, T., Dorr, M., Ittermann, T., Schwahn, C., Volzke, H., Ruppert, J., et al. (2010). Low serum magnesium concentrations predict increase in left ventricular mass over 5 years independently of common cardiovascular risk factors. *Atherosclerosis, 213*, 563-9.
6. Evans, S. J., Levi, A. J. & Jones, J. V. (1996). Low magnesium enhances the pro-arrhythmic effect of low potassium in the hypertrophied rat heart but not in the normal rat heart. *Journal of Hypertension, 14*, 635-44.

7. Laurant, P. & Touyz, R. M. (2000). Physiological and pathophysiological role of magnesium in the cardiovascular system: implications in hypertension. *Journal of Hypertension, 18*, 1177-91.

8. Mubagwa, K., Gwanyanya, A., Zakharov, S. & Macianskiene, R. (2007). Regulation of cation channels in cardiac and smooth muscle cells by intracellular magnesium. *Archives of Biochemistry and Biophysics, 458*, 73-89.

9. Leicht, M., Greipel, N., Rassler, B. & Zimmer, H. G. (2002). Catecholamine-induced cardiac hypertrophy in rats is associated with the activation of p70 kinase and c-Jun NH(2)-terminal kinase 2. *Experimental and Clinical Cardiology, 7*, 131-7.

10. Meszaros, J., Khananshvili, D. & Hart, G. (2001). Mechanisms underlying delayed afterdepolarizations in hypertrophied left ventricular myocytes of rats. *American Journal of Physiology: Heart and Circulatory Physiology, 281*, H903-14.

11. Young, L., Bercute-Dammann, A. & Weis, M. T. (2002). Mg^{2+} efflux from the isolated perfused rabbit heart is mediated by two states of the beta1-adrenergic receptor. *Naunyn-Schmiedebergs Archives of Pharmacology, 366*, 431-9.

12. Romani, A. M. (2012). Modulation of cellular Mg^{2+} content in cardiac cells by alpha1-adrenoceptor stimulation and anti-arrhythmic agents. *Recent Pat Biotechnol, 6*, 212-22.

13. Howarth, F. C., Waring, J., Hustler, B. I. & Singh, J. (1994). Effects of extracellular magnesium and beta adrenergic stimulation on contractile force and magnesium mobilization in the isolated rat heart. *Magnesium Research, 7*, 187-97.

14. Takaki, M. (2012). Cardiac mechanoenergetics for understanding isoproterenol-induced rat heart failure. *Pathophysiology, 19*, 163-70.

15. Kitagawa, Y., Yamashita, D., Ito, H. & Takaki, M. (2004). Reversible effects of isoproterenol-induced hypertrophy on in situ left ventricular function in rat hearts. *American Journal of Physiology: Heart and Circulatory Physiology, 287*, H277-85.

16. Sameshima, H., Ota, A. & Ikenoue, T. (1999). Pretreatment with magnesium sulfate protects against hypoxic-ischemic brain injury but postasphyxial treatment worsens brain damage in seven-day-old rats. *American Journal of Obstetrics and Gynecology, 180*, 725-30.

17. Ferrera, R., Benhabbouche, S., Bopassa, J. C., Li, B. & Ovize, M. (2009). One hour reperfusion is enough to assess function and infarct size with TTC staining in Langendorff rat model. *Cardiovascular Drugs and Therapy, 23*, 327-31.

18. Samsamshariat, S. A., Samsamshariat, Z. A. & Movahed, M. R. (2005). A novel method for safe and accurate left anterior descending coronary artery ligation for research in rats. *Cardiovascular Revascularization Medicine, 6*, 121-3.

19. Curtis, M. J., Hancox, J. C., Farkas, A., Wainwright, C. L., Stables, C. L., Saint, D. A., et al. (2013). The Lambeth Conventions (II): guidelines for the study of animal and human ventricular and supraventricular arrhythmias. *Pharmacology and Therapeutics, 139*, 213-48.

20. Miller, L. E., Hosick, P. A., Wrieden, J., Hoyt, E. & Quindry, J. C. (2012). Evaluation of arrhythmia scoring systems and exercise-induced cardioprotection. *Medicine and Science in Sports and Exercise, 44*, 435-41.

