Calcium handling and cell contraction in rat cardiomyocytes depleted of intracellular magnesium

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Abstract

Objectives: Depressed levels of cardiac Mg have been found in patients with ischaemic heart disease or heart failure, but it is not known whether low intracellular free [Mg] ([Mg]i) is a causal factor in such myocardial dysfunction. The aims for the present study were to develop a method of lowering [Mg]i in myocytes isolated from normal rat hearts, and so to determine whether a low [Mg]i, itself would cause abnormalities of intracellular Ca (i.e., [Ca2+]i) homeostasis or myocyte contractile function in absence of any cardiac disease. Methods: Rat ventricular myocytes were loaded with mag-indo-1/AM or indo-1/AM for determination of total [Mg]i and [Ca2+]i, respectively. Mitochondrial [Ca2+]i was determined by selective loading of indo-1/AM into the mitochondria. Cell contraction was measured using an edge-tracking device. Myocytes were depleted of [Mg2+], by incubation in absence of external Mg. This resulted in a decrease in [Mg2+]i, from about 1.3 to 0.3 mM. In subsequent experiments, 1.2 mM MgCl2 was again present in the superfusate. Results: Under basal conditions (low rate of stimulation, 0.2 Hz, and 1 mM external [Ca2+]), the Mg-depleted cells showed very similar changes in [Ca2+]i to control cells, despite an increase in the amplitude of cell contraction. But in presence of high external [Ca2+] (4 mM) and 5 Hz stimulation rate, the Mg-depleted cells showed defects in systolic Ca handling and in cell contraction; in particular, they were unable to increase systolic [Ca2+]i in response to the stimulus, unlike control cells. Despite these alterations in total [Ca2+], mitochondrial Ca2+ uptake was unchanged in the Mg-depleted cells. Conclusions: A low [Mg2+], can itself cause significant cardiomyocyte dysfunction in absence of any contributing disease state. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Calcium (cellular); Ion transport; Mitochondria; Myocytes

1. Introduction

Magnesium is essential for many aspects of myocardial function, and depressed levels of cardiac Mg have been found in patients with ischaemic heart disease or heart failure. Despite its importance, very little is known about the regulation of intracellular free [Mg2+] ([Mg2+]i) in the heart, or whether it is a causal factor in the myocardial dysfunction seen in disease states.

A reduced level of cardiac total Mg has been observed in patients with congestive heart failure, acute myocardial infarction, and in patients undergoing cardiac surgery [1,2]. In addition, a reduced Mg has been observed in diseases associated with aging, such as hypertension and non-insulin-dependent diabetes mellitus [3]. In experimental studies, animals fed on a low-Mg diet develop defects in cardiac function; for example, cardiomyopathies [4,5], depressed myocardial oxidative metabolism [6], and a higher incidence of free radical production upon reperfusion following ischaemia [7]. Free radical scavengers such as vitamin E and captopril can protect against myocardial injury in animal models [5,7,8] and in hypertensive patients [9,10], supporting a role for free radicals in the pathogenesis of cardiomyopathy related to Mg-deficiency [8].

However, there has been conflicting evidence regarding the efficacy of Mg-supplementation in protecting against ischaemic disease or cardiac arrhythmias following an ischaemic episode: in animal models of ischaemia/reperfusion injury, adding Mg to the perfusate decreased the number of reperfusion-induced ventricular arrhythmias in...
the rat [11], but did not reduce infarct size in the dog [12]. In human patients with acute myocardial infarction, Mg infusion suppressed ventricular arrhythmias over a 24 h period, but did not reduce the number of ischaemic episodes or peak creatine kinase release [13]. Mg is routinely included in cardioplegic solutions in patients undergoing cardiac surgery, with beneficial effects in terms of preventing substrate derangement upon reperfusion [14], and reducing the incidence of ventricular arrhythmias [15]. However, a study using cardioplegia on a rat model of ischaemia/reperfusion injury concluded that, although Mg-supplementation was protective, it depended heavily on the corresponding concentration of Ca\(^{2+}\) in the solution, and suggested that an appropriate ionic balance was the most important factor, rather than individual ion concentrations [16].

