ANTI-INFARCT EFFECT OF MAGNESIUM IS NOT MEDIATED BY ADENOSINE A₁ RECEPTORS IN RAT GLOBALLY ISCHAEMIC ISOLATED HEARTS

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SUMMARY

1. The aim of present study was to investigate the effects of magnesium (Mg) on cardiac function and infarct size and to compare it effects with those of adenosine. The mechanism of Mg-mediated cardioprotection was explored by combined use of Mg and a selective adenosine A₁ receptor antagonist.

2. Rat isolated hearts were used for Langendorff perfusion. Hearts were either non-preconditioned or preconditioned with Mg (6 mmol/L) or adenosine (1 mmol/L) before 30 min sustained ischaemia followed by 120 min reperfusion. Within each of these protocols, hearts were divided into two groups; one group was exposed to the A₁ receptor antagonist 8-cyclopentyl-1,3-dipropylxanthine (DPCPX; 200 nmol/L). Infarct size was measured by the triphenyltetrazolium chloride method. Left ventricular function was assessed by left ventricular developed pressure (LVDP), the product of heart rate × LVDP and coronary flow (CF).

3. The administration of Mg had an anti-infarct effect independent of its effect on postischaemic functional recovery in rats. Both Mg and adenosine equipotently reduced infarct size, but this effect of Mg was not blocked by the simultaneous administration of DPCPX. Cardiac function was improved by both adenosine and Mg and blockade of adenosine A₁ receptors attenuated these effects for both agents.

4. In conclusion, the results of the present study indicate that stimulation of adenosine A₁ receptors is not responsible for the anti-infarct effect of Mg in ischaemic myocardium in rats, but that the Mg-mediated protection of postischaemic functional recovery in rats is mediated by these receptors.

Key words: adenosine, infarction, ischaemia, magnesium, reperfusion.

INTRODUCTION

There is growing interest in a beneficial role for Mg in the pathophysiology of cardiovascular disorders. A number of cardiovascular disorders have been associated with low extracellular or intracellular concentrations of Mg, including myocardial infarction, arrhythmias and congestive heart failure. Elevated extracellular Mg concentrations are used in cardioplegic solutions, where Mg improves myocardial recovery. Given the variety of clinical uses of elevated Mg concentrations and the implication of imbalances in Mg in cardiovascular pathophysiology, the clinical application of Mg therapy remains controversial in acute myocardial infarction. It has been reported that Mg therapy reduced the mortality rate in acute myocardial infarction in the Leicester Intravenous Magnesium Intervention Trial. However, a significant effect on mortality rate for Mg could not be demonstrated in the International Study of Infarct Survival. Despite these conflicting results, several experimental myocardial infarction studies have suggested that Mg treatment is potentially effective in reducing infarct size in different animal species. However, the mechanism of for the efficacy of Mg is not fully understood.

Magnesium is an important cofactor for many enzymatic reactions and could stimulate purine nucleoside formation in the intact myocardium. Adenosine is known to confer significant protection to the myocardium. The main pathway of adenosine synthesis in ischaemic myocardium is decomposition of adenosine monophosphate by ecto-5'-nucleotidase. Interestingly, Mg is an important cofactor of ecto-5'-nucleotidase. Therefore, we hypothesized that Mg potentiates 5'-nucleotidase activity and protects the ischaemic myocardium.

The aim of the present study was to determine whether the administration of Mg limits the infarct size in acute myocardial infarction in rats and, if so, whether the mechanism is mediated by adenosine. To accomplish these goals, we examined the effect of the administration of Mg, with or without a selective A₁ receptor antagonist, in experimental acute myocardial infarction in rats.

METHODS

Animals
A total of 42 male Wistar rats (250–300 g) was used. Animals were anaesthetized by pentobarbital sodium (40 mg/kg bodyweight, i.p.).