21. Garson, C., Kelly-Laubscher, R., Blackhurst, D. & Gwanyanya, A. (2015). Lack of cardioprotection by single-dose magnesium prophylaxis on isoprenaline-induced myocardial infarction in adult Wistar rats. *Cardiovascular Journal of Africa, 26*, 1-9.

22. Buwa, C. C., Mahajan, U. B., Patil, C. R. & Goyal, S. N. (2015). Apigenin Attenuates beta-Receptor-Stimulated Myocardial Injury Via Safeguarding Cardiac Functions and

Escalation of Antioxidant Defence System. *Cardiovascular Toxicology*. doi:10.1007/s12012-015-9336-9.

23. Dimitrow, P. P., Undas, A., Bober, M., Tracz, W. & Dubiel, J. S. (2008). Obstructive hypertrophic cardiomyopathy is associated with enhanced thrombin generation and platelet activation. *Heart, 94*, e21.

24. Ying, S. Q., Fang, L., Xiang, M. X., Xu, G., Shan, J. & Wang, J. A. (2007). Protective effects of magnesium against ischaemia-reperfusion injury through inhibition of P-selectin in rats. *Clinical and Experimental Pharmacology and Physiology, 34*, 1234-9.

25. Du, C. S., Yang, R. F., Song, S. W., Wang, Y. P., Kang, J. H., Zhang, R., et al. (2010). Magnesium Lithospermate B Protects Cardiomyocytes from Ischemic Injury Via Inhibition of TAB1-p38 Apoptosis Signaling. *Frontiers in Pharmacology, 1*, 111.

26. Garcia, L. A., Dejong, S. C., Martin, S. M., Smith, R. S., Buettner, G. R. & Kerber, R. E. (1998). Magnesium reduces free radicals in an in vivo coronary occlusion-reperfusion model. *Journal of the American College of Cardiology, 32*, 536-9.

27. Kusniec, F., Fischer, G., Sela, B. A., Ashkenazy, Y., Feigel, D., Moshonov, S. & Zor, U. (1994). Magnesium protects against anaphylactic shock and cardiac myolysis in guinea-pigs. *Journal of Basic and Clinical Physiology and Pharmacology, 5*, 45-58.

28. Barros, L. F., Chagas, A. C., da Luz, P. L. & Pileggi, F. (1995). Magnesium treatment of acute myocardial infarction: effects on necrosis in an occlusion/reperfusion dog model. *International Journal of Cardiology, 48*, 3-9.

29. Schlack, W., Bier, F., Schafer, M., Uebing, A., Schafer, S., Borchard, U. & Thamer, V. (1995). Intracoronary magnesium is not protective against acute reperfusion injury in the regional ischaemic-reperfused dog heart. *European Journal of Clinical Investigation, 25*, 501-9.

30. Christensen, C. W., Rieder, M. A., Silverstein, E. L. & Gencheff, N. E. (1995). Magnesium sulfate reduces myocardial infarct size when administered before but not after coronary reperfusion in a canine model. *Circulation, 92*, 2617-21.

31. Herzog, W. R., Schlossberg, M. L., MacMurdy, K. S., Edenbaum, L. R., Gerber, M. J., Vogel, R. A. & Serebruany, V. L. (1995). Timing of magnesium therapy affects experimental infarct size. *Circulation, 92*, 2622-6.

32. Roberts, R., Husain, A., Ambos, H. D., Oliver, G. C., Cox, J. R., Jr. & Sobel, B. E. (1975). Relation between infarct size and ventricular arrhythmia. *British Heart Journal, 37*, 1169-75.

33. Nguyen, T. P., Qu, Z. & Weiss, J. N. (2014). Cardiac fibrosis and arrhythmogenesis: the road to repair is paved with perils. *Journal of Molecular and Cellular Cardiology, 70*, 83-91.

34. Kies, S. J., Pabelick, C. M., Hurley, H. A., White, R. D. & Ackerman, M. J. (2005). Anesthesia for patients with congenital long QT syndrome. *Anesthesiology, 102*, 204-10.