Another area of conflict is whether measurements of total Mg, for example in serum, are an indicator of cardiac Mg levels. Haigney et al. [2] concluded that the levels of total Mg measured in a sublingual epithelial sample did correlate with cardiac Mg whereas levels of total serum Mg did not. They subsequently found, using a canine model of heart failure [17], that sublingual Mg also correlated with cardiac free \([\text{Mg}^{2+}]_i\), in myocytes subsequently isolated from the hearts. In the dogs with heart failure, both sublingual Mg and myocyte \([\text{Mg}^{2+}]_i\), were depressed, so sublingual Mg could be used as a prognostic indicator of cardiac disease in this case. Again, however, it could not be determined whether the reduced \([\text{Mg}^{2+}]_i\) itself was causing the cardiac abnormalities.

So important questions remain regarding the role of Mg in contributing to and treating cardiovascular diseases. For example, the role of intracellular versus extracellular actions of Mg\(^{2+}\), and whether Mg-deficiency itself is a causal factor in inducing myocardial dysfunction. The aims for the present study were (i) to develop a method of lowering \([\text{Mg}^{2+}]_i\) in myocytes isolated from normal rat hearts, and (ii) to determine whether low \([\text{Mg}^{2+}]_i\) itself would cause abnormalities of intracellular Ca\(^{2+}\) (\([\text{Ca}^{2+}]_i\)) homeostasis and myocyte contractile function, in presence of a normal concentration of extracellular Mg, and in absence of any cardiac disease. Although other studies have attempted to manipulate \([\text{Mg}^{2+}]_i\), within cardiac cells [18,19], the present study is the only one to date which has addressed the issue of the effects of Mg-depletion on cardiac cell function. Part of this work has been published in an abstract [20].

## 2. Materials and methods

### 2.1. Myocyte isolation

Single cardiac myocytes were isolated from rat ventricles by collagenase digestion [21]. The investigation conforms with the ‘Guide for the Care and Use of Laboratory Animals’ published by the US National Institute of Health (NIH Publication No. 85-23, revised 1996). Male Wistar rats (200–250 g) were killed by cervical dislocation and the heart removed and placed in ice-cold ‘isolation buffer’ plus 0.75 mM CaCl\(_2\). Isolation buffer contained, in mM: 20 sodium N-hydroxyethylpiperazine-N’-2-ethanesulfonic acid (Hepes), 130 NaCl, 4.5 KCl, 5 MgCl\(_2\), 1 NaH\(_2\)PO\(_4\), 21 glucose, 5 Na-pyruvate, pH 7.25 with NaOH. The heart was perfused with isolation buffer plus 0.75 mM CaCl\(_2\) at 37°C for 4 min before switching to Ca\(^{2+}\)-free buffer (isolation buffer plus 90 \mu M EGTA) for 4 mm. The perfusate was then switched to ‘enzyme solution’ consisting of 50 ml isolation buffer plus 50 mg collagenase (Worthington, type I), 5 mg protease (Sigma, type XIV) and 15 \mu M CaCl\(_2\). The enzyme solution was continued until the tissue felt soft; approximately 15 min. The heart was then washed with isolation buffer plus 150 \mu M CaCl\(_2\), ventricles removed, sliced approximately 10 mm and shaken approximately 1 min and 30 s at 37°C in 20-25 ml isolation buffer plus 150 \mu M CaCl\(_2\). After filtration the cells were allowed to sediment in this buffer for 7 min. The supernatant was removed and cells resuspended in 0.5 mM CaCl\(_2\), the process repeated and cells finally resuspended in approximately 15 ml of isolation buffer containing 2 mM CaCl\(_2\).

### 2.2. Measurement of intracellular \([\text{Ca}^{2+}]_i\) and \([\text{Mg}^{2+}]_i\)

Total intracellular \([\text{Ca}^{2+}]_i\) (\([\text{Ca}^{2+}]_i\)) and mitochondrial \([\text{Ca}^{2+}]_i\) (\([\text{Ca}^{2+}]_i\)_m) were determined using indo1/AM, and \([\text{Mg}^{2+}]_i\), using mag-indo-1/AM (indicators from Molecular Probes Inc.). Loading conditions for the indicators were as follows: 3 ml cell suspension was incubated with 10 \mu M indo-1/AM for 15 min at 30°C when the dye partitions approximately equally between cytosolic and mitochondrial compartments [21]. The cells were centrifuged for 1 mm at 500 rpm and resuspended in 5 ml isolation buffer (containing 1 mM CaCl\(_2\)). For measurement of total \([\text{Ca}^{2+}]_i\), 1 mM probenecid was added to the cell suspension to prevent dye leakage from the cytosol and the cells stored at room temperature until use.