Perfusion protocol
Rats were anaesthetized and anticoagulated with heparin. Heparin (1000 IU/kg) was injected i.p. 30 min before anaesthesia was induced. Hearts were immediately excised after anaesthesia had been induced and immersed in cold perfusion buffer (0°C) before being mounted on a non-recirculating Langendorff apparatus for retrograde perfusion. The ischaemic time between excision and mounting was less than 1 min. Hearts...
were perfused with oxygenated (95% O₂–5% CO₂) normothermic (37°C) Krebs–Henseleit bicarbonate (KHB) buffer at a constant pressure of 80 mmHg. The KHB buffer had the following composition (in mmol/L): NaHCO₃ 25; KCl 4.7; NaCl 118.5; MgSO₄ 1.2; KH₂PO₄ 1.2; glucose 11; CaCl₂ 2.5 (pH 7.4). The perfusion apparatus was water-jacketed to maintain a constant perfusion temperature of 37°C and, during prolonged ischaemic periods, hearts were immersed in KHB buffer at 37°C. Hearts were allowed to beat spontaneously throughout the experiments. Ischaemia was achieved by clamping the aortic perfusion catheter so that coronary flow was reduced to zero. To determine left ventricular pressure, a latex balloon was inserted into the left ventricle through an incision in the left atrial appendage. The balloon was tied securely into place and filled with water until a stable left ventricular end-diastolic pressure of 4–6 mmHg was obtained. The adjusted volume remained constant throughout the experiments. The balloon was connected to a pressure transducer via water-filled polyethylene tubing. Heart rate (HR) and the left ventricular developed pressure (LVDP) were monitored continuously and recorded on a four-channel physiograph. Developed pressure is defined as the peak of systolic minus end-diastolic pressure. Left ventricular function was assessed by LVDP, the rate pressure product (RPP = HR × LVDP) and coronary flow (CF). Coronary flow rate was determined by collecting the coronary effluent in a graduated cylinder.

**Experimental protocol**

Hearts were perfused for 20 min to establish equilibrium haemodynamics. Equilibration was ceased when HR, LVDP and CF were maintained at the same level for three continuous measurement periods timed 5 min apart. Baseline measurements were recorded at the end of this time. Control hearts were perfused without global ischaemia (GI) for 180 min. Non-preconditioned (NP) hearts were subjected to 30 min ischaemia followed by 120 min reperfusion; the Mg group received a 10 mL bolus injection of Mg sulphate (6 mmol/L) 15 min before the 30 min sustained ischaemia (see below); the Mg/DPC group was administered the A₁ receptor-selective antagonist DPCPX (200 nmol/L) as a bolus 10 mL injection 5 min before administration of the Mg sulphate. To compare the effects of Mg with those of adenosine, one group of hearts received a 10 mL bolus injection of adenosine (1 mmol/L) 15 min before the 30 min ischaemia; the adenosine/DPC group received DPCPX 5 min before the administration of adenosine. Bolus injections were made into the aortic root via the side arm of a cannula located proximal to the perfusion cannula. The concentration of DPCPX used in the present series of experiments was derived from the previous investigation of Headrick et al., whereas the concentration of adenosine used was derived from the study of McCully et al.; and the concentration of Mg concentration was selected following preliminary dose–response studies for Mg sulphate. Another preliminary experiment was performed to determine the effects of ethanol on cardiac function.