35. Kaumann, A., Bartel, S., Molenaar, P., Sanders, L., Burrell, K., Vetter, D., et al. (1999). Activation of beta2-adrenergic receptors hastens relaxation and mediates phosphorylation of phospholamban, troponin I, and C-protein in ventricular myocardium from patients with terminal heart failure. *Circulation, 99*, 65-72.

36. Jin, Y. T., Hasebe, N., Matsusaka, T., Natori, S., Ohta, T., Tsuji, S. & Kikuchi, K. (2007). Magnesium attenuates isoproterenol-induced acute cardiac dysfunction and beta-adrenergic desensitization. *American Journal of Physiology: Heart and Circulatory Physiology, 292*, H1593-9.

37. Suzuki, N., Tanabe, K., Osada, N., Yamamoto, A., Nakayama, M., Yokoyama, Y., et al. (2000). Magnesium dynamics and relation to left ventricular function in acute myocardial infarction. *Japanese Circulation Journal, 64*, 377-81.

38. Hayashi, Y., Ohtani, M., Hiraishi, T., Kobayashi, Y. & Nakamura, T. (2006). "Initial, continuous and intermittent bolus" administration of minimally-diluted blood cardioplegia supplemented with potassium and magnesium for hypertrophied hearts. *Heart, Lung & Circulation, 15*, 325-31.

39. Wexler, B. C. (1979). Isoprenaline-induced myocardial infarction in spontaneously hypertensive rats. *Cardiovascular Research, 13*, 450-8.

Figure Legends

Fig. 1 Treatment protocols and general parameters

a *In-vivo* treatment protocols for different groups of rats [Control, ISO, ISO + Mg^{2+} (ISO + Mg), and Mg^{2+} (Mg)].

b Initial body weight measured on the first day of treatment.

c Ratios of heart weight-to-initial body weight in different treatment groups.

Values are presented as mean ± SEM; n = 9-10 rats per group; ***$p < 0.001$ *vs*. control.

Fig. 2 Effects of ISO and Mg^{2+} treatments on ischaemia/reperfusion (I/R)-induced infarcts

a Representative images of TTC-stained ventricular slices of four different rats treated with various drugs. Viable myocardium stains red (TTC-positive), whereas areas of irreversible infarcts appear white (TTC-negative) and the area-at-risk (AAR) is outlined outside the dark (Evans blue-stained) region.

b AAR, expressed as a percentage (%) of total ventricular area.

c Infarct size, expressed as % of AAR. Values are presented as mean ± SEM; n = 7-10 rats per group; **$p < 0.01$ *vs*. control; $^{\#\#}p < 0.01$ *vs*. ISO; $^{\#\#\#}p < 0.001$ *vs*. ISO.

Fig. 3 Effects of various treatments on I/R-induced arrhythmogenesis

Number of episodes of (**a**) ventricular premature beats (VPB), **(b)** ventricular tachycardia (VT), and (**c**) ventricular fibrillation (VF) counted during I/R in the different treatment groups.

d The overall arrhythmia score in the various treatment groups. Values are presented as mean ± SEM; n = 6-10 rats per group; *$p < 0.05$, **$P < 0.01$ *vs*. control; $^{\#\#}p < 0.01$, $^{\#\#\#}P < 0.001$ *vs*. ISO.

Fig. 4 Effects of ISO and Mg^{2+} treatments on hemodynamic function

Pre- and post-I/R hemodynamic parameters recorded in hearts from different treatment rat groups. The parameters include (**a**) left ventricular (LV) developed pressure (LVDP), (**b**) LV end-diastolic pressure (LVEDP), (**c**) maximal rate of LV pressure rise ($+dP/dt_{max}$), and (**d**) maximal rate of LV pressure decline ($-dP/dt_{max}$). The baseline (pre-I/R) values were measured at the end of the stabilisation period, whereas post-I/R values were measured at the end of reperfusion. Values are presented as mean ± SEM; n = 6-10 rats per group; *$p < 0.05$, *vs*. control; $^{\#}p < 0.05$ *vs*. ISO.

Fig. 5 Effects of ISO and Mg^{2+} on histological characteristics

Representative images of ventricular slices of the different treatment groups of rat hearts stained with H&E (**a**, magnification x40) or Masson's trichrome (**b**, magnification x10). The labelled bars on the images represent scales of length. Note the larger width of myocytes (H&E), and the presence of more prominent connective tissue (Masson's trichrome) in ISO-treated hearts compared to non-ISO-treated hearts.

c Arbitrary score of histological changes in hearts of the different treatment groups. (-) depicts none, whereas (++) depicts moderate. n = 3-7 rats per group.

