Reduction of perifusate magnesium alters inotropic response of papillary muscle to ion channel modulators

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Abstract. Magnesium has a significant role in the regulation of ion transport. Marginal deficiency of Mg can therefore affect myocardial excitability and contractility. This study was taken up with the objective of examining the inotropic response of the myocardium to variation in extracellular [Mg]o and identifying the ion channels and pumps mediating the inotropic changes. Electrically stimulated rat papillary muscle was used as the experimental model and mechanical changes were recorded using a physiograph. Channel specific antagonists were used to identify the channels mediating the functional changes. Diastolic Ca2+ levels were determined in isolated myocytes by the ratiometric method using the fluorescent indicator Fura2-AM. A negative association was observed between the level of [Mg]o and force of contraction, with a peak at 0.48 mM Mg. The force of contraction in Mg deficient medium (0.48 mM) was 158% of control (1.2 mM Mg) (p < 0.001). Inotropic response to the L-type channel antagonist (verapamil–1 lM) and NaK ATPase inhibitor (Ouabain–0.3 mM) was augmented in Mg deficiency (p<0.005), indicating activation of the channel and the pump. The response to T-type channel inhibitor (NiCl2–40 lM) was attenuated in Mg deficiency (p < 0.05). The response to the sarcoplasmic reticular Ca pump inhibitor (caffeine-10 mM) and the SR Ca2+ release channel inhibitor (ryanodine-1 lM) were not significantly affected by Mg deficiency. Diastolic level of Ca2+ increased with a decrease in Mg (p < 0.05). The observations of the study lead to the conclusion that the positive inotropic response in Mg deficiency is mediated by an increase in basal Ca2+ combined with Ca-induced-Ca release consequent to Ca2+ influx through L-type Ca channel. Variation in sensitivity to Ca channel blockers and NaK ATPase inhibitor in Mg deficiency can have pharmacological implications.

Keywords: cardiac inotropy, Mg deficiency, papillary muscle, ion channel modulators

Studies during the past two decades have revealed the importance of magnesium in the maintenance of cardiac function. Magnesium deficiency has been identified as a risk factor for cardiovascular diseases such as cardiac arrhythmias and coronary heart disease, as well as hypertension and diabetes mellitus [1-3]. Epidemiologists observed that patients dying suddenly from ischemic heart disease had lower concentrations of myocardial tissue Mg and potassium than controls and that there was a greater proportion of deaths in places with soft water [4, 5]. Experimental and clinical studies indicate that chronic suboptimal intake of the element results in marginal Mg deficiency characterized by decrease in extracellular Mg with maintenance of tissue levels. Magnesium concentration in serum, erythrocytes and urine were lower when dietary magnesium was low [6]. Hypomagnesemia, defined as suboptimal levels of extra-
cellular Mg, is common in regions with soft water [7, 8]. In Kerala (India), where Mg in potable water is low, a significant difference was observed in the serum and erythrocyte Mg of children from low socioeconomic groups, indicating insufficient intake [9]. Experimental studies in rats have shown that with insufficient intake serum magnesium levels are reduced by 40-60% while the cardiac as well as aortic tissue levels are maintained [10-12].

Though there are innumerable studies to show the beneficial effects of Mg supplementation, marginal Mg deficiency has not received the attention it deserves, probably due to the absence of clinical symptoms. The effect of extracellular Mg [Mg]o on the contractile properties of the myocardium involves modulation of ion movements, which themselves are regulated by many intra- and extracellular factors. Significant increases in supraventricular and supraventricular plus ventricular beats were reported when the dietary magnesium concentration was low [6]. A negative correlation between extracellular Mg levels and the extent of contraction was observed in ventricular preparations and isolated cardiac myocytes [13-15]. Magnesium has a key role in regulating ion transport. As magnesium is known to be a weak Ca2+ channel blocker, it is presumed that decrease in the level of [Mg]o will be accompanied by greater influx of Ca2+, mediating a positive inotropic response. Magnesium modulates Ca2+ flux through the voltage gated Ca2+ channels and also activates transport by binding directly to the transporters, as in the case of Na and Ca pumps [16]. Magnesium may also alter the pharmacokinetics and pharmacodynamics of some cardiovascular drugs like Ca2+ antagonists and cardiac glycosides.