**Measurement of infarct size**

At the end of the reperfusion period, hearts were frozen and kept in a –20°C freezer to facilitate slicing of 2 mm transverse sections across the long axis. All hearts were approximately the same size (1.2 cm; atria and great vessels

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**Table 1** Baseline haemodynamic characteristics

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>LVDP (mmHg)</th>
<th>CF (ml/min)</th>
<th>HR (b.p.m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7</td>
<td>110.56 ± 4.48</td>
<td>11.3 ± 1.2</td>
<td>274 ± 15</td>
</tr>
<tr>
<td>NP</td>
<td>7</td>
<td>108.32 ± 2.76</td>
<td>12.4 ± 0.9</td>
<td>277 ± 13</td>
</tr>
<tr>
<td>Mg</td>
<td>7</td>
<td>109.17 ± 3.48</td>
<td>10.5 ± 1.71</td>
<td>270 ± 12</td>
</tr>
<tr>
<td>Mg/DPC</td>
<td>7</td>
<td>112.6 ± 3.1</td>
<td>11.59 ± 0.85</td>
<td>278 ± 16</td>
</tr>
<tr>
<td>Adenosine</td>
<td>7</td>
<td>107.43 ± 2.89</td>
<td>12.5 ± 1.3</td>
<td>296 ± 10</td>
</tr>
<tr>
<td>Adenosine/DPC</td>
<td>7</td>
<td>108.36 ± 5.88</td>
<td>10.68 ± 1.02</td>
<td>274 ± 16</td>
</tr>
</tbody>
</table>

Values are the mean ± SEM. n, number of hearts in each group; LVDP, left ventricular developed pressure; CF, coronary flow; HR, heart rate; NP, non-preconditioning; DPC, DPCPX.

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**Chemicals**

Adenosine, DPCPX, MgSO₄ and TTC were obtained from Sigma-Aldrich (Deisinhofen, Germany). General laboratory chemicals were from Merck (Darmstadt, Germany). Stock solutions of DPCPX, adenosine and MgSO₄ were prepared separately and then diluted to appropriate concentrations in KHB equilibrated with 95% O₂–5% CO₂ (pH 7.4 at 37°C). Adenosine and MgSO₄ were dissolved in KHB and DPCPX was dissolved in ethanol. Control hearts received a 10 mL bolus injection of KHB.

**Statistical analysis**

Statistical analysis was performed using SPSS (version 11.5 for Windows; SPSS, Chicago, IL, USA). Data are expressed as the mean ± SEM. To account for interanimal variability, the functional indices measured during treatment periods and the 120 min reperfusion period were expressed as a percentage of the control value recorded for each heart before any test intervention was made. Groups were compared by one-way ANOVA. If a significant F-value was obtained, the Tukey test was used to identify individual group differences and differences were considered statistically significant at P < 0.05.

**RESULTS**

Baseline and function during interventions

Table 1 summarizes the baseline haemodynamics of all groups. No significant differences were observed between or within groups after the 20 min stabilization period for the parameters examined (LVDP, RPP, HR and CF). Administration of all drugs caused temporary changes in haemodynamic parameters that returned to baseline levels by the beginning of GI.

**Postischaemic functional recovery**

Postischaemic haemodynamic data are given in Table 2. Postischaemic left ventricular function was assessed by LVDP

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both adenosine and Mg similarly improved recovery of LVDP during the 120 min reperfusion. The results given in Table 2 indicate that significantly less (P < 0.01) than that achieved with Mg throughout the 120 min reperfusion. The results given in Table 2 indicate that both adenosine and Mg similarly improved recovery of LVDP (except in the first minute of reperfusion), but that HR recovered better in the Mg group compared with the adenosine group during 20–100 min reperfusion. It seems that the difference in RPP between groups is due, at least in part, to differences in HR. According to these results, it cannot be concluded that Mg is absolutely superior to adenosine in the protection of postischaemic cardiac function. Blockade of A1 receptors significantly depressed postischaemic recovery of RPP in the adenosine/DPC and Mg/DPC groups (P < 0.001 vs adenosine and Mg groups). With the onset of ischaemia, CF has fallen to zero and, at the start of reperfusion, CF has recovered to between 95 and 100% of pre-ischaemic levels in all groups.

Infarct size
There were no significant differences in left ventricular area between the groups. Infarct size of different groups is summarized in Fig. 2. Infarct size in the control group was 0.37 ± 0.02% and, at the start of reperfusion, CF has recovered to between 95 and 100% of pre-ischaemic levels in all groups.

