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Brief Reviews

Magnesium in Heart Muscle

By Philip I. Polimeni and Ernest Page

■ In physiological studies of the major inorganic constituents of heart muscle cells, magnesium (Mg) has until recently been the neglected stepchild. There are several reasons for this neglect. Chemical analysis for Mg was for many years difficult and tedious. The only available radioactive isotope of Mg, ^{28}Mg , is expensive and has a short half-life. Moreover, Mg acts on relatively inaccessible intracellular processes some of which have been identified and measured only in tissue fractions or in purified proteins extracted from cells; the intracellular processes in which Mg is implicated are multiple and themselves poorly understood. The ionized Mg concentration ($[\text{Mg}^{2+}]$) in the cardiac cell cannot at present be directly determined, and the rate at which Mg is transported into and out of myocardial cells is so much slower than the rates for potassium, sodium, calcium, and chloride that isotopic or electrophysiological measurements of ion transport in the usual *in vitro* preparations of heart muscle become insensitive or inconvenient.

Nevertheless, there are compelling reasons for reexamining the role of Mg in heart muscle at this time. First, recent experiments on skeletal muscle indicate that $[\text{Mg}^{2+}]$ may be a critical modulator of the tension with which the contractile apparatus of striated muscle responds to the prevailing ionized calcium concentration ($[\text{Ca}^{2+}]$) (1-5); at the same time, the Mg complex with adenosine triphosphate (MgATP) is the substrate for the enzymatic reactions that underlie the sliding filament mechanism for myofibrillar contraction and relaxation. Second, work with subcellular model systems indicates that Mg participates in many of the most vital oxidative, synthetic, and transport processes of the myocardial cell. Finally, advances in the microchemical and radioactive measurement of Mg as well as in the

diversity and suitability of available heart muscle preparations have greatly simplified the problems of working with this element.

CONTENT AND INTRACELLULAR CONCENTRATION OF MAGNESIUM

The high Mg content of mammalian ventricular muscle is an old observation (6). The Mg content of plasma and interstitial space is relatively small; most of the Mg is therefore inside myocardial cells. It has recently become possible to correct for extracellular Mg in rat ventricular muscle with increased accuracy, because more reliable measurements of extracellular volume can now be made (7). The cellular Mg content of rat ventricular muscle obtained with the help of such a measurement is 43.4 ± 0.4 mmoles/kg dry weight, a value that is in good agreement with previous estimates. The calculated intracellular Mg concentration is 17.3 ± 0.2 mmoles/kg cell water, a value that is roughly comparable to that of 16.1 mM for rat skeletal muscle (8).

INTRACELLULAR DISTRIBUTION

The calculated intracellular Mg concentration is a nominal value, which does not take into account the intracellular distribution of Mg among the various cellular subcompartments and organelles. Surprisingly little is known about this distribution in heart muscle; by contrast, a systematic attempt to determine the distribution of Mg among the various components of mammalian skeletal muscle was made by Hasselbach as early as 1957 (9). Until recently, attempts to quantify the distribution of Mg among intracellular organelles in heart muscle seemed unpromising, since the contributions of the various ultrastructural components to the volume and mass of the myocardial cell were not accessible to precise measurement. Newly developed techniques now make it possible to determine these contributions by quantitative measurements on tissue electron micrographs of heart muscle (10-12).

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Conditions are therefore favorable for a reexamination of the intracellular distribution of Mg. Such a reexamination ought ideally to be done in intact specimens of heart muscle, using physical or other relatively nondestructive techniques (13). Pending the perfection of such techniques, it has been necessary to make do with measurements on subcellular fractions isolated from a tissue homogenate. This technique has two drawbacks: first it is difficult to be sure that the distribution of Mg is not changed during the fractionation and second, if a cellular component is purified sufficiently to minimize cross-contamination with other components, the yield is usually low. The low yield leads to an underestimation of the amount of the component (and thus of the fraction of total Mg associated with that component) in the intact cell.