This study was therefore taken up with the objective of examining the contractile response of the myocardium to decrease in [Mg]o, and to assess the inotropic response to ion channel modulators. Variation in response to the modulators with change in [Mg]o will help to identify the ion channels and pumps mediating the mechanical variation.

**Methods**

The experimental studies were carried out with the approval of the Institutional Animal Ethics Committee. Mechanical response of the myocardium to variation in [Mg]o, and the response to ion channel modulators were recorded in rat papillary muscle. Diastolic calcium levels were determined in isolated cardiac myocytes. Adult female Sprague-Dawley rats weighing 200 ± 25 g were used for the study.

**Isolation of left ventricular papillary muscle and measurement of myocardial contraction**

A thoracotomy was performed in anaesthetized animals and the heart was rapidly excised into modified Krebs Ringer Hanseli (KRH) buffer containing (in mM): NaCl-133, KCl-3.6, CaCl2-1.5, MgCl2-1.2, glucose-16 and HEPES-3 and pH 7.4. Papillary muscles were isolated carefully from the left ventricle into oxygenated KRH buffer. The mural end of the muscle was clamped and tendinous end connected to a force transducer. Muscles were superfused with the buffer and stimulated electrically to contract isometrically at 0.5 Hz, at a voltage 10% above the threshold, by pulses of 5ms duration delivered through two platinum electrodes. Temperature was maintained at 32 °C. After an initial equilibration period of 1 h in KRH buffer, the muscles were gradually stretched in a stepwise manner until the developed force was maximal. The baseline contraction was recorded using a physiograph. The muscles were then exposed to different experimental interventions and steady state contraction in response to the treatments was recorded.

**Isolation of cardiomyocytes and measurement of intracellular calcium**

Cardiomyocytes were isolated by the enzymatic perfusion method standardized in the laboratory [17]. Hearts were perfused retrogradely with KRH buffer containing 1 mM Ca and 25 IU/ml heparin to flush out the blood, followed by calcium free buffer containing 0.1 mM EGTA and 20 mM taurine for 5 minutes. EGTA was washed out by perfusion with EGTA free buffer. The same Ca free solution containing 0.06% (w/v) collagenase type I (Sigma) and 0.1% (w/v) fatty acid free fraction V bovine serum albumin (BSA) was perfused with the buffer and stimulated electrically to contract isometrically at 0.5 Hz, at a voltage 10% above the threshold, by pulses of 5ms duration delivered through two platinum electrodes. Temperature was maintained at 32 °C. After an initial equilibration period of 1 h in KRH buffer, the muscles were gradually stretched in a stepwise manner until the developed force was maximal. The baseline contraction was recorded using a physiograph. The muscles were then exposed to different experimental interventions and steady state contraction in response to the treatments was recorded.
Role of ion channels influencing mechanical variation in marginal magnesium deficiency

To identify the ion channels/pumps mediating the inotropic variation, the muscle was treated with the channel modulators for 30 min. and the force of contraction in Mg sufficient (1.2 mM) and deficient (0.48 mM) media were compared. Variable response in Mg sufficient and deficient media is an indication of the involvement of the channel/pump in mediating the mechanical change. The channels and pumps selected for study and the antagonists used are listed in Table 1.

Table 1. Channels and pumps examined and antagonists used.

<table>
<thead>
<tr>
<th>Channel/pump</th>
<th>Antagonist</th>
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<tbody>
<tr>
<td>Sarcolemmal L type Ca channel</td>
<td>1 μM verapamil [18]</td>
</tr>
<tr>
<td>Sarcolemmal T type Ca channel</td>
<td>40 μM NiCl₂ [19]</td>
</tr>
<tr>
<td>Sarcolemmal Na K ATPase</td>
<td>0.3 mM ouabain [20]</td>
</tr>
<tr>
<td>Sarcoplasmic reticular Ca pump</td>
<td>10 mM caffeine [21]</td>
</tr>
<tr>
<td>Sarcoplasmic reticular Ca release</td>
<td>1 μM ryanodine [22]</td>
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Figure 1. Inotropic response of papillary muscle to variation in extracellular Mg. Values are mean ± SD; n = 10-13 preparations. *$p < 0.05$ versus baseline, # $p < 0.001$ versus baseline.