Figures

Fig. 1

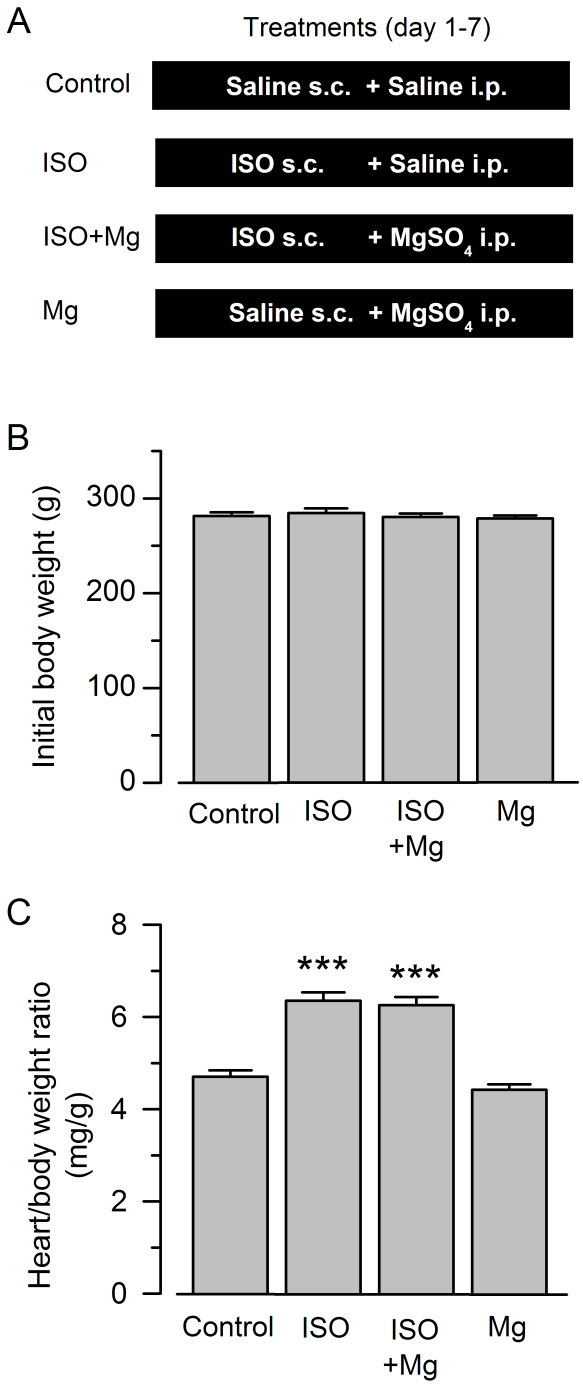


Fig. 2

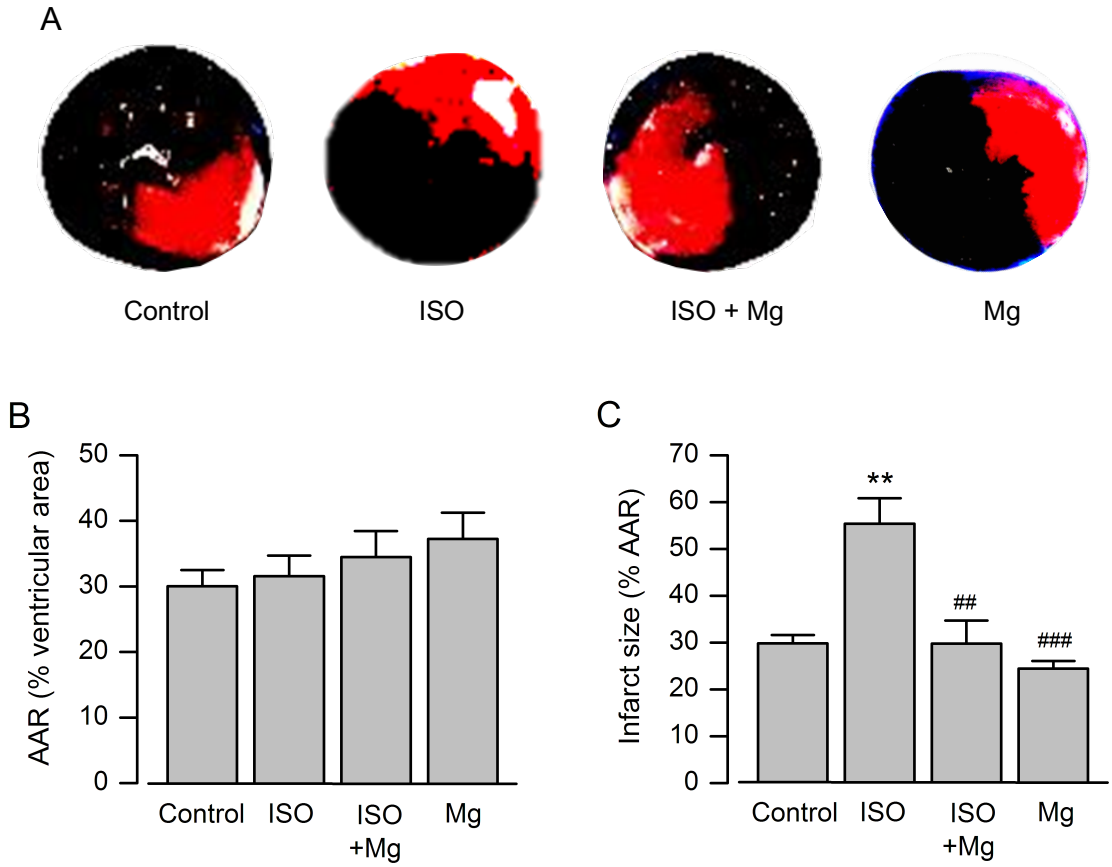

A
Control
ISO
ISO + Mg
Mg
B
AAR (% ventricular area)
0
10
20
30
40
50
Control
ISO
ISO +Mg
Mg
C
Infarct size (% AAR)
0
10
20
30
40
50
60
70
**
##
###
Control
ISO
ISO +Mg
Mg

