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pH-dependent modulation of intracellular free magnesium ions with ion-selective electrodes in papillary muscle of guinea pig

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A change in pH can alter the intracellular concentration of electrolytes such as intracellular Ca^{2+} and Na^+ ($[Na^+]_i$) that are important for the cardiac function. For the determination of the role of pH in the cardiac magnesium homeostasis, the intracellular Mg^{2+} concentration ($[Mg^{2+}]_i$), membrane potential and contraction in the papillary muscle of guinea pigs using ion-selective electrodes changing extracellular pH ($[pH]_o$) or intracellular pH ($[pH]_i$) were measured in this study. A high CO_2-induced low $[pH]_o$ causes a significant increase in the $[Mg^{2+}]_i$ and $[Na^+]_i$, which was accompanied by a decrease in the membrane potential and twitch force. The high $[pH]_o$ had the opposite effect. These effects were reversible in both the beating and quiescent muscles. The low $[pH]_o$-induced increase in $[Mg^{2+}]_i$ occurred in the absence of $[Mg^{2+}]_o$. The $[Mg^{2+}]_i$ was increased by the low $[pH]_i$ induced by propionate. The $[Mg^{2+}]_i$ was increased by the low $[pH]_i$ induced by NH_4Cl-prepulse and decreased by the recovery of $[pH]_i$ induced by the removal of NH_4Cl. These results suggest that the pH can modulate $[Mg^{2+}]_i$ with a reverse relationship in heart, probably by affecting the intracellular Mg^{2+} homeostasis, but not by Mg^{2+} transport across the sarcolemma.

Key words: ion-selective electrodes, guinea pig, magnesium, papillary muscle

Although the biophysiological importance of magnesium has long been recognized [28] since it was first demonstrated to play an essential role in mammals 75 years ago [19], the mechanism for how magnesium is transported across the cell membrane and how the cells regulate its intracellular level is unclear. Nowadays, a magnesium deficiency is believed to be a major contributory factor to many diseases and the role of magnesium as a therapeutic agent has been tested in many large clinical trials [28]. The problem associated with the use of magnesium ion (Mg^{2+}) in a clinical setting is the fundamental lack of understanding of Mg^{2+} transport and homeostasis [27].

In the mammalian heart, there should be several processes that act in concert to maintain the intracellular Mg^{2+} concentration ($[Mg^{2+}]_i$) constant below 1 mM in the bulk cytosol because the total Mg^{2+} concentration has been reported to be up to 25 mM [23]. These processes include membrane transport systems and intracellular Mg^{2+} buffering systems including uptake/release from subcellular organelles [8].

Changes in intracellular pH ($[pH]_i$) have been shown to occur in many cell types under various pathophysiological conditions [17]. Therefore, changes in $[pH]_i$ may affect the cytosolic electrolyte concentrations including changes in the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) [12,24], Na^+ concentration ($[Na^+]_i$) [5,13] and possibly $[Mg^{2+}]_i$, which can cause various pathophysiological conditions.

An increase in the $[Mg^{2+}]_i$ evoked by intracellular acidosis has been reported in isolated chicken heart cells [9], rat cardiomyocytes [20] and cortical neurons [26], while no change was reported in ferret ventricular muscles [6] and a decrease in amnion cells [22] and leech Retzius neurons [11]. Although few studies at the cellular level have addressed the role of $[pH]_i$ in cardiac Mg^{2+} homeostasis, its role at the tissue level is unclear and particularly controversial.

Therefore, the aim of this study was to examine the role of pH in cardiac Mg^{2+} regulation by the simultaneous measurements of the $[Mg^{2+}]_i$, membrane potential (Em) and twitch force (TF) in papillary muscle of guinea pig using ion-selective electrodes changing the extracellular pH ($[pH]_o$) or $[pH]_i$.