Statistical analysis

A minimum number of 6 replicates were analyzed for each sample. Data are presented as mean ± SD. Statistical significance was analyzed by Student’s t test and a value of $p < 0.05$ was considered significant.

Results

Inotropic response of papillary muscle to variation in extracellular magnesium

The contractile response of papillary muscle to variation in $[\text{Mg}]_\text{o}$ was studied by exposing the papillary muscle to different concentrations of Mg. A positive inotropic response was observed with a decrease in the concentration of $[\text{Mg}]_\text{o}$ (Figure 1). Baseline contraction was recorded at 1.2 mM Mg. With a decrease in the level of Mg in the superfusate, an increase in the force of contraction was observed. Steady state contraction was seen at 30 min. and the change was maintained even after 1h. The increase in the force of contraction was maximal at 0.48 mM Mg, the value being 158% of the control (1.2 mM Mg). Hence, for further studies 0.48 mM Mg was taken as Mg deficient. The positive inotropic effect of Mg deficiency was reversed in Mg sufficient medium. The time for peak contraction was comparable in Mg sufficient (0.254 ± 0.008s) and deficient media (0.252 ± 0.007s). When $[\text{Mg}]_\text{o}$ was 0.48 mM, the time for 1/2 relaxation (0.232 ± 0.007s) was significantly lower compared to that in sufficient Mg (0.272 ± 0.009, $p < 0.001$). The
diastolic level of calcium was also found to be higher at 0.48 mM Mg compared to 1.2 mM and the difference was statistically significant as observed by paired t-test (figure 2).

Myocardial response to ion channel modulators

The force of contraction was recorded 30 min after the addition of channel antagonists as steady state contraction was attained by that time. Sarcolemmal ion flux appears to have a significant influence on Mg induced contractile variation (figure 3). Decrease in contraction in the presence of verapamil was 8.6% in Mg sufficient medium and 20% in Mg deficient medium, suggesting a significant increase in Ca\(^{2+}\) influx through voltage gated L-type Ca channel in Mg deficiency. The negative inotropic response to NiCl\(_2\) was significantly less in Mg deficiency (7.2%) compared to control (15%). The positive inotropic response to ouabain was augmented in Mg deficiency. The force of contraction increased from 130% in Mg sufficient medium to 150% in Mg deficient medium, suggesting activation of the NaK ATPase. The response to caffeine, the SR Ca\(^{2+}\) pump blocker was unaffected by variation in [Mg\(^{2+}\)]\(_o\) but the force of contraction being 133-135% of baseline. Decrease in contraction in response to ryanodine was 42% in the presence of 1.2 mM Mg and 49% in 0.48 mM Mg, suggesting increased Ca\(^{2+}\) release. Though the difference was not statistically significant, greater influx of Ca\(^{2+}\) through L-type Ca channel is expected to stimulate Ca\(^{2+}\) release from the sarcoplasmic reticulum due to Ca-induced-Ca release.

Discussion

Small changes in [Mg\(_o\)] can have significant effects on vascular tone and also cardiac excitability, contractility and reactivity. [Mg\(_o\)] is reported to exert negative inotropic and chronotropic effects in cardiac tissue [14, 23-27]. The observations of this study indicate that decrease in [Mg\(_o\)] is accompanied by an
increase in contractile force (figure 1). This observation is supported by earlier reports [13-15]. Positive inotropy with a decrease in [Mg]o attained a peak at 0.48 mM Mg, followed by a decline in amplitude on further reduction in Mg. This pattern is comparable to that observed in isolated cardiomyocytes [15].