Fig. 3

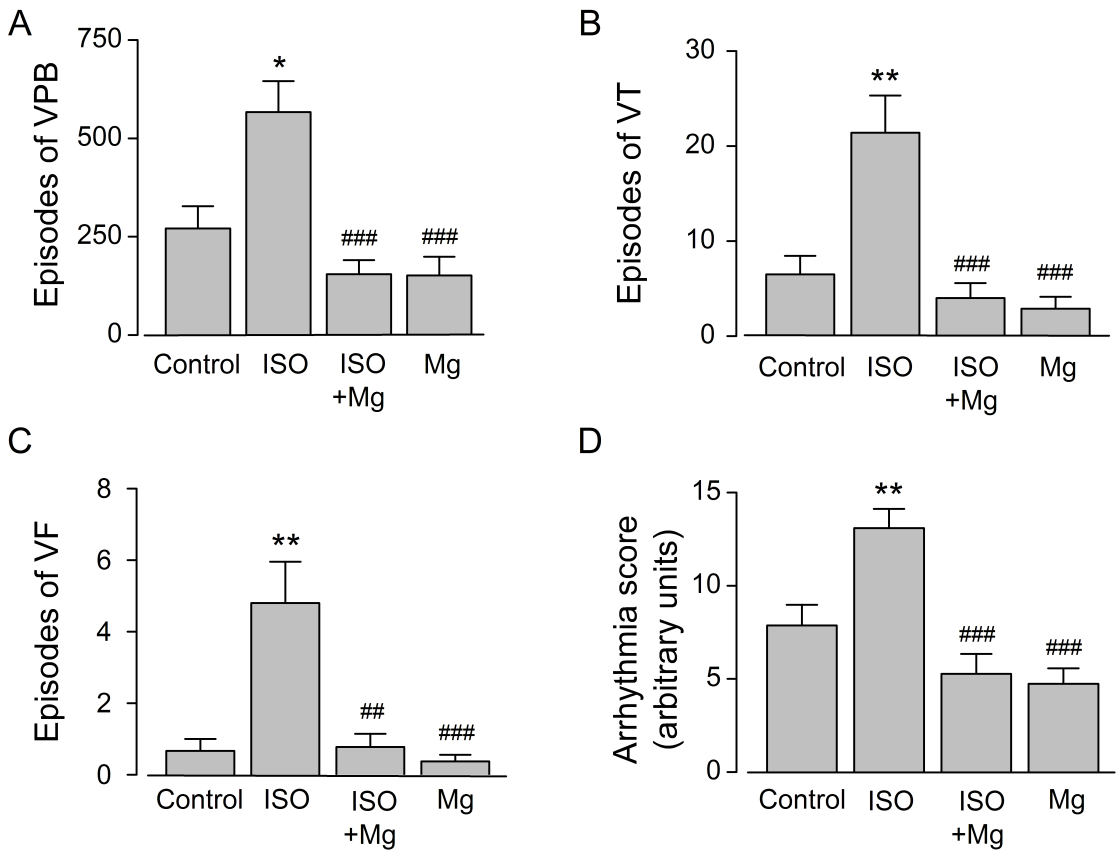

A
Episodes of VPB
750
500
250
0
*
###
###
Control
ISO
ISO +Mg
Mg
B
Episodes of VT
30
20
10
0
**
###
###
Control
ISO
ISO +Mg
Mg
C
Episodes of VF
8
6
4
2
0
**
##
###
Control
ISO
ISO +Mg
Mg
D
Arrhythmia score (arbitrary units)
15
10
5
0
**
###
###
Control
ISO
ISO +Mg
Mg

