Journal of Clinical and Basic Cardiology

An Independent International Scientific Journal

Journal of Clinical and Basic Cardiology 2005; 8 (1-4), 29-32

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Indexed in
Chemical Abstracts
EMBASE/Excerpta Medica

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Free Intracellular Magnesium Remains Uninfluenced by Changes of Extracellular Magnesium in Cardiac Guinea Pig Papillary Muscle

S. Gasser[1], N. Bareza[1], E. Scheer[1], E. Toferer[1], D. Pruthi[1], R. Gasser[1], U. E. Spichiger-Keller[2]

It has been argued that a rise in free, unbound, intracellular Magnesium (Mg^{2+}_i) could be beneficial during myocardial ischaemia, effecting the regulation of certain enzymes, the rectification of channels, intracellular Na^+ and Ca^{2+}, Mg^{2+} is a co-factor of the Na/K-ATP-ase, competes with Ca^{2+} at the contractile apparatus etc. However, all of these properties are confined to the intracellular site. Large trials, such as LIMIT 2, ISIS 4 and MAGIC have been conducted in order to prove a positive effect upon the outcome of patients treated with i. v. Mg during early myocardial ischaemia. In summary, the trials have failed to prove such an effect. The present study investigates the effect of extracellular Mg-elevation upon intracellular concentrations of Mg^{2+}. Here we studied Mg^{2+}_i with Mg^{2+}-selective microelectrode (ETH 7025) in isolated guinea pig papillary muscle. Resting Mg^{2+}_i in 7 guinea pig papillary muscles assessed with these microelectrodes amounted to 1.06 + 0.11 mM. In 7 papillaries, varying extracellular Mg^{2+} levels between 0 and 8 mM produced no significant change in measured free intracellular Mg^{2+}. Our results show that Mg^{2+}_i remains unaffected by changes of extracellular Mg. Hence, we conclude that favourable actions of intracellular Mg^{2+} will not be achieved by elevating serum Mg levels using Mg infusion. These data may partially explain the negative results of ISIS 4 and MAGIC.
J Clin Basic Cardiol 2005; 8: 29–32.

Key words: *magnesium, myocardial, ion-selective*

Intracellular Mg^{2+} (Mg^{2+}_i) plays an essential role in numerous intracellular processes. Cardioprotective actions of Mg^{2+} have been postulated and large clinical trials have investigated the therapeutic use of i. v. Mg during myocardial ischaemia with variable success [1–3]. As we have shown earlier, free intracellular Mg^{2+} rises during myocardial ischaemia [4]. This is likely to be a result of Mg^{2+} dissociating from the MgATP-complex during ATP-hydrolysis. ADP, on the other hand, binds Mg^{2+} less effectively, thus leading to a rise of free, unbound, ionised Mg^{2+} in the cytoplasm. It has, however, been shown that total intracellular Mg falls [5]. It has been argued that a rise in free, unbound, intracellular Magnesium could be beneficial during myocardial ischaemia, effecting the regulation of certain enzymes, the rectification of channels, intracellular Na^+ and Ca^{2+}, Mg^{2+} is a co-factor of the Na/K-ATP-ase, competes with Ca^{2+} at the contractile apparatus etc. However, all of these properties are confined to the intracellular site. Large trials, such as LIMIT 2, ISIS 4 and MAGIC have been conducted in order to prove a positive effect upon the outcome of patients treated with i. v. Mg during early myocardial ischaemia [1–3]. In summary, the trials have failed to prove such an effect. It has been assumed, that raising Mg^{2+} in the extracellular fluid must be beneficial in myocardial infarction. However, it has not been shown whether extracellularly added magnesium ultimately will get to the intracellular site within a reasonable amount of time in order to be effective. Hence, we measure intracellular Mg^{2+} during changes of extracellular magnesium concentrations in guinea pig papillary muscle using a ETH 7025 design of Mg-selective microelectrodes, which has been given to us as a present by Dr. Spichiger's group from Zürich [6].

Material and Methods

Preparations

Guinea pig and rat papillary muscles from the right ventricle (animals of approximately 350 grams of either sex) were used in our experiments. Animals were gently put asleep by cervical dislocation and exsanguination. The heart was quickly removed and washed in Tyrode's solution equilibrated with 100 % O_2. The right ventricle was opened near the interventricular septum and a suitable uniform papillary muscle chosen of a diameter of 1–2 mm and length of 3–5 mm was taken from it [7]. The base of the preparation was electrically isolated by a tight knot of surgical thread used also for pinning the preparation in the experimental chamber. The preparation was partially immobilised by stretching it lightly over a narrow bridge made of 4 fine micropins (100 µm diameter, see [8]). The free tendinous end of the papillary muscle was connected via a hook to a force transducer (based on an AKERS strain gauge). Papillary muscles were stretched to a length of which optimal contractile force was achieved. A conventional microelectrode was then inserted into a superficial cell in the region of the muscle above this narrow bridge. A Mg-selective microelectrode was impaled within a circle of 100 µm according to the design of the experiments. The perfusion chamber had a total volume of approximately 1.5 mm. For all experiments shown, n = 7.

Solutions

The preparations were continuously superfused with Tyrode's solution containing (in mM): NaCl 140, KCl 4.5, $CaCl_2$ 2.5, $MgCl_2$ 1.0, glucose 10, HEPES 20. Solutions were adjusted to a pH of 7.4 by titration with 4 M NaOH and equilibrated with 100 % O_2 at 37 °C. Solutions with 0 Mg were produced using 1 mM EDTA (in these solutions pH was readjusted after 1 hour of equilibration).

Microelectrodes

Conventional Microelectrodes

These microelectrodes were pulled from filamented borosilicate tubing, 1.5 mm outer diameter, 1 mm inner diameter and bevelled to a resistance of 4–6 MΩ (which equals an outer tip diameter of about l µm). Conventional microelectrodes, which were used to measure membrane potential, were invariably filled with 3 M KCl. Tip potentials devel-

Received and accepted: June 13th, 2005.
From the [1]WG of Experimental Cardiology, Department of Cardiology, University Department of Internal Medicine, Graz, Austria, and the [2]Centre for Chemical Sensors/Biosensors and Bio-Analytical Chemistry (CCS), ETH Swiss Federal Institute of Technology Zurich, Switzerland.
Correspondence to: Prof. Robert Gasser MD, PhD (Oxon), WG of Experimental Cardiology, Department of Cardiology, University Department of Internal Medicine, Auenbruggerplatz 15, A-8036 Graz, Austria; e-mail: robert.gasser@meduni-graz.at

oped rarely during such an experiment. If this was the case, electrodes were discarded and the experiment repeated.

Mg-Selective Microelectrodes

Electrodes were pulled from non-filamented borosilicate tubing, 1.5 mm outer diameter, 1 mm inner diameter with a conventional microelectrode puller. For technical details of making ion selective microelectrodes see earlier publications [7–9]. Briefly, electrodes were silanised using trimethyl-dimethyl-silyl-arnin (Fluka) after preheating (250 °C) and were then bevelled dry to an outer tip diameter of approximately l µm. The bevelled electrode was then filled retrogradely with a reference solution, consisting of (in mM): NaCl 140, KCl 4.5, HEPES 10 and MgCl 1, buffered to a pH of 7.4 with NaOH. The extracellular solution consisted of (in mM): NaCl 140, KCl 4.5, $CaCl_2$ 2.5, $MgCl_2$ 1.0, glucose 10, HEPES 20. Filling was made under pressure until one could see the filling solution escape from the tip. The Mg-selective liquid was sucked anterogradely into the tip, using a vacuum applied to a tubing sealed to the back end of the electrode. Short column electrodes were used to obtain low noise recordings [10]. For Mg measurements, we used ETH 7025 [6]. Electrodes were calibrated before and after the experiments and those which produced less than 28 mV between 0.4 mM and 4 mM Mg were discarded. For further details see Schaller et al. [6] as well as Buri et al. [11].

Results

Mg-Selective Microelectrodes

A general description of the Mg-selective microelectrodes used here as well as a detailed description of their fabrication, of microelectrode construction, silanisation, potentiometric measurements, selectivity coefficients, the use of PVC, testing for voltage uniformity and other membrane components has been given elsewhere [6–13] and shall therefore not be described here again. The V_{diff} with the logarithm of the Mg^{2+}-ion concentration of the typical calibration curves of the Mg^{2+}-selective ligands ETH 7025 microelectrode shows only in very fine tipped ion-selective microelectrodes a moderate super-Nernstian behaviour. We generally used tips that are not extremely fine but still easy to impale, as seen in Figure 1. The Mg^{2+}-selective properties of ETH 7025 in the presence of intracellular ion compositions is not satisfactory. The tip diameter of our electrodes lies within the submicron region (Fig. 1). Calibrations have been made before and after each experiment using intracellular fluid compositions and only those experiments have been used in which the electrode readings did not differ for more than 1 mV before and after the experiment. The experiment shown in Figure 2 was conducted in guinea pig papillary muscle. One can see that the ETH 7025 Mg^{2+}-selective microelectrodes give stable readings under experimental conditions over longer periods of time. In this experiment, stable signals have been obtained over more than three hours in a resting papillary muscle.

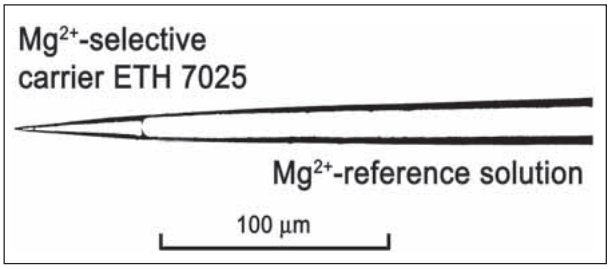

Figure 1: Mg^{2+}-ion-selective microelectrode photographed by electrone microscopy. The ion-selective ligand can be seen in the tip of the microelectrode. The liquid junction potential indicating the individual Mg^{2+}-concentration measured, arises at the surface between the reference solution and the ion-selective resin.

Free Intracellular Resting Mg^{2+}_{i}

The resting levels of intracellular ionised Mg^{2+}_{i} found in literature varies by a factor of 30 and largely depends on the method used. Hess, Metzger and Weingart [14] measure an Mg^{2+}_{i} of 3.0 mM in ferret ventricular muscle. We measured a free Mg^{2+}_{i} in guinea pig papillary muscle using ETH 7025 and Mg^{2+}_{i} was 0.73 ± 0.08 (n = 7; ± SEM) in the same type of tissue. These values are similar to those assessed earlier in sheep Purkinje fibre [4, 15, 16] and ferret papillary muscle [17]. The values measured for Mg^{2+}_{i} amount to approximately the same values as those recommended as "within the normal range" in the serum (0.7–1.0 mM) [18]. Calculating from the Nernst equation, the equilibrium potential would be around 0 mV, thus indicating equal distribution of Mg in both extracellular and intracellular space. For our experiments, it would be between +2 mV and +4 mV. Changes of pH do not affect ETH 7025 Mg-selective electrode readings at cytoplasmatic Mg-concentrations shown earlier [6]. In the present experiments we changed extracellular Mg from 1.0, 2.0, 5.0, 0.0 and 8.0 mM as can be seen in Figure 2. No effect upon intracellular levels of Mg^{2+}_{i} can be noted. Mg^{2+}_{i} seems to be an extremely well buffered intracellular ion, the transmembrane Mg-transport is known to act at an extremely low rate and the cell membrane itself is practically impermeable to magnesium ions. In seven papillary muscles we found the same result. There was no statistically significant difference between Mg^{2+}_{i} at different extracellular Mg concentrations.

Discussion

Theoretical Basis

Free intracellular ionised magnesium plays a decisive role in over 300 cellular biochemical reactions and effects ion-channel gating properties as well as transmembrane ion pumps. It also plays its part in the regulation and feed-back of cellular high energy metabolism. Approximately 5 % of the cells total intracellular Mg^{2+} is available in its chemically unbound ionised and thus biologically active form. There is experimental evidence that Mg-free diet leads within months to severe parenchymal necrosis invariably followed by death of the animal. Although we know much about the biochemical function of Mg^{2+}_{i} little or almost nothing is known about its

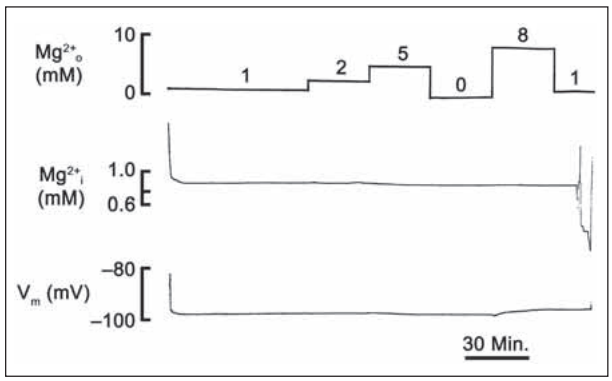

Figure 2: Changing extracellular concentrations of Mg^{2+} does not influence intracellular magnesium levels. One can see a typical experiment lasting approximately 3 hours. The signal stabilises some minutes after impalement. The electrode is equilibrated at the end of the experiment. Upon changing extracellullar Mg^{2+} a small depolarisation of the membrane potential can be seen without influencing the Mg^{2+}-subtraction potential.

regulation and membrane transport. There is even disagreement about its intracellular concentration and the values for Mg^{2+}_{i} found in the literature vary by a factor of 30 (0.2–6 mM). Magnesium has been used empirically in the treatment of various types of arrhythmias, heart failure and coronary syndromes. Since the myocardial regulation of Mg^{2+}_{i} is by no means well understood, the design of large studies on the therapeutic use of Magnesium like LIMIT 2, ISIS 4 and MAGIC hence suffered from an adequate theoretical basis and brought about conflicting evidence [1–3]. In order to study the mechanisms of cellular Mg^{2+}_{i} metabolism one needs to know its actual cytoplasmic concentration and find an adequate technique to study its concentration changes over time upon various maneuvres. Here we use ETH 7025 Mg-selective microelectrodes and test their usefulness in the assessment of myocardial Mg^{2+}_{i} in guinea pig and rat papillary muscle microelectrodes. In order to interpret our results we have to consider the validity of our experiments: ion-selective microelectrodes have been fabricated and successfully used in our laboratories for practically two decades. ETH 7025 suffers little from interference of other ions. ETH 7025 has offered significant improvement because the interference of sodium and potassium is negligible at the physiological concentrations of these ions. The electrode response between 4 mM and 0.4 mM Mg shows almost "Nernstian behaviour". The interference of calcium does not hamper intracellular measurements, since intracellular calcium concentrations (normal: $\leq 2 \times 10^{-7}$ M) seen in the cytoplasm usually do not reach values which could cause significant disturbances on the electrode. A change of Ca^{2+} from 0 (EGTA, 1 mM) to 5×10^{-6} M in the calibration solution, for example, produces a shift of approximately 1 mV in the electrode reading of 1 mM Mg with ETH 7025. Note that the highest physiologically occurring, intracellular steady state calcium concentrations never reach concentrations in the millimolar range [18–20]. Detailed accounts of the properties of the ligand and its potentiometric selectivity coefficient and ohmic properties has been given earlier. Mg-selective microelectrodes have been used successfully by us in sheep-Purkinje fibres and guinea pig papillary muscles before. Preparations, solutions and experimental assembly belong to established experimental techniques [6–17].

Intracellular Free Ionised Mg

A fundamental requirement for the investigation of the physiological role of Mg^{2+}_{i} is a method which allows for the assessment of its intracellular concentration. At this point already difficulties arise: each of the techniques so far employed in the quest for an accurate determination of Mg^{2+}_{i} in muscle cells harbours serious limitations. ETH 5214 has been developed 15 years ago [21] and has been used and tested by Frey et al. [20] and by Buri [11] in guinea pig hearts, who measured a free Mg^{2+}_{i} of slightly below 1 mM. Our own measurements in sheep Purkinje fibre amounted to 0.9 mM [4]. Here, using the same resin, we found free intracellular Mg^{2+} at 0.84 mM in guinea pig papillary muscle. These measurements compare well with the result of fluorometric measurements [22]. In the present paper we show measurements of free intracellular Mg^{2+} in guinea pig papillary muscle using the new Mg-selective neutral carrier ETH 7025 and find Mg^{2+}_{i} at slightly lower than a mean value (1.4 ± 0.2 mM) which we have calculated from 18 different publications using various techniques (references with the author). These results are in agreement with measurements of Gupta et al. who measured intracellular magnesium in guinea pig muscle with 31p-NMR at 0.8 mM [5]. Hu, using the same method, in rat found 0.5 mM [21]. Interestingly, values of intracellular magnesium of the same magnitude have already been calculated for rat myocardium by England et al. [23] using the Mg-dependence of citrate/isocitrate, whereas later studies using Mg-efflux and microelectrodes [14, 24] found values between 3 mM and 4 mM. The use of fluorescent dyes [22] showed results similar to ours.

Effect of Mg_o-Changes on Mg^{2+}_{i}

Figure 2 shows that changes of extracellular Mg_o up to 8 mM do not effect intracellular concentrations of ionised Mg. We do not know however, whether Mg entry into the cell is so little or access Mg^{2+}_{i} is actively transported out of the cell or bound in intracellular stores. It is a noteworthy observation that changing Mg_o between 0 and very high levels (much higher than those which can be achieved for in patients by acute Mg-infusion) has practically no effect on the intracellular concentrations of biologically active Mg^{2+}_{i}. From literature one can deduce that the beneficial mechanisms of Mg-treatment in myocardial infarction are to a large extent confined to the intracellular site: Mg^{2+}_{i} is an important cofactor in over 300 intracellular biochemical reactions, Mg^{2+}_{i} regulates rectification and gating properties of numerous ion channels, whereas extracellular Mg does not [25], intracellular regulates the activity of several ion pumps (e.g. Na/K-ATP-ase), it competes with Ca-ions for binding sites with troponin C, deactivates Ca-dependent proteases, decreases Ca-sensitivity of myofibrils, thus affecting contractility and oxygen consumption, intracellular ATP, regulates the activity of hexokinase in an ATP-dependent manner, affects cAMP, G-protein, SR-calcium ATP-ase etc. [26]. The finding that intracellular Mg remains unchanged over hours despite changing extracellular Mg raises the question if any of the intracelluar effects of Mg can be brought about by a simple Mg-infusion. This is a strong argument in favour of the negative results of ISIS 4 and MAGIC [2, 3].

In summary, intracellular magnesium homoeostasis is relatively well understood, transmembrane Mg-transport is not. However, from the present observations, one can deduce that Mg^{2+}_{i} is well buffered and remains relatively uninfluenced by extracellular maneuvres. Hence, it seems unlikely that intracellular action sites of magnesium can be effected by intravenous magnesium infusion.

This research project has been supported by a research grant of the Austrian National Bank (OENB Nr. 5563).

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