An analysis of subcellular fractions isolated by a conventional fractionation at 0°C from a homogenate of rat ventricular muscle indicates that less than 15% of the cellular Mg remains associated with the mitochondria or myofibrils (14). Of the remaining 85% a major fraction is presumably present as Mg complexes of the adenine nucleotides adenosine triphosphate (ATP), adenosine diphosphate (ADP), and adenosine monophosphate (AMP). (The nominal concentration of total adenine nucleotide in the rat ventricle can be calculated from the data of Williamson [15] to be about 10 mM, as compared with 17.3 mM for the nominal cellular Mg concentration.) The intracellular localization of Mg is thus intimately bound up with the intracellular localization of ATP and ADP. The total amounts and the nominal intracellular concentrations of adenine nucleotides in the hearts of different mammals have been repeatedly determined (15-18) and are similar; however, as for the case of Mg, reliable information about the intracellular distribution of adenine nucleotides is scarce. On a priori grounds it seems reasonable that most of the adenine nucleotide (and therefore much of the Mg) in myocardial cells should be associated with the myofibrils, whose total energy consumption greatly exceeds that of other cellular components. If this conclusion is accepted, it is necessary to inquire how the Mg-adenine nucleotide complexes are presented to the myofibrils. Are the complexes dispersed in an aqueous solution or gel throughout the spaces between the myofilaments or is there a more discrete localization? If there is a more discrete localization, it seems desirable to determine how it is related to the longitudinal myofibrillar

striation pattern and also to the critical distance for ATP supply (under aerobic conditions, the distance from the geometrical center of a unit of myofibrillar volume to the nearest mitochondrion).

In rat left ventricles about 12% of cellular Mg is firmly associated with the mitochondria (14), but even this fraction is probably inhomogeneous. Thus Bogucka and Wojtczak (19) have found that in liver mitochondria 50% of the Mg is in the compartment between the inner and the outer mitochondrial membranes, 41% is in the matrix, and 4% and 5%, respectively, are bound to the outer and the inner mitochondrial membranes.

About 2% of cellular Mg is firmly bound to the myofibril. This fraction will not exchange with labeled extracellular Mg and cannot be extracted from glycerinated heart muscle by a combination of chelating agents, KCl, and nonionic detergents (20). On the basis of experiments in skeletal muscle (21-23) it seems probable that this fraction represents Mg sequestered within the thin filament during the polymerization of globular actin to filamentous actin at the time the thin filament is assembled. The sequestered myofibrillar Mg is useful as an assay for the content of myofibrils in heart muscle under conditions where myofibrillar content changes (12, 20, 24).

IONIC ACTIVITY OF INTRACELLULAR MAGNESIUM

Even if the distribution of Mg within myocardial cells were known, a more difficult problem, that of the ionic activity of Mg in each cellular subcompartment, would remain. Within each subcompartment Mg may be expected to form complexes of different stability with ATP, ADP, AMP, inorganic phosphate, and other intracellular compounds as well as with various negatively charged sites on cellular macromolecular constituents. The stability constants for the complexes of Mg and Ca with adenine nucleotides (25) and the kinetic constants for the formation and decomposition of these complexes (26, 27) have been measured in aqueous systems of defined chemical composition, ionic strength, and pH. For the subcompartments of the myocardial cell the chemical composition, ionic strength, and pH as well as the concentration and chemical nature of macromolecular binding sites are at best speculative. Moreover, as pointed out by Botts et al. (28), the calculation of $[Mg^{2+}]$ in the presence of adenine nucleotides, ionized potassium, Ca^{2+} , and cellular binding sites is complex even when the properties of the binding sites are known or assumed. Since the quantities necessary for such

a computation are not accessible to exact measurement for any of the cellular subcompartments of the myocardial cell, a calculation of $[Mg^{2+}]$ for this cell based solely on physical-chemical principles seems premature. An alternative approach would be the insertion into myocardial cells and cellular subcompartments of ion-selective electrodes with a high selectivity for Mg^{2+} . Although ion-selective chloride and potassium electrodes small enough to be introduced into myocardial cells have recently been developed (29, 30), sufficiently small elec-

trodes which are selective for Mg^{2+} are not yet available.