Materials and Methods

General

The papillary muscles (2~3 mm long, ~0.5 mm diameter) were isolated from the right ventricle of guinea pigs (either

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gender weighing 250~350 g, Bio-Safety Research Institute of Chonbuk National University) according to the Guide for the Care and Use of Laboratory Animals (ILAR, USA). Mounted in a superfusion chamber, the muscle was stimulated by 1 Hz rectangular pulses with a 1 msec duration at 1.2 times the threshold voltage using an electronic stimulator (Narco Biosystem, USA). The isometric tension that developed in the preparation was measured using a force transducer (Cambridge Technology, USA) and recorded on a physiograph (Gould, USA).

Solutions

The muscle preparation was superfused continuously at 50 ± 2 ml/10 min with a Tyrode solution containing (in mM): NaCl 137, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 1.1, NaH_2PO_4 0.45, glucose 5, $NaHCO_3$ 11.9; the pH was adjusted to 7.4 ± 0.05 by aeration with 95 % O_2 and 5 % CO_2 and the temperature was maintained at 38 ± 0.5°C. The $[pH]_o$ of the Thyroid solution was altered by modifying the percentage of CO_2 in the air mixture. No $MgCl_2$ was added to the nominally Mg^{2+}-free solution. The contaminative magnesium concentration of the Mg^{2+}-free solution (nominally free of extracellular Mg^{2+}) was less than 0.9 nM using atomic absorption spectroscopy (Analab, Korea). The NaCl in the Tyrode solution was replaced with Na^+-propionate on an equimolar basis. For the experiments controlling the NH_4Cl concentration, an equimolar amount of choline chloride was added to the Thyroid solution.

Measurements of $[Na^+]_i$ or $[Mg^{2+}]_i$ in papillary muscles

Beveling, silanization and calibration of microelectrode was carried out with a slight modification of the methods reported elsewhere [3,6,16] Briefly, the microelectrodes were pulled from borocilicate glass capillaries (Richland, USA and World Precision, USA) using a vertical puller (David Kopf, USA). Conventional microelectrodes were used as the internal reference electrodes and the electrical resistance was 20~30 MW when filled with 3 M KCl. The tributylchlorosilane-silanized microelectrodes were backfilled with 100 mM NaCl for the Na^+-selective microelectrodes and 100 mM $MgCl_2$ for the Mg^{2+}-selective microelectrodes, and then beveled to increase the tip diameter to ~1 mm. The microelectrodes were filled with the Na^+ neutral carrier, ETH 227, and the Mg^{2+} neutral carrier, ETH 5214, respectively. Calibration was carried out with a fixed ionic background to mimic intracellular conditions and thereby to minimize effects of interfering ions (unorthodox calibration). The possibility of the interference to Mg^{2+}-selective electrodes by H^+, Na^+ was excluded by the use of ETH 5214 [3]. The Mg^{2+}-selective electrodes used in these experiments had 0.15 mM of detection limit and more than 52 mV of the slope between 10 and 0.1 mM $MgCl_2$. The Na^+-selective electrodes used in these experiments had more than 61 mV of the slope between 100 and 1 mM NaCl [16]. As the electrodes did not behave linearly in the physiological range, their calibration curves were fitted with the Nicolsky-Eiseman equation. Electrodes were only used when their slope in the linear range of the electrode was at least 90 % of the Nernstian slope. After every successful experiment, the electrodes were recalibrated. While the two calibration curves for Na^+ electrodes were usually in good agreement, most Mg^{2+} electrodes showed a considerable loss in sensitivity during an experiment. An experiment was used for quantitative evaluation when the values calculated from the two calibration curves did not differ by more than 0.4 mM [6].

The ion-selective and conventional electrodes were inserted into the beating papillary muscle within a 1 mm distance. The intracellular membrane potential (E_m) was recorded using a conventional electrode and the intracellular potential (E_{Na} or E_{Mg}) was recorded by each of the ion-selective electrodes. These electrodes were referenced to the potential of a reference electrode placed in a superfusing solution close to the impaling sites. The potential sensitivity to intracellular Na^+ (E_{Na}-E_m) and Mg^{2+} (E_{Mg}-E_m) was converted to $[Na^+]_i$ and $[Mg^{2+}]_i$, respectively, using an individual calibration curve for each electrode.