Fig. 4

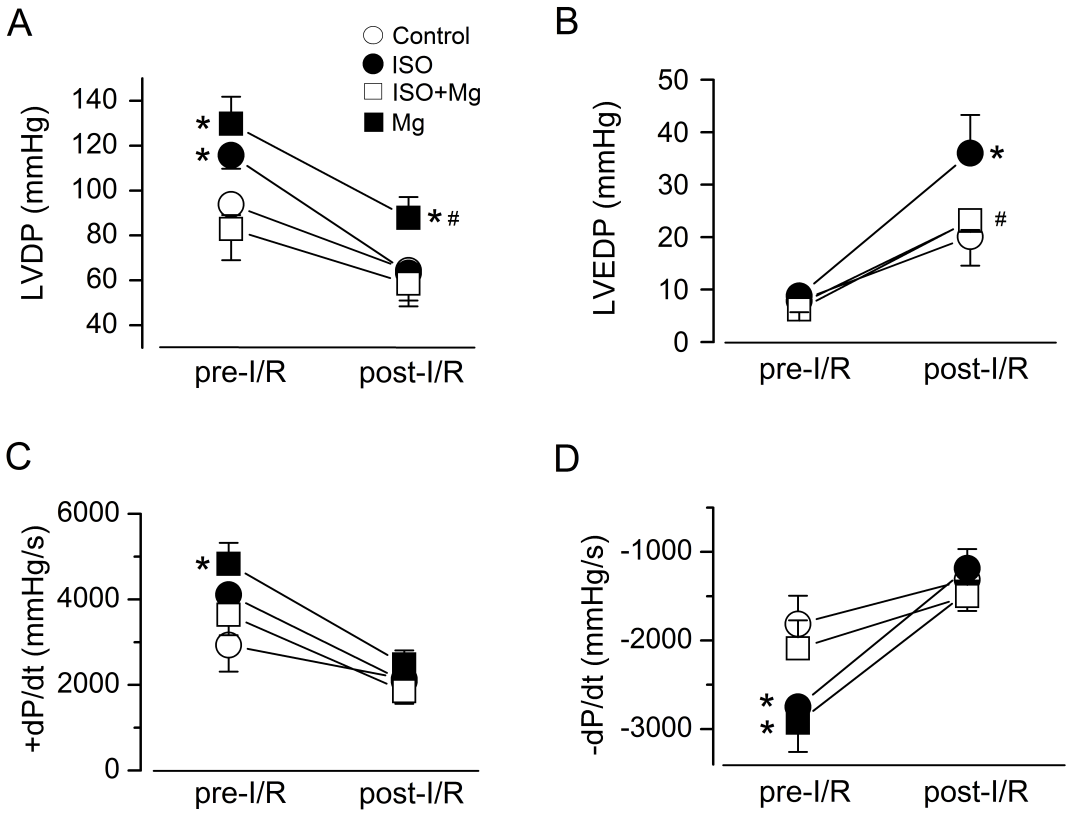
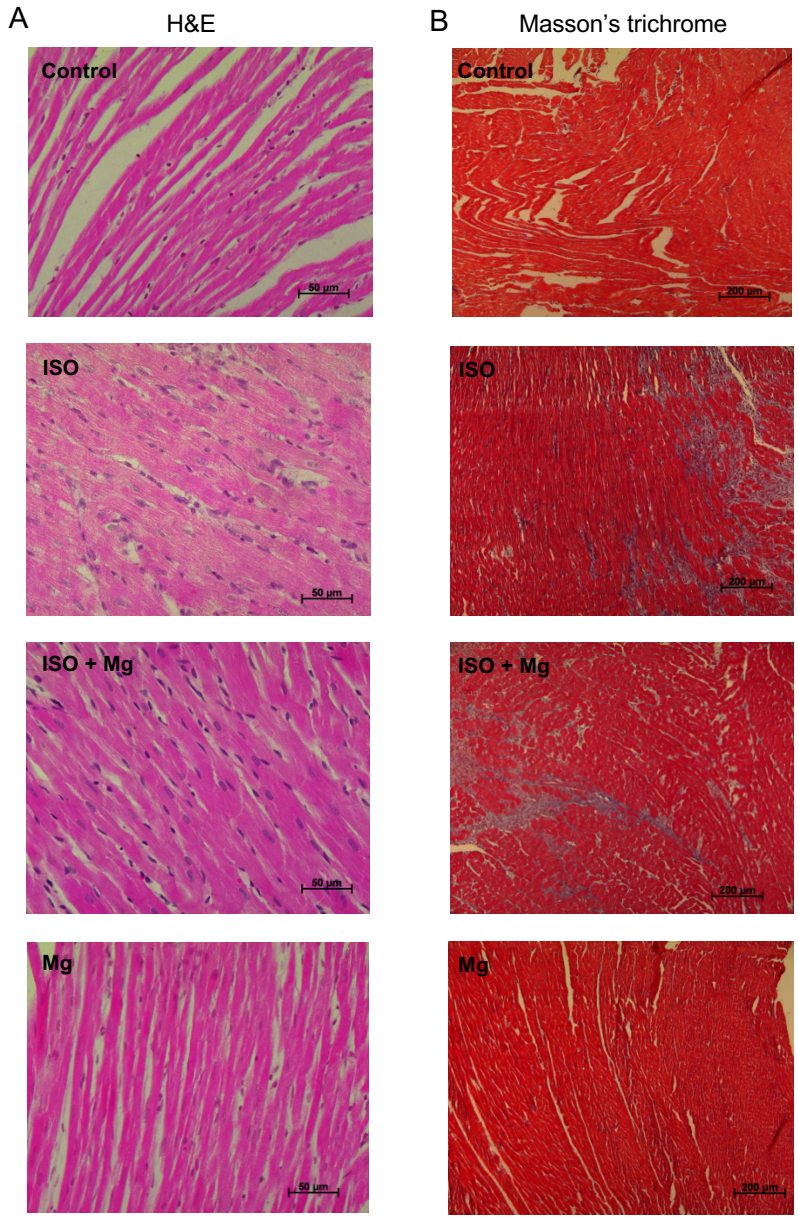

A
Control
ISO
ISO+Mg
Mg
LVDP (mmHg)
140
120
100
80
60
40
pre-I/R
post-I/R
B
LVEDP (mmHg)
50
40
30
20
10
0
pre-I/R
post-I/R
C
+dP/dt (mmHg/s)
6000
4000
2000
0
pre-I/R
post-I/R
D
-dP/dt (mmHg/s)
-1000
-2000
-3000
pre-I/R
post-I/R

Fig. 5



C

	Myocyte enlargement	Fibrosis
Control	-	-
ISO	++	++
ISO+Mg	++	++
Mg	-	-