Similarity of observation in isolated myocytes and papillary muscle excludes the role of cardiac endothelium in the contractile changes induced by variation in [Mg]o. Magnesium is considered as the physiological Ca channel blocker [23]. The inverse relation between [Mg]o and the contractile force may therefore be related to Ca2+ availability. Decrease in [Mg]o was associated with increase in diastolic calcium (figure 2). Single channel studies have shown that the presence of [Mg2+]o converts long lasting channel openings to briefer events without altering the unitary conductance, with hyperpolarization increasing the degree of block [28]. It has also been reported that Mg2+ could prevent Ca2+ influx in the resting cell [29]. These observations support the increase in diastolic Ca2+ in Mg deficiency. Diastolic Ca2+ levels can also be raised when enhanced Ca2+ influx is not accompanied by a parallel increase in the activity of Ca2+ pump, as observed by the response to caffeine (figure 3). Katholi et al. [30] studied the dual dependency of heart cells on both Ca2+ and Mg2+ for electrical stability. According to them, Ca2+ participates in the generation of action potential, promoting electrical stability and initiating myocardial contraction, while Mg2+ has importance as an activator of cation transport through the sarcolemma. The significant increase in the negative inotropic response to verapamil in Mg deficiency implies that the L-type calcium channel activity is augmented in Mg deficiency (figure 3). The L-type Ca2+ channel plays a key role in transmembrane Ca2+ influx and Mg was shown to modulate the flux through this channel [31]. Relaxation of the blocking effect of Mg with reduction of [Mg]o, can increase the influx of initiator Ca associated with proportionate increase in the release of Ca from sarcoplasmic reticular Ca stores leading to an increase in contractile force. Though not significant statistically, increased sensitivity to ryanodine in Mg deficiency suggests activation of Ca2+ release channels. The inotropic response to T-type channel antagonist NiCl2 indicates down regulation of the channel, and augmentation of the ouabain-induced positive inotropic response signifies increase in activity of NaK ATPase in Mg deficiency (figure 3). Increase in NaK ATPase activity (influx of K+ and efflux of Na+) in Mg deficiency is supported by the decrease in relaxation time (1/2). T-type channels help in generating pacemaker potentials in the SA node [32] but in the working cells of the atria and ventricles they are not concentrated in the T-tubules, which makes it unlikely that they play an important role in opening the SR Ca2+ release channels [33]. In cells of the atria, ventricle and His-Purkinge system, the transient currents carried by T-type channels occur at the same time as the much larger Na currents, so that the former play little or no role in membrane depolarization [34].

Increase in NaK ATPase activity is expected to be associated with a corresponding increase in Na influx. Increase in activity of the Na channel may be responsible for the decrease in activity of the T-type Ca channel due to the overlap of the depolarization potentials at which the Na and the T-type Ca channels are active.

Increase in the force of contraction with decrease in [Mg]o may therefore be due to increased Ca2+ availability consequent to raised basal Ca2+ level accompanied by an increase in Ca2+ influx through voltage gated Ca2+ channels, which in turn induces SR Ca release.

Observation of variation in response to verapamil, ouabain and NiCl2 suggest that Mg status can affect the response to drugs. Calcium antagonists like verapamil are commonly used in the treatment of hypertension. T-type calcium channel blockers are also found to have a therapeutic application, in preventing electrical remodeling caused by atrial tachycardia [35]. Hypomagnesaemia precipitated ouabain-induced arrhythmia in dogs [36]. Magnesium acting as an indirect antagonist of digoxin at the sarcolemmal Na/K ATPase pump decreases cardiac arrhythmias in digoxin toxicity [37]. The interaction between Mg and some cardiovascular drugs indicates the importance of monitoring Mg status to prevent unfavorable reactions.

**Conclusion**

The observations of this study lead to the conclusion that positive inotropic response to decrease in [Mg]o is mediated by the additive action of increase in basal calcium and enhanced influx of initiator Ca2+ through the L-type Ca2+ channels associated with increase in Ca-induced Ca release from intracellular stores. Variation in sensitivity to Ca channel antagonists and NaK ATPase inhibitors in Mg deficiency have pharmacological implications.
Acknowledgements

The authors thank the Department of Science and Technology (India) for their financial support. The authors are grateful to the Director of Sree Chitra Tirunal Institute for Medical Sciences and Technology, India for the facilities and the permission to publish the paper.

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