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COMMENTARY

MAGNESIUM IONS IN CARDIAC FUNCTION

REGULATOR OF ION CHANNELS AND SECOND MESSENGERS

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Over the last decades our understanding of the role of cations in cardiac physiology has grown dramatically. The major discoveries have centered on Ca^{2+}, K^+, and Na^+, but relatively little attention has been paid to the importance of Mg^{2+}. The importance of Mg^{2+} as a metabolic cofactor, particularly in reactions involving transfer of phosphate groups, has long been recognized but, until recently, possible regulatory roles of Mg^{2+} have been neglected. This is due, at least partly, to the fact that techniques for studying Mg^{2+} have lagged behind those for other ions. For example, ^{28}Mg has a very short half-life (21 hr) and has not been readily available. Mg^{2+}-selective electrodes lack selectivity and sensitivity. Furthermore, because older estimates placed the concentration of intracellular free Mg^{2+} ($[Mg^{2+}]_i$) in the range of 3–10 mM, it seemed that Mg^{2+}-dependent systems would normally be saturated by Mg^{2+} and not subject to regulation by this ion. However, as techniques for the study of Mg^{2+} have been refined, it has been realized that $[Mg^{2+}]_i$ is lower than previously suspected (very likely in the submillimolar range), and that several systems have K_m values for Mg^{2+} within this range. This opens the possibility that $[Mg^{2+}]_i$ may vary physiologically and might be a physiological modulator. This suggestion has been strengthened by the discovery of specific Mg^{2+} transport systems in cells, several of which are regulated hormonally. Because Mg^{2+} is an essential component in the coupling of cell surface receptors (e.g. β-adrenoceptors) to G-proteins, second messenger systems are attractive candidates for sites of Mg^{2+} action. Furthermore, recent advances in cardiac electrophysiology, particularly in the development and refinement of the patch clamp technique (which makes it possible to perfuse cells internally with different solutions), have uncovered interesting effects of physiological concentrations of Mg^{2+} on the function of both K^+ and Ca^{2+} channels in the cardiac sarcolemma. Given the importance of these ion channels in the initiation and maintenance of the heart beat, these results suggest an important regulatory role for intracellular Mg^{2+}. Finally, there is considerable evidence relating changes in magnesium concentration to alterations in normal cardiac physiology or pathology at the clinical level [1–5].

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In this commentary, we will discuss the more recent developments concerning the role of intracellular Mg^{2+} in cardiac cells. Given the effects of Mg^{2+} on the biochemistry and electrophysiology of cardiac cells and its rather unique qualities compared to other ionic species, we propose that $[Mg^{2+}]_i$ is regulated in cardiac cells and functions as a long-term regulator of cardiac physiology. For more extensive treatment of the general role of magnesium in cell processes, the reader is referred to excellent reviews by Flatman [6] and Gupta *et al.* [7].

Concentration of internal free Mg^{2+}

Mg^{2+} is the second or third most prevalent ionic species of the intracellular milieu and is the major divalent ion present in the cytoplasm. Total magnesium concentration in cardiac muscle has been estimated to be 17.3 mmol/kg cell water [8]. The concentration of $[Mg^{2+}]_i$, however, remains to be agreed upon. This may be due to physiological variations in $[Mg^{2+}]_i$ or may reflect technical or species differences. Recent estimates range from 0.2 to 6.0 mM (Table 1). Various techniques have been employed in the quest for an accurate determination of $[Mg^{2+}]_i$ in muscle cells, but each of these techniques has rather serious limitations. Mg^{2+}-sensitive dyes have been used by Baylor *et al.* [9], who reported $[Mg^{2+}]_i$ ranges from 0.2 to 6.0 mM, depending on the particular dye employed and the pH. This variability was attributed to the tendency of these dyes to bind to various cellular components. This binding alters reaction stoichiometry, absorbance spectra, dissociation constants, and, possibly, the velocity of Mg^{2+}-dye reactions. Gupta's laboratory has made significant contributions to estimates of $[Mg^{2+}]_i$ using NMR. The ^{31}P-NMR technique for measuring $[Mg^{2+}]_i$ is based upon a measurement of the frequency difference between αP and βP resonances in the ^{31}P-NMR spectrum of intracellular ATP [7]. These resonances depend on the extent of complexation between Mg^{2+} and ATP. Therefore, accurate determinations of $[Mg^{2+}]_i$ depend upon selection of the appropriate stability constant for MgATP, which has been a matter of some discussion [7]. Furthermore, variations in intracellular ion concentrations or pH that are not recognized would influence the determination. Accurate estimates of $[Mg^{2+}]_i$ with Mg^{2+}-sensitive microelectrodes are hampered by a lack of selectivity of the currently

Table 1. Determinations of intracellular $[Mg^{2+}]$

$[Mg^{2+}]_i$	Tissue	Method	Ref.
2.0–3.0 mM	Squid axon	$^{28}Mg^{2+}$	[36]
0.5–6.0 mM	Frog skeletal muscle	Metallochromic dyes	[9]
0.8–1.6 mM	Frog skeletal muscle	Mg^{2+}-sensitive electrode	[11]
0.5–0.6 mM	Skeletal muscle	NMR	[13]
0.2 mM	Frog skeletal muscle	Electron probe	[14]
3.0–3.5 mM	Mammalian ventricle	Mg^{2+}-sensitive electrode	[107]
0.4 mM	Mammalian ventricle	Mg^{2+}-sensitive electrode	[12]
2.4 mM	Mammalian ventricle	Mg^{2+}-sensitive electrode	[15]

available ion exchangers. The best available ion exchanger, ETH-1117, has only a 25-fold greater sensitivity for Mg^{2+} over K^+ and only a 12-fold greater sensitivity for Mg^{2+} over Na^+ [10]. Because intracellular K^+ may be twenty to fifty times higher than Mg^{2+}, significant errors can be introduced. Recent studies which have corrected for interference by Na^+ and K^+ have estimated $[Mg^{2+}]_i$ to lie between 0.4 and 1.6 mM in striated muscle (Table 1, [11, 12]). These determinations agree well with the values of 0.5 to 0.6 mM reported from NMR studies of skeletal and cardiac muscle by Gupta and Moore [13] and Gupta *et al.* [7].

Recently, Maughan [14] estimated total diffusible magnesium