For determination of \([\text{Ca}^{2+}]_i\)_m, following indo-1/AM loading, the cells were incubated at room temperature for 2 h, shaken gently at 37°C for 1.5 h, sedimented by centrifugation, resuspended and stored at room temperature. This process, termed ‘heat-treatment’, promotes loss of cytosolic, but not mitochondrial, indo-1 through sarcosomal anion channels, and can be inhibited by probenecid. Full details of the method and experiments to confirm the mitochondrial origin of the remaining fluorescence are given in Griffiths et al. [21].

For measurement of total \([\text{Mg}^{2+}]_i\), cells were incubated with 5 \mu M mag-indo-1/AM for 15 min at 30°C and stored at room temperature. Although mag-indo also binds to Ca\(^{2+}\), the Kd is about 23 \mu M [22], which is well below the
physiological range of [Ca\(^{2+}\)], hence Ca\(^{2+}\) binding to mag-indo would not be expected to interfere with measurements of [Mg\(^{2+}\)], in the present study. Mag-indo has been reported to compartmentalize into both cytosol and mitochondria in rat myocytes [22]. However, unlike the case for the mitochondrial Ca\(^{2+}\) transporters, there are no known inhibitors of mitochondrial Mg\(^{2+}\) transport, and so proving a mitochondrial localisation of mag-indo would have been difficult in the present study. Therefore, only measurements of total [Mg\(^{2+}\)], are presented. It has been shown in isolated mitochondria that there is no net gradient of Mg\(^{2+}\) across the mitochondrial membrane [23], unlike that for Ca\(^{2+}\) [24], so it is likely that mitochondrial [Mg\(^{2+}\)] and cytosolic [Mg\(^{2+}\)] are in equilibrium within the cell.

2.3. Measurement of fluorescence and cell length

A small portion of the loaded cells was placed in an experimental chamber which was mounted on the stage of an inverted microscope (Nikon Diaphot 300). The normal superfusate contained, in mM: 137 NaCl, 5 KCl, 1.2 MgSO\(_4\), 1.2 NaH\(_2\)PO\(_4\), 16 D-glucose, 1 CaCl\(_2\), 20 Hepes pH 7.4 (using NaOH), temperature 37°C. The myocyte to be studied was illuminated with a red light and its image visualized with a TV camera and monitor. Indo-1 was excited at 340–390 nm and emission detected at 410±5 nm and 490±5 nm, corresponding to the peak emissions of the Ca\(^{2+}\) bound and Ca\(^{2+}\) free forms of the indicator, respectively. Detection of mag-indo-1 was by identical means. Fluorescence of the whole cell was collected on-line by a Newcastle Photometric Systems Photon Counting System (Newcastle, UK). Light was collected at a rate of up to one data point/ms from a single myocyte following subtraction of background fluorescence.

Cell length changes were monitored using a Crescent Electronics Video Edge Motion Detector.

2.4. Depletion of [Mg\(^{2+}\)]

In preliminary experiments, it was found that simply altering extracellular Mg levels had no acute effect on [Mg\(^{2+}\)]. So cells were incubated for longer periods of time in buffer containing no Mg to determine whether this protocol could lower [Mg\(^{2+}\)]. A previous study suggested that 16 h of Mg-depletion was necessary to reduce [Mg\(^{2+}\)]\(_{i}\) in chick heart cells [18], whereas another study found that less time was required in rat myocytes, but that depletion was affected by presence or absence of both external Na and Ca [19]. After trying buffers containing various combinations of extracellular ions, and incubating cells for various periods of time, the following protocol was found to be optimal for reducing [Mg\(^{2+}\)]\(_{i}\) in the present study: Following the complete isolation procedure (including Ca-repletion) described above, myocytes were allowed to sediment again. Cells were resuspended in HEPES buffer (see ‘Methods’) but containing 0 mM MgCl\(_2\) and 0.1 mM CaCl\(_2\). Cells were then incubated in a shaking water bath at 37°C for 90 min before being allowed to sediment again, and resuspended in buffer containing 1 mM CaCl\(_2\) (0 Mg). Control cells underwent the same protocol except that buffer contained 1.2 mM MgCl\(_2\) throughout. Cells were then loaded with mag-indo as described above. 1.2 mM MgCl\(_2\) was present in subsequent experimental protocols.