Table 2 Functional parameters at the end of the 120 min reperfusion period

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>% LVDP</th>
<th>CF (mL/min)</th>
<th>RPP (b.p.m × mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7</td>
<td>98.2 ± 0.8</td>
<td>11.0 ± 2.1</td>
<td>98.41 ± 1.09</td>
</tr>
<tr>
<td>NP</td>
<td>7</td>
<td>49.16 ± 3.45†</td>
<td>13.4 ± 1.1</td>
<td>18.3 ± 1.8†</td>
</tr>
<tr>
<td>Mg</td>
<td>7</td>
<td>96.34 ± 1.44*</td>
<td>12.8 ± 1.3</td>
<td>95.3 ± 3.1</td>
</tr>
<tr>
<td>Mg/DPC</td>
<td>7</td>
<td>31.93 ± 3.36†</td>
<td>11.47 ± 1.05</td>
<td>19.8 ± 0.9†</td>
</tr>
<tr>
<td>Adenosine</td>
<td>7</td>
<td>91.22 ± 2.89*</td>
<td>11.98 ± 0.85</td>
<td>76.39 ± 2.22†</td>
</tr>
<tr>
<td>Adenosine/DPC</td>
<td>7</td>
<td>46.66 ± 5.88†</td>
<td>10.85 ± 1.23</td>
<td>22.87 ± 2.01†</td>
</tr>
</tbody>
</table>

Values are the mean ± SEM. *P < 0.001 compared with the non-preconditioned (NP) group; †P < 0.001 compared with the control group. n, number of hearts in each group; LVDP, left ventricular developed pressure; CF, coronary flow; HR, heart rate; DPC, DPCPX.
adenosine groups, respectively). Treatment with DPCPX significantly abolished the effect of adenosine in reducing infarct size, resulting in an increase in infarct size in the adenosine/DPC group (45.8 ± 2.1%). 8-Cyclopentyl-1,3-dipropylxanthine was not able to block the reduction in infarct size produced by Mg.

**DISCUSSION**

Although there is no drug known that completely prevents myocyte necrosis, some agents can slow the rate of cell death. The administration of Mg has been reported to protect the myocardium against ischaemia and reduce reperfusion injury. It has been reported that Mg therapy started early after reperfusion is effective in reducing infarct size in a swine model. In contrast, Mg therapy reduced infarct size when administered before, but not after, reperfusion in a canine model. Thus, the optimum time for the prevention of Mg remains to be determined. Previous studies have addressed the involvement of Mg in cardioprotection using postischaemic functional recovery and/or myocardial infarct size as an end-point of cardiac protection. However, there is some controversy regarding the results of these studies. This may be due to differences in species, end-point, experimental models or intervention used. In the present study, we assessed the preconditioning effect of Mg in the protection of the heart with respect to both postischaemic functional recovery and infarct size. This is the first report of the preconditioning effect of Mg. All previous studies have used steady state elevations in extracellular Mg concentration. The mechanism by which Mg prevents myocardial injury during ischaemia is not fully elucidated. One of the aims of the present study was to clarify the role of A1 adenosine receptor stimulation in the protection afforded by Mg. Matsusaka et al. were the first to report the mechanism of the infarct size-limiting effect of Mg in acute myocardial infarction. They reported that this effect of Mg was abolished by the use of an adenosine receptor antagonist and suggested that the effect was attributable, at least in part, to augmentation of adenosine mechanism. The first important finding of the present study is that blockade of A1 receptors does not abolish the protective effect of Mg on infarct size. This shows that activation of these receptors alone is not a crucial step in the mechanism behind the anti-infarct effect of Mg in rat heart. One possible explanation for the difference between the results of Matsusaka et al. and the present study is the fact that protection mechanisms are redundant and not necessarily identical between species. Experiments in animal models of myocardial infarction have provided evidence that early Mg infusion can limit infarct size. One mechanism that has been postulated to be of importance is protection of the cardiomyocyte against calcium overload during or after ischaemia. It is well recognized that prolonged ischaemia in hearts causes defects in the ability of mitochondria to generate ATP. This dysfunction is mediated, at least in part, by an increase in calcium overload in the cytosol and/or mitochondria, leading to necrotic or apoptotic cell death in the ischaemic–reperfused heart. Under normal physiological conditions, the mitochondrial inner membrane is impermeable to all but a few selected metabolites and ions. However, under conditions of stress, a non-specific pore known as the mitochondrial permeability transition pore (MPTP) can be opened in the inner membrane of mitochondria. There is increasing evidence that opening of the MPTP may be critical in the transition from reversible to irreversible cell injury in response to ischaemia–reperfusion. The key factor leading to the opening of the MPTP is mitochondrial calcium overload. Activation of the MPTP by calcium is totally selective for calcium and pore opening is strongly inhibited by Mg overload. Although we have not determined the effect of Mg on the MPTP, we speculate that the anti-infarct effect of Mg may be mediated by inhibition of the MPTP.