Drugs and statistics

The NaCl, KCl, $CaCl_2$, $MgCl_2$, NaH_2PO_4, glucose, $NaHCO_3$, choline chloride, Na^+-propionate and NH_4Cl were purchased from the Sigma-Aldrich (USA). The N-tributyl-chlorosilane, ETH 5214 (Magnesium ionophore II-cocktail A), ETH 227 (Sodium ionophore I-cocktail A), Mg^{2+} stock and Na^+ stock solutions were purchased from Fluka (Switzerland). All the chemicals were prepared as concentrated stock solutions and diluted with the Tyrode solution or a proper solvent. The final solvent concentration in experimental solutions did not exceed 0.1 %.

The results are presented as a mean ± SD. The data was analyzed using a Student's *t*-test and repeated measures analyses of variance, one-way ANOVA. A p value less than 0.05 was considered significant.

Results

Effects of $[pH]_o$ on $[Mg^{2+}]_i$, $[Na^+]_i$, membrane potential (E_m) and twitch force (TF)

In the papillary muscles, lowering the $[pH]_o$, which was achieved by increasing the partial pressure of CO_2, had a negative inotropic effect, and depolarization of Em. In addition, it evoked a significant increase in the $[Mg^{2+}]_i$ and $[Na^+]_i$ Figs. 1 and 2 show a typical experimental result. In 22 beating muscles, the extracellular acidosis from pH 7.4 to 6.4 induced depolarization of the Em by 5.5 ± 1.8 mV from the control value of 83.3 ± 2.6 mV, and decreased the TF by 19.0 ± 3.5% of control level over a 5 min period. It also led to a definite reversible increase in the $[Mg^{2+}]_i$ by 0.36 ± 0.03

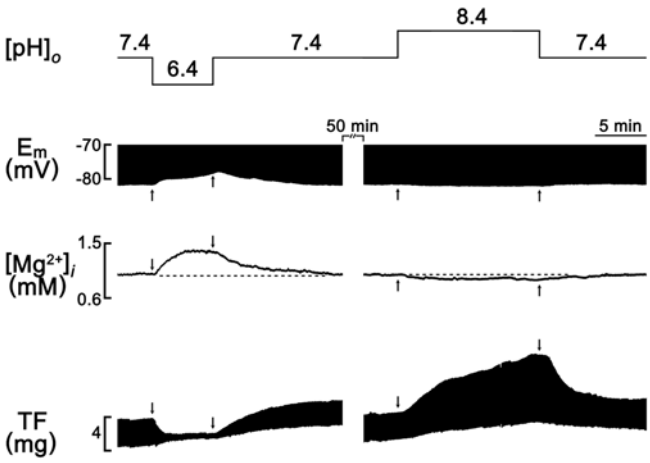

Fig. 1. Effects of changes in $[pH]_o$ on $[Mg^{2+}]_i$ in papillary muscle. Typical recordings of the membrane potential (E_m), intracellular Mg^{2+} concentration ($[Mg^{2+}]_i$) and twitch force (TF) during the change in extracellular pH ($[pH]_o$) in the beating state. Extracellular acidification was induced by regulating the CO_2/O_2 composition.

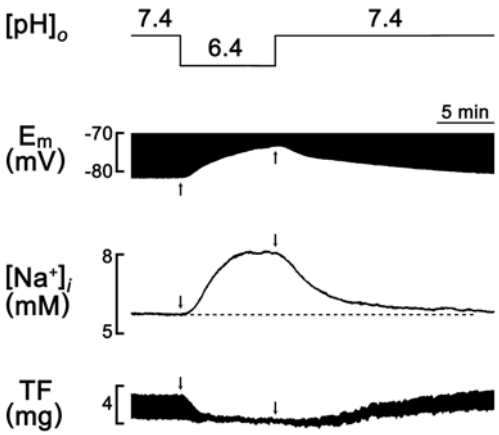

Fig. 2. Effects of low $[pH]_o$ on the $[Na^+]_i$. Typical recordings of the E_m, $[Na^+]_i$ and TF during a change in $[pH]_o$ in the beating state. Extracellular acidification was induced by regulating the CO_2/O_2 composition.