2.5. Expression of results and statistical analyses

[Ca\(^{2+}\)] is expressed as indo-1 ratio, calibration of the indo-1 signal in terms of absolute values of calcium was not attempted since this would provide no advantage over ratio values in the present experiments. However, calibration of mag-indo was performed since it was necessary to determine the actual level of Mg-depletion achieved to compare it with that measured in models of heart failure [17]. Calibration was achieved as described in Silverman et al. [22]. Briefly, mag-indo loaded cells were superfused with varying concentrations of Mg\(^{2+}\) (0, 0.5, 1, 5, 10, 20, and 50 mM) in presence of 1 mM EGTA, 1 μM FCCP, 123 mM KCl 10 mM HEPES, 10 ng/ml valinomycin, 2 μM nigericin, and 50 μM ionomycin. EDTA was added to the 0 mM Mg\(^{2+}\) solution to obtain a minimum value. Results are presented as means±S.E. unless raw data tracings are shown.

Statistical analyses were performed using Student’s t-test, paired where appropriate.

3. Results

3.1. Depletion of [Mg\(^{2+}\)]

In preliminary experiments, it was found that simply altering extracellular Mg levels had no acute effect on [Mg\(^{2+}\)], despite greatly altering cell contractility: if the external Mg level was increased above the normal 1.2 mM, then cell contraction decreased, whereas lower levels of Mg increased cell contraction. A similar result was obtained by Silverman et al. [22], and so the results are not presented here.

However, cells could be depleted of [Mg\(^{2+}\)], following prolonged incubation in Mg-free media (see ‘Methods’ for details). Fig. 1 shows that the Mg-depleting protocol resulted in a small but highly significant (P<0.0001) lowering of the mag-indo ratio. When the resulted were expressed in terms of actual concentration of Mg\(^{2+}\) (Fig. 1), this revealed a substantial drop in [Mg\(^{2+}\)], from about 1.3 to 0.3 mM. Since this concentration of Mg was similar to that observed by in a canine model of heart failure, 0.4 mM [17], this protocol was used in subsequent experiments.
3.2. Effects of Mg-depletion on $[Ca^{2+}]_i$ and cell contraction

Fig. 2A shows raw data tracings of changes in $[Ca^{2+}]_i$ (indo-1 ratio) and simultaneous measurements of cell contraction in myocytes stimulated to contract at a basal level of 0.2 Hz. 1.2 mM external MgCl$_2$ was present throughout to ensure that any differences were due to the decreased $[Mg^{2+}]_i$. Mg-depletion had no effect on the amplitude of the Ca$^{2+}$ transient but did significantly increase the amplitude of cell contraction ($P=0.017$; Figs. 2B and 2C).

To determine the effect of more physiological rates of stimulation and of challenging the cells with a higher external $[Ca^{2+}]$, cells were exposed to 4 mM external $[Ca^{2+}]$ followed by increasing the pacing frequency to 5 Hz. Fig. 3 shows the effects of this protocol on the amplitude of cell contraction. Under basal conditions of 0.2 Hz stimulation rate and 1 mM external $[Ca^{2+}]$, the Mg-depleted cells showed an increased contractile amplitude. When the external Ca was raised to 4 mM (‘high-Ca buffer’), control cells showed an increase in amplitude, as expected, but the Mg-depleted cells all underwent frequent spontaneous contractile oscillations and no longer responded to the electrical stimulus, making measurements of cell length unreliable (indicated by the question mark in Fig. 3). Typically in these cells the oscillations appeared to be of smaller amplitude but greater duration than normal contractions. When cells were then paced at 5 Hz, the Mg-depleted cells regained their ability to respond in time with the stimulus. However, under these conditions the
Fig. 3. Contractile response of myocytes to high external [Ca\(^{2+}\)] and 5 Hz stimulation rate. Amplitude of cell contraction in control (n=9) and Mg-depleted (n=10) cells stimulated at 0.2 Hz (basal), upon exposure to 4 mM external [Ca\(^{2+}\)] (high Ca), and upon exposure to high-Ca and 5 Hz stimulation rate. Note that for the Mg-depleted cells upon exposure to high-Ca all cells underwent frequent spontaneous oscillations, making measurements of cell length unreliable (hence the question mark in the figure). *, \(P=0.014\); **, \(P=0.017\) vs. control (unpaired \(t\)-test).