Another finding of the present study is that administration of DPCPX before ischaemia completely abolishes the effects of Mg on postischaemic functional recovery, with haemodynamic parameters in the Mg/DPC group being similar to those observed in NP hearts. Adenosine is important for the control of cardiac function and in protecting the heart from ischaemic injury. The formation of adenosine is considered to be partially Mg dependent owing to the Mg sensitivity of S'-nucleotides. It is possible that the beneficial effects of Mg could stem, in part, from adenosine receptor activation subsequent to Mg-dependent increases in adenosine. This has been demonstrated previously in the rat isolated heart subjected to sustained GL. The findings of the present study are in agreement with this hypothesis, which raises the possibility that the protective effect of Mg on postischaemic function could also result from enhanced adenosine formation.

Excess intracoronary Mg elevates adenosine concentrations in the interstitial compartment, which is in close proximity to adenosine A1 receptors. In contrast with the findings of the present study, Headrick et al. showed no increases in myocardial adenosine release with steady state changes in extracellular concentrations of Mg. It seems that transient changes in Mg elicit transient changes in adenosine to induce preconditioning. In the present study, blocking A1 receptors eliminated all protection afforded by adenosine pretreatment. This result is in contrast with the results of many prior studies on the A1 receptor. Recent studies suggest that, in addition to the A1 receptors, A3 receptors are also involved in cardioprotection. Kilpatrick et al. reported that the reduction in infarct size with both A1 and A3 receptor agonists was completely blocked by DPCPX and suggested that A3 receptor agonists protect the ischaemic myocardium via A1 receptor activation. In addition, preliminary studies using A3 receptor knock-out mice have suggested the opposite (i.e. that A1 receptor activation can enhance ischaemic injury) because deletion of this receptor increases the resistance to contractile dysfunction or cell necrosis caused by myocardial ischaemia. In summary, the majority of studies support a protective role mediated by A1 receptors and the involvement of these receptors in the cardioprotection afforded by preconditioning. In contrast, discordant results have been reported for the role of A2 receptors and more work is needed to determine the exact role of A2 receptors in different animal species and under different experimental conditions.

Intrinsic to the development of new myoprotective protocols in cardiac surgery are the requirements of new methods to enhance postischaemic functional recovery and to decrease myocardial infarct size. Our results indicate that the administration of adenosine and Mg significantly decreases infarct size and improves postischaemic function in the rat heart. It is worth mentioning that Mg supplementation is inexpensive, easy to administer and relatively free of side-effects. In conclusion, the administration of Mg to rat heart resulted in improved postischaemic functional recovery, as well as a reduction in infarct size. These results may be
important clinically to reduce morbidity and mortality in myocardial disease.

REFERENCES