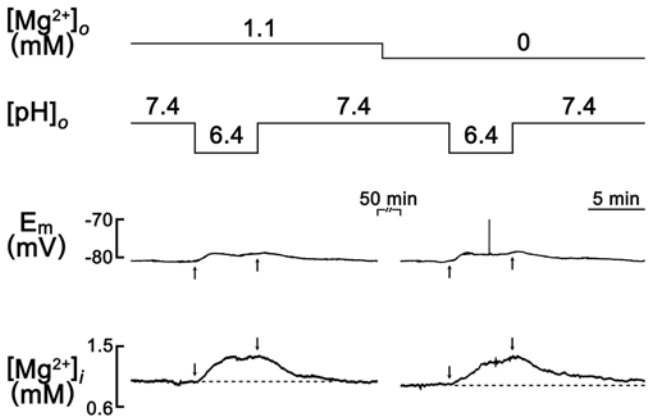

Fig. 3. Effects of $[Mg^{2+}]_o$ on low $[pH]_o$-induced increase in $[Mg^{2+}]_i$. Typical recordings of the E_m and $[Mg^{2+}]_i$ during a change in $[pH]_o$ in the absence and presence of $[Mg^{2+}]_o$ in a quiescent muscle. Extracellular acidification was induced by regulating the CO_2/O_2 composition. No $MgCl_2$ was added to the Mg^{2+}-free solution (nominally absence of extracellular Mg^{2+}).

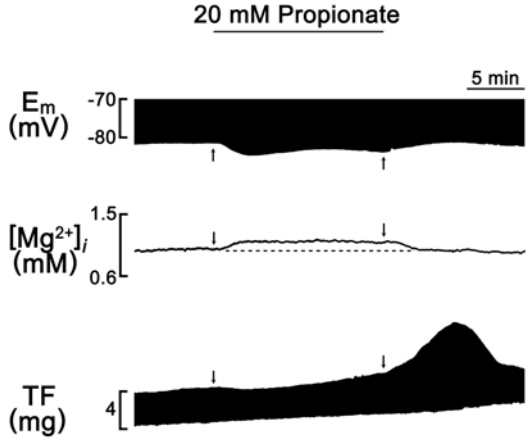

Fig. 4. Effects of propionate on the $[Mg^{2+}]_i$. Typical recordings of the E_m, $[Mg^{2+}]_i$ and TF during the application of 20 mM Na^+-propionate. All experiments were at constant $[pH]_o$ of 7.4. The NaCl in the Tyrode solution was replaced Na^+-propionate.

mM (n = 22) and an enormous increase in $[Na^+]_i$ by 2.36 ± 0.12 mM (n = 6). There were no significant differences between the beating and quiescent states in these results.

Increasing the $[pH]_o$ had opposite effects as illustrated in Fig. 1. During 10 min, the extracellular alkalosis from the pH 7.4 to 8.4 slightly decreased the $[Mg^{2+}]_i$ by 0.12 ± 0.03 mM (n = 12), hyperpolarized the Em by 1.3 ± 0.5 mV and increased the TF by 257 ± 22.4%.

Effect of extracellular Mg^{2+} concentration ($[Mg^{2+}]_o$) on the low $[pH]_o$-induced increase in $[Mg^{2+}]_i$

The $[pH]_o$ was altered in an absence of $[Mg^{2+}]_o$ in order to determine if the change in the $[Mg^{2+}]_i$ caused by the extracellular acidification was due to Mg^{2+} influx from the extracellular Mg^{2+}. As shown in Fig. 3, the low $[pH]_o$-induced increase in the $[Mg^{2+}]_i$ in the absence of $[Mg^{2+}]_o$ was similar to that in the presence of a $[Mg^{2+}]_o$. In addition, the low $[pH]_o$-induced increase in the $[Mg^{2+}]_i$ in 20 mM $[Mg^{2+}]_o$ was similar to that in 1.1 mM $[Mg^{2+}]_o$ (data not shown.).