Fig. 4 shows the corresponding changes in [Ca\(^{2+}\)]\(_i\). Under basal conditions, Mg-depleted cells had a slightly higher diastolic [Ca\(^{2+}\)] than controls, but no differences were observed upon exposure to high-Ca or increased pacing (Fig. 4A). In contrast, although there was no difference between basal levels of systolic Ca\(^{2+}\), upon exposure to high-Ca and 5 Hz, the Mg-depleted cells failed to increase their systolic Ca\(^{2+}\), unlike the control cells (Fig. 4B). This is shown more clearly in the amplitude of the Ca\(^{2+}\) transient; in control cells this progressively increased throughout the protocol, whereas in the Mg-depleted cells it remained unchanged (Fig. 4C).

3.3. Effects of Mg-depletion on mitochondrial [Ca\(^{2+}\)]

Alterations in intracellular Ca\(^{2+}\) handling in response to Mg-depletion, such as those described above, may be reflected in abnormalities of mitochondrial Ca\(^{2+}\) homeostasis. [Ca\(^{2+}\)]\(_m\) does not change in rat myocytes during a single contraction [21,25]. However, when the cells are stimulated to contract rapidly in presence of an adrenergic agonist, or high external Ca\(^{2+}\), then a slow increase in [Ca\(^{2+}\)]\(_m\) does occur [21,25].

In the present study, [Ca\(^{2+}\)]\(_m\) was measured (as described in ‘Materials and Methods’) in myocytes stimulated under the conditions described above, i.e. at 5 Hz in
presence of 4 mM external CaCl₂, in order to compare results with the changes in total [Ca²⁺]. Fig. 5 shows that [Ca²⁺]ₐ increased significantly in both control and Mg-depleted cells. But, importantly, there were no differences in the values of [Ca²⁺]ₐ achieved between control and Mg-depleted cells.

4. Discussion

This paper has shown that a lower [Mg²⁺], can itself cause significant cardiomyocyte dysfunction in absence of any contributing disease state. Although previous studies described methods of lowering [Mg²⁺], in myocytes [18,19], this is the first to examine the consequences of Mg-depletion on cell contractility and intracellular Ca²⁺ homeostasis. Under basal conditions (low rate of stimulation, 0.2 Hz, and 1 mM external [Ca²⁺]), the Mg-depleted cells showed very similar changes in [Ca²⁺]ₐ to control cells, despite an increase in the amplitude of cell contraction. However, when challenged with high external [Ca²⁺] and when paced at a rate of 5 Hz, the Mg-depleted cells all showed defects in systolic Ca²⁺ handling and in cell contraction. In particular, they were unable to increase systolic [Ca²⁺] in response to the stimulus, unlike control cells. Despite these alterations in total [Ca²⁺], mitochondrial Ca²⁺ uptake was unchanged in the Mg-depleted cells.

The [Mg²⁺], in control cells reported here, approximately 1.3 mM, is within the range of those reported previously in cardiomyocytes, 0.8–1.3 mM [22,26,27]. The [Mg²⁺], following the Mg-depleting protocol, 0.3 mM, was similar to that found in the only study so far which has measured [Mg²⁺], in myocytes from diseased hearts: Haigney et al. [17], using a canine model of rapid-pacing induced heart failure, reported an [Mg²⁺], of 0.4 mM in myocytes isolated from the failing heart compared with 1.2 mM [Mg²⁺], in control myocytes.

Most [11,13,28,29], but not all [30], studies have found Mg-supplementation to be protective in animal experiments and clinical studies. One possible reason for the differences may be the confusion between intracellular versus extracellular actions of Mg²⁺. The present study has shown that a low intracellular [Mg²⁺] can induce abnormalities of contraction and Ca²⁺ homeostasis in absence of any underlying disease. So it is possible that in cases of cardiac disease it is the reduced [Mg²⁺] per se that is at least partly responsible for cardiac dysfunction. However, what the present study and previous ones have also found is that simply increasing extracellular [Mg²⁺] ([Mg²⁺]ₑ) does not result in an elevation of [Mg²⁺]ᵢ (at least in the short term). Conversely, reducing [Mg²⁺], had very similar effects on cell contractility and Ca²⁺ homeostasis, at least under basal conditions, to those of a reduced [Mg²⁺], (present study and 22); this effect again must have been due to extracellular actions of Mg²⁺, since [Mg²⁺], was unchanged. There is a consensus from both experimental and clinical studies that increasing [Mg²⁺]ₑ is anti-arrhythmic (see, for example, [11,13]) suggesting an extracellular site of protection; however, increasing [Mg²⁺], could not attenuate the prevailing ischaemia [13]. This, therefore, may be one explanation for the above conflicting results; external Mg may be able to correct some of the arrhythmias, but will not correct remaining dysfunctions due to a reduced [Mg²⁺]ᵢ.