In the absence of direct measurements, the magnitude of $[Mg^{2+}]$ must be inferred from indirect evidence. The indirect evidence is the dependence of various intracellular processes and enzymatic reactions on $[Mg^{2+}]$ in the presence of adenine nucleotides under conditions which resemble, more or less, those prevailing in situ. Table 1 is a partial list of the very large number of such intracellular processes and enzymatic reactions. It is

TABLE 1

Some Mg-Dependent Processes and Reactions in Heart Muscle

Process or chemical reaction	Experimental preparation	Comments
Force development by and relaxation of myofibrils in situ	Single, isolated skeletal muscle cells of crayfish (2, 3) or frog (5) perfused after partial removal of sarcolemma	When cytoplasmic $[MgATP]$ is progressively increased at constant cytoplasmic $[Ca^{2+}]$, tension (active state) rises to a maximum then decreases. Stepwise increments in cytoplasmic $[Ca^{2+}]$ from 10^{-8} to $3 \times 10^{-6}M$ (while $[MgATP]$ is held constant at a value appropriate for tension development) produce stepwise increments in tension (3). At $[Ca^{2+}]$ lower than the concentration required for maximum tension, an increase in $[Mg^{2+}]$ from 0.3 mM to 1 mM decreases the developed tension (5).
Superprecipitation, syneresis, ATP hydrolysis	Actomyosin gels or suspended myofibrils prepared from mammalian skeletal and heart muscle (model systems for the behavior of the contractile mechanism in suspension or solution) (1, 32, 33, 35, 37, 57-61)	Display bell-shaped dependence on $[MgATP]$ at a given $[Ca^{2+}]$ and are partially inhibited by raising $[Mg^{2+}]$ in a manner which parallels the behavior of tension (active state) in the intact cell.
Release of Ca from sarcotubules	Vesicles of fragmented sarcotubular membrane from frog and rabbit skeletal muscle (62)	Raising $[Mg^{2+}]$ from 0.2 mM to 4 mM slows rate and reduces magnitude of Ca release from sarcotubules.
Binding and release of Ca by sarcotubules	Vesicles of fragmented sarcotubules from dog heart (ventricles) (63)	Raising $[Mg^{2+}]$ from 1 mM to higher values increases binding of Ca to sarcotubules but inhibits restimulation of the sarcotubular Ca binding-release cycle.
Na^{+} - and K^{+} -stimulated ATP hydrolysis by sarcolemma (presumptive component of Na-K pump)	Suspensions of sarcolemmal fragments from guinea pig ventricles (64, 65)	Raising $[Mg^{2+}]$ from 0.5 mM to 1.5 mM markedly stimulates activity; higher $[Mg^{2+}]$ is inhibitory.
Mitochondrial ATPase related to oxidative phosphorylation	Mitochondrial suspensions isolated from guinea pig and beef ventricles (66)	Mg^{2+} is required for and $[Mg^{2+}]$ affects rate of oxidative phosphorylation.
Phosphorylase kinase activity	Purified enzyme from rabbit skeletal muscle (67)	Mg^{2+} is required for activity and therefore for regulation of glycogen breakdown (apparent $K_a = 0.6$ mM Mg^{2+}).
Adenyl cyclase activity	Fragmented membrane particles (presumably predominantly sarcolemma) prepared from pig and rabbit ventricles (68)	Mg^{2+} binds to sites on the enzymes thereby increasing the velocity of the reaction in which adenyl cyclase catalyzes the conversion of its substrate, MgATP, to adenosine 3', 5'-monophosphate (cyclic AMP).

probably significant for the cellular physiology of heart muscle that many of these processes and reactions proceed rapidly when $[Mg^{2+}]$ is in the range of 1 mM or less.

INTRACELLULAR FUNCTIONS OF MAGNESIUM

The relative importance of the reactions and processes listed in Table 1 depends on one's point of view. Within the scope of this brief review we chose to stress the dependence of myofibrillar contraction and relaxation on $[MgATP]$ and $[Mg^{2+}]$ and the dependence of the uptake and the release of Ca from the sarcotubules on $[Mg^{2+}]$. We made this choice because these dependences illustrate how fluctuations in the prevailing concentrations of Mg-adenine nucleotide complexes and Mg^{2+} within fairly narrow limits can profoundly affect the contractile performance of the muscle cell.