Effects of propionate on $[Mg^{2+}]_i$, E_m and TF

In order to determine if a change in $[pH]_i$ can alter $[Mg^{2+}]_i$, the $[pH]_i$ was manipulated at a constant $[pH]_o$ and the subsequent effect on the $[Mg^{2+}]_i$ in the heart was examined. As shown in Fig. 4, 20 mM propionate, which elicited intracellular acidosis in the cardiac myocytes [6,18], caused a rapid increase in the $[Mg^{2+}]_i$ (0.16 ± 0.03 mM, n = 6) at a constant $[pH]_o$ of 7.4. After the muscle was superfused with the normal Thyroid solution, the $[Mg^{2+}]_i$ promptly recovered to the control level. There was a rapid hyperpolarization of

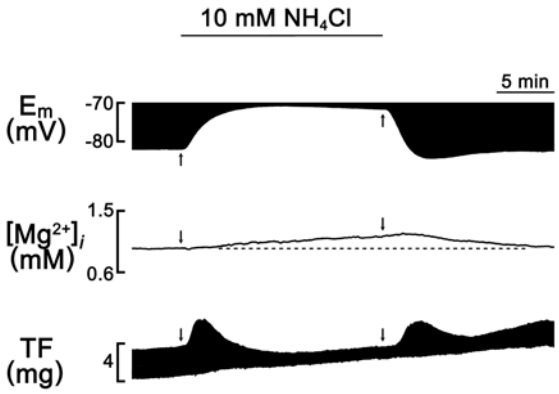

Fig. 5. Effects of NH_4Cl on $[Mg^{2+}]_i$. Typical recordings of the E_m, $[Mg^{2+}]_i$ and TF during the application of 10 mM NH_4Cl. All the experiments were at constant $[pH]_o$ of 7.4. For the control, the choline chloride was added to the Thyroid solution.

the E_m (2.8 ± 0.4 mV) followed by a slower partial recovery with the treatment with propionate. After withdrawing the propionate, the E_m rapidly depolarized (1.7 ± 0.3 mV) and slowly recovered. The TF was increased slightly by propionate but not significantly. After withdrawing the propionate, the TF was enforced twofold (196.8 ± 13.3% of control).

Effects of NH_4Cl on $[Mg^{2+}]_i$, E_m and TF

Perfusion with 20 mM NH_4Cl causes transient intracellular alkalinization, and the removal of NH_4Cl elicits transient intracellular acidification [9,17]. Fig. 5 shows the time course of the changes in the $[Mg^{2+}]_i$, E_m and TF evoked by NH_4Cl in the papillary muscle. During perfusion with 20 mM NH_4Cl, there were a sustained increase in $[Mg^{2+}]_i$ (0.23 ± 0.05 mM, n = 6), enormous depolarization (11.0 ± 1.0 mV) and a transient increase (146.8 ± 11.3% of control) in the TF following a significant sustained decrease (36.7 ± 5.4% of control). After removing the NH_4Cl, the $[Mg^{2+}]_i$ increased transiently and then recovered to the control level accompanied by a transient increase in the TF, which was below the control level.

Discussion

Magnesium is the 4th most common mineral salt in vertebrates after phosphorus, calcium, and potassium. Mg^{2+} is also the 2nd most common intracellular ion after K^+ and the 4th most common plasma ion after Na^+, K^+ and Ca^{2+} [21]. It is an essential cofactor in the activation of more than 320 enzyme systems in many organisms [21], involved in carbohydrates, lipid, proteins and the DNA metabolism, interacting either with the substrate or with the enzyme directly. Despite the abundance of Mg^{2+} within all cells and its importance in animal life, its general roles in the cellular function are not well understood for a variety of reasons [23].

It has been reported that submillimolar $[Mg^{2+}]_i$ significantly influence many intracellular processes in the cardiac muscles, including the adenylate cyclase activity [21], Na^+ pathways [6], K^+ pathways [1], excitation-contraction coupling [12], Ca^{2+} sensitivity of myofilaments [2] and Ca^{2+} binding to intracellular sites [14]. $[Mg^{2+}]_i$ is maintained in a relatively narrow concentration range to ensure proper functioning of the cells.

Intracellular pH is an important modulator of cardiac function, influencing processes as varied as contraction [4,13], excitation [30] and electrical arrhythmia [25]. Protons are produced metabolically within the heart, they are highly reactive with cellular proteins, and they must be removed if cardiac function is to be maintained. A sophisticated system for regulating pH_i has therefore evolved in heart cells. Steady-state pH_i is typically 7.1-7.3 [17]. It can decline modestly with an increase in heart rate [4,7] and, more dramatically, during myocardial ischemia [10]. Although few studies at the cellular level have addressed the role of $[pH]_i$ in cardiac Mg^{2+} homeostasis, its role at the tissue level is unclear and particularly controversial.

This study found that extracellular acidification led to a definite reversible increase in $[Mg^{2+}]_i$ in the papillary muscles from guinea pigs. The high $[pH]_o$ had opposite effect. The low $[pH]_i$-induced increase in $[Mg^{2+}]_i$ might be induced by an increase in Mg^{2+} influx or the modulation of the intracellular Mg^{2+} homeostasis. The extracellular acidification also caused an huge increase in $[Na^+]_i$ in the papillary muscles. This might result in a decrease in the driving force for the Na^+/Mg^{2+} exchange and cause the decrease in $[Mg^{2+}]_i$. An acidification-induced increase in $[Mg^{2+}]_i$ was also observed whilst exposing the muscles to a nominally Mg^{2+}-free bath solution. In addition, the low $[pH]_o$-induced increase in the $[Mg^{2+}]_i$ in 20 mM $[Mg^{2+}]_o$ was similar to that in 1.1 mM $[Mg^{2+}]_o$. These results suggest that acidification did not cause Mg^{2+} influx such as transport through Na^+/Mg^{2+} exchange but possibly a redistribution of the intracellularly bound Mg^{2+}. Such redistribution might involve the Mg^{2+} binding within the cytoplasm or the Mg^{2+} storing in organelles [8].

If the extracellular acidification directly caused Mg^{2+} influx, Mg^{2+}/H^+ exchange and/or Mg^{2+}/HCO^{3-} cotransport would be a strong candidate for involvement in the influx. If Mg^{2+}/H^+ exchange were present in the cardiac sarcolemma as in the epithelium [18], extracellular acidification would increase its driving force and cause a decrease rather than an increase in $[Mg^{2+}]_i$ until the $[pH]_i$ is reduced to electrochemical equivalent level of $[pH]_o$. However, the low $[pH]_o$ immediately caused an increase in $[Mg^{2+}]_i$. If the exchange worked as a H^+-exporting and Mg^{2+}-importing mechanism, it should have caused an increase in $[Mg^{2+}]_i$ during $[pH]_i$ recovery from pH 6.4 to 7.4 when reperfusing. However, a decrease in $[Mg^{2+}]_i$ was observed during $[pH]_i$ recovery. Therefore, Mg^{2+}/H^+ exchange plays no role in the acidification-induced

increase in $[Mg^{2+}]_i$. Nevertheless, the acidification-induced modulation in the $[Mg^{2+}]_i$ coupled with HCO^{3-} such as Mg^{2+}/HCO^{3-} cotransport in sarcolemma and mitochondrial membrane [29] and with H^+ such as Mg^{2+}/H^+ exchange in the mitochondrial membrane cannot be excluded [15].

It is well known that a high CO_2-induced extracellular acidification can produce a low $[pH]_i$ [4]. The decrease in $[pH]_i$ results from the intracellular hydration of CO_2 and the subsequent dissociation to produce H^+ and HCO^{3-}. Changing the $[pH]_o$ from 6.4 to 8.4 caused a linear change in $[pH]_i$ in same direction of 0.085 $[pH]_i$ units/$[pH]_o$ units in ferret ventricular muscle [3]. In the papillary muscle of guinea pigs, the $[pH]_o$ was reduced from 7.4 to 6.5, and $[pH]_i$ would be expected to decrease by only 20~40% of the $[pH]_o$ change [4]. Therefore, it is possible that the extracellular acidification increased the $[Mg^{2+}]_i$, which is dependent upon a decrease in $[pH]_i$.

In order to clarify this possibility, the changes in $[pH]_i$ while maintaining a constant $[pH]_o$ of 7.4 were examined. Intracellular acidification by Na^+-propionate results from the influx of uncharged propionate and the subsequent intracellular dissociation [17]. Intracellular acidification can occur even when the $[pH]_o$ is neutral, because only the concentration gradient of the neutral form is the driving force. In this study, the $[Mg^{2+}]_i$ in the papillary muscle was increased as a result of propionate exposure, which can cause a low $[pH]_i$ at neutral $[pH]_o$. The addition and removal of NH_4Cl can lead to large transient changes in the $[pH]_i$ [9,17]. There are four phases to this process: 1) phase 1; rapid alkalinization by addition, 2) phase 2; slow acidification, 3) phase 3; rapid acidification by removal and 4) phase 4; recovery of $[pH]_i$. This study found that the $[Mg^{2+}]_i$ was increased by the subsequent acidification (phase 2) by NH_4Cl in the papillary muscle, which was enforced by the rapid acidification (phase 3) and recovered to the control level by the recovery of $[pH]_i$ (phase 4). Because the $[Mg^{2+}]_i$ had not been affected by the intracellular alkalinization (phase 1), the size and time of alkalinization could not sufficiently alter the $[Mg^{2+}]_i$. Indeed, the effect of alkalinization on the $[Mg^{2+}]_i$ was lower than that of acidification. Therefore, changes in $[pH]_i$ can affect the $[Mg^{2+}]_i$ in the heart; acidification increases the $[Mg^{2+}]_i$ and alkalinization decreases the $[Mg^{2+}]_i$. Similar conclusions were also reported by Feudenrich *et al*. [9], who used Mag-fura 2 AM to determine the $[Mg^{2+}]_i$ of single chick cardiomyocytes, and by Li and Quamme [20], who examined isolated rat cardiomyocytes. Feudenrich *et al.* showed acidification (0.89 pH units) with the use of 10 mM NH_4Cl increases the $[Mg^{2+}]_i$ from the basal levels of 0.35 to 0.47 mM. Alkalinization of the heart cells from pH 7.11 to 8.32 modestly decreased the Mg^{2+} activity by 0.02 mM. Li and Quamme reported that the $[Mg^{2+}]_i$ within a single cell decreased by 129 ± 13 mM with rapid alkalinization from the basal levels of pH 7.1 to 7.6 following a NH_4^+ pulse. The removal of the NH_4Cl bathing solution caused cytosolic acidification, pH 6.9, and an increase in the $[Mg^{2+}]_i$, from 467 ± 47 to 569 ± 41 mM. Rajdev and Reynolds [26] induced intracellular acidification of 2.46 pH units by the withdrawal of 25 mM NH_4Cl and observed a mean increase in the $[Mg^{2+}]_i$ of 0.62 mM in the cortical neurons. Therefore, the pH can regulate the $[Mg^{2+}]_i$ with a reverse relationship at tissue level as well as at cell level in the heart.

In conclusion, pH could regulate the $[Mg^{2+}]_i$ in guinea pig heart; acidification increases the $[Mg^{2+}]_i$ and alkalinization decreases the $[Mg^{2+}]_i$. The modulation of $[Mg^{2+}]_i$ might be the result of a change in the intracellular Mg^{2+} buffering, but not by the transportation of Mg^{2+} across the sarcolemma.

Acknowledgments

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