Mechanisms of the anti-arrhythmic effect of Mg are not understood. Song et al. [28], using guinea-pig ventricular myocytes, found that changing external Mg had no significant effect on the inward Ca²⁺ current, but that high external Mg did reduce the number of spontaneous oscillations in response to a Ca-overloading procedure, and suggested an external non-specific charge-effect on the cell membrane. Aomine et al. [29] also found that high external Mg (5–10 mM) inhibited the number of after contractions following overdrive stimulation in both rat papillary muscle and guinea-pig myocytes. But whereas 10 mM Mg also inhibited the underlying Ca²⁺ transient, 5 mM Mg had no effect on this. They suggested that Mg was shifting the threshold for activation of various ion channels to less negative potentials, and possibly also blocking either the Ca-channel, Na/Ca exchanger and various K channels. Thus little is known about the interactions of Mg²⁺ with ion transporters of the sarcosomal membranes. However, Mg²⁺ is known to affect sarcoplasmic reticular (SR) Ca²⁺ transport [31]; for example, in absence of Mg²⁺, ryanodine receptors take seconds, rather than milliseconds, to adapt.
to an increase in [Ca²⁺]. [32]. The SR Ca²⁺-ATPase also requires Mg²⁺, the optimal concentration being about 1 mM [33]; concentrations of Mg²⁺ above or below this are inhibitory. So effects of a low [Mg²⁺], on SR Ca²⁺ transport could well explain the results presented here, where, in the Mg-depleted cells, systolic Ca²⁺ could not be increased when cells were challenged with a high external Ca²⁺ and rapid stimulatory rate.

Another area that needs clarification is the mechanism of Mg transport across the cell membrane. The myocyte membrane has a low permeability for Mg²⁺; if [Mg²⁺], were in equilibrium with [Mg²⁺]o, and given a membrane potential of −80 mM, then [Mg²⁺]o would be around 200 mM [34]. Mg²⁺ is not transported into the cell on the L-type Ca²⁺ channel, although it is a weak blocker of these [33]. There is some evidence for a Na/Mg exchanger, but evidence for this in the heart is controversial [31,34]. One reason may be that it differs between species: increasing [Mg²⁺]o caused a drop in [Na⁺] in guinea-pig [35] but not ferret [36] papillary muscle. In chick and rat heart cells, absence of [Na⁺], resulted in an increase in [Mg²⁺], [18,34], but in the chick cells this still occurred when [Mg²⁺]o was removed, suggesting the increase in [Mg²⁺], was due to release from intracellular stores [34].

Finally, in the present study it seems likely that mitochondrial Ca²⁺ uptake was actually enhanced in the Mg-depleted cells since they showed a similar uptake of Ca²⁺ to control cells, but in the face of a reduced total [Ca²⁺]. Mg is known to inhibit Ca²⁺ uptake in isolated mitochondria [24], thus it is possible that reducing Mg²⁺ in the cytosol of myocytes would have a stimulating effect on mitochondrial Ca²⁺ uptake. However, in a hamster model of cardiomyopathy, mitochondrial Ca²⁺ transport was found to be depressed [37]. [Mg²⁺], was not measured in this study but it seems likely, in view of the present results, that the mitochondrial dysfunction observed in this model was due to other facets of the disease. Taken together, these results suggest that impaired coupling of ATP supply and demand seen in cases of heart disease may well be due to defects in mitochondrial Ca²⁺ transport, but a reduction in [Mg²⁺], is not the primary cause of this aspect of the disease.

In conclusion, only by further understanding of regulation of Mg²⁺ transport in the heart and its effects on Ca²⁺ homeostasis, contraction and energy metabolism under both normal and diseased conditions, and the relative effects of extracellular versus intracellular Mg²⁺, will we be able to maximise its effectiveness during cardiac surgery, and in both treating and preventing cardiovascular diseases.

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References

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