The interior of otherwise intact single skeletal muscle cells can be perfused with test solutions after part of the sarcolemma has been dissected away ("skinning" the cell) (31). This experimental system has been used to examine the interrelated effects of the concentrations of MgATP, Mg^{2+} , and Ca^{2+} to which the myofibrils are exposed, on the tension produced by the myofibrillar contractile apparatus (2, 3, 5). In skinned skeletal muscle cells of the crayfish, Reuben et al. (2) showed that the tension increased to a maximum as $[MgATP]$ was raised. When $[MgATP]$ was raised further, the tension progressively decreased. Such a bell-shaped dependence of tension on $[MgATP]$ could be demonstrated even when $[Ca^{2+}]$ was much lower (10^{-8} - $10^{-10}M$) than the range (10^{-7} to $10^{-5}M$) thought to prevail in the cytoplasmic environment of the myofibril under physiological conditions. The interactions of MgATP with myofibrillar sites which lead to development of tension at lower $[MgATP]$ and to relaxation at higher $[MgATP]$ are therefore not directly localized to troponin, the Ca-binding protein at which the cytoplasmic $[Ca^{2+}]$ exerts its well-known control on myofibrillar tension development. Instead, MgATP reacts with the globular portions of the myosin molecule that make up the cross-bridges between the myosin and actin of the thick and thin filaments. Myosin (or actomyosin) is an enzyme which catalyzes the hydrolysis of its substrate MgATP. Thus, the bell-shaped dependence of tension on $[MgATP]$ suggests that the relaxation which occurs at high $[MgATP]$ corresponds to the well-known phenomenon of substrate inhibition, the inhibition of an enzyme by an excess of its substrate.

Although MgATP can elicit tension development, hydrolysis of MgATP, and relaxation in the virtual absence of Ca^{2+} , the physiological events of contraction and relaxation take place in the presence of a finite $[Ca^{2+}]$. The normal, critical range for cytoplasmic $[Ca^{2+}]$ is $10^{-7}M$ to $10^{-5}M$. The dependence of tension on $[MgATP]$ in skinned crayfish skeletal muscle cells was therefore re-examined at progressively higher $[Ca^{2+}]$ over the range of 10^{-7} to $10^{-5}M$. In this way it was found that the peak of the bell-shaped plot of tension vs. $[MgATP]$ was shifted to the right by an increase in $[Ca^{2+}]$; moreover, the maximum tension developed for a given $[MgATP]$ approximately doubled as $[Ca^{2+}]$ was raised from $10^{-8}M$ to $3 \times 10^{-6}M$ (3). Within this range of $[Ca^{2+}]$, raising $[Ca^{2+}]$ causes binding of Ca to troponin with stimulation of myofibrillar ATP hydrolysis, and lowering $[Ca^{2+}]$ causes release of Ca from troponin with inhibition of myofibrillar ATP hydrolysis. In vivo, $[MgATP]$ in muscle cells is high while $[Ca^{2+}]$ is low. In the resting, unstimulated or diastolic state the muscle is therefore relaxed. The Ca released by the sarcotubules into the cytoplasm in response to an action potential brings into play the control mechanism which is turned on by the binding of Ca to troponin. This control mechanism suppresses the inhibition of the contractile event by excess MgATP. Under these conditions raising cytoplasmic $[Ca^{2+}]$ over the physiological range ($10^{-7}M$ to $10^{-5}M$) shifts the MgATP concentration required for maximum tensions from about 1 mM to about 10 mM. Analogous observations have been reported with less intact assay systems including the superprecipitation of actomyosin gels (32, 33) and the contraction of glycerinated muscle (34).

Is the response of the myofibrillar contractile mechanism to MgATP and its modulation by the Ca-troponin system dependent on $[Mg^{2+}]$? Observations by Kerrick and Donaldson (5) in skinned frog skeletal muscle cells suggest such a dependence. They reported a striking decrease in isometrically developed tension when $[Mg^{2+}]$ was raised from 0.3 mM to 2 mM; a similar effect could not be produced by raising the concentrations of either MgATP or free ATP. The experiments were carried out in a range of $[Ca^{2+}]$ below that which elicits maximum developed tension. The adenosinetriphosphatase (ATPase) activity of a purified myofibrillar suspension prepared from rabbit skeletal muscle also shifts its dependence on $[Ca^{2+}]$ when $[Mg^{2+}]$ is increased (35).

The site or sites on the myofibril to which Mg binds in exerting this effect have not been identified with certainty. Dreizen and Richards (36) have recently reported that one or more of the light chains associated with the globular heads of the myosin molecule bind Mg, presumably to acidic or imidazole residues. The relation of this binding to the ATPase activity of myosin is still unclear. At 6°C the rate-limiting step for the hydrolysis of ATP by myosin is the dissociation of ADP from the active sites (37–40); at 25°C a different rate-limiting process, as yet unidentified, may become important (41). The rate-limiting process at 6°C is sensitive to Mg^{2+} . Divalent cations increase the affinity of ADP for the enzymatically active portion of the myosin molecule; the affinity constants diminish in the order $Mn > Mg > Zn > Ni > Co > Ca \approx Sr$ (42). It has been suggested (41) that Mg serves as a bridge linking the hydrolytically active portion of the myosin molecule and the nucleotide phosphates (ATP or ADP) together in a ternary complex (enzyme-metal-substrate).

In addition to these direct effects of $[Mg^{2+}]$ on the myofibril, there is some evidence that $[Mg^{2+}]$ affects the release of Ca into the cytoplasm from storage sites in the sarcotubules. Ford and Podolsky (4) examined this effect of $[Mg^{2+}]$ in skinned frog skeletal muscle cells. In these cells the release of Ca from the sarcotubules is regenerative, i.e., the release of Ca is itself triggered by an increase in $[Ca^{2+}]$ within the myofilament space which bathes the cytoplasmic or outer face of the sarcotubular membrane. This Ca-stimulated release of Ca from the sarcotubules is a process which propagates throughout the muscle cell, presumably along the sarcotubular system. When Ford and Podolsky (4) raised $[Mg^{2+}]$ in the myofilament space from 0.02 mM to 1.4 mM, they observed an inhibition of the quick contraction usually elicited by perfusion of the skinned fiber with Ca^{2+} . They concluded that the Ca-stimulated release of Ca from sarcotubular Ca stores could take place in the presence of a high $[Mg^{2+}]$ but that the propagation of the sarcotubular Ca-release process throughout the cell was inhibited by the high $[Mg^{2+}]$. In addition, Ford and Podolsky (4) speculated that an increased $[Mg^{2+}]$ may affect the rate at which Ca is taken up by the sarcotubules.

MAGNESIUM TRANSPORT IN HEART MUSCLE

Observations on skinned skeletal muscle cells and other experimental models indicate that small changes in $[Mg^{2+}]$ and $[MgATP]$ within the cell

can directly affect the functioning of the myofibrils and the sarcotubules. Since important physiological processes depend on cellular $[Mg^{2+}]$ and $[MgATP]$, it is logical to investigate whether there exist mechanisms by which the heart muscle cell can regulate its content of Mg and perhaps thereby set and maintain $[Mg^{2+}]$ and $[MgATP]$ at physiologically appropriate values. Two such mechanisms have been described to date, one localized in the mitochondria, the other in the plasma membrane. The mitochondrial mechanism has been extensively studied in suspensions of mitochondria isolated from heart muscle by Brierley and co-workers (43, 44). Under in vitro conditions this mechanism can cause the accumulation of large amounts of Mg by transporting it from the suspending medium across the inner mitochondrial membrane into the mitochondrial matrix. This ability of the mitochondria to accumulate Mg could be useful to the heart muscle cell in regulating $[Mg^{2+}]$ in the cytoplasm. It must be emphasized, however, that there is to date no evidence about whether and to what extent this mechanism is used in vivo. The only information about mitochondrial Mg content of heart muscle in vivo is that in rat ventricles all of the mitochondrial Mg appears to exchange with isotopic Mg administered intraperitoneally to the animal (45).

The plasma membrane of rat left ventricular myocardial cells is the locus of a transport mechanism by which about 98% of cellular Mg exchanges at a very slow rate (45). This slow exchange takes place at a single rate both in vivo and in vitro. At 37°C and at the near-physiological extracellular Mg concentration of 0.56 mM, the rate is 0.15 ± 0.02 mmole Mg exchanged/min kg^{-1} dry left ventricle or about 0.21 ± 0.02 picomoles exchanged/sec cm^{-2} of plasma membrane. To within the range and the precision of the available measurements, the rate of exchange is independent of the frequency of contraction and the external work done by the ventricle (14). Measurements of the unidirectional influx and efflux of Mg demonstrate saturation kinetics and the phenomenon of countertransport described by Wilbrandt and Rosenberg (46); these experiments therefore suggest a carrier-mediated transport of Mg by the plasma membrane, a conclusion reinforced by the very low passive permeability of myocardial cells to Mg. Assuming that the mean value of $[Mg^{2+}]$ for the cytoplasm is 1 mM or less, the electrochemical gradient would favor the diffusion of Mg into the cell. A carrier-mediated transport of Mg out of the

cell would thus be capable of preventing a physiologically inappropriate rise in $[Mg^{2+}]$ within the cell. The data available so far do not permit conclusions about the relation of the Mg carrier to the carrier and noncarrier transport mechanisms for Ca, sodium, and potassium.

ACCUMULATION OR LOSS OF MAGNESIUM BY MYOCARDIAL CELLS

In vivo, myocardial cells accumulate Mg in response to stimuli which cause the cells to grow or hypertrophy (20). Two examples are the growth induced in rat left ventricular myocardial cells by constriction of the ascending aorta and the growth response to thyroxin in immature animals whose growth has previously been arrested by thyroidectomy. In both cases the total left ventricular Mg content increases markedly and in proportion to the increase in ventricular dry mass. This response can be demonstrated within 24 hours after aortic constriction. The mechanism remains to be worked out, but the uptake is significant as an example of the coupling of transport of an ion to a growth stimulus. In this connection it is of interest that the time course of the Mg accumulation by myocardial cells in response to aortic constriction is similar to the time course of de novo adenine nucleotide synthesis (47).

A net loss of Mg by myocardial cells can occur under many pathophysiological conditions. In evaluating a reduced total Mg content in a sample of heart muscle it is necessary to distinguish between a deletion of groups of myocardial cells because of focal processes (as described, for example, by Lehr [48]) and a more-or-less uniform loss of Mg from all the cells in the sample because of a process affecting all cells. One such diffuse process, which has been studied in some detail in isolated rat ventricles (14), is the well-known net loss of Mg associated with oxygen deficiency (49-53). In perfused left ventricles contracting under hypoxic conditions the unidirectional Mg influx decreased and the calculated unidirectional Mg efflux increased.

MAGNESIUM AND CARDIAC CELLULAR ELECTROPHYSIOLOGY

Different phases of the cardiac action potential are characterized by selective increases in ionic permeability for sodium, potassium, Ca, and chloride with the activation of ionic currents which are more-or-less specific for a particular ion (54). Conditions under which the cell membrane of heart muscle preparations becomes selectively permeable to Mg have not been described, but the question

has not yet been exhaustively investigated. In particular, the question of Mg permeability in heart muscle needs to be reexamined by voltage clamp techniques while the pH and the Ca concentration are systematically varied. The experimental design should take into account the principles governing selectivity for cations (including Mg) as described by Eisenman (55). In this connection, the study of squid giant axons provides a precedent for a selective increase in Mg permeability during electrical activity. In this tissue there is an increased entry of Mg when the nerve is repetitively stimulated (56); the increased entry is inhibited by manganese ion, which is known to interfere with the tetrodotoxin-insensitive (late) channel for Ca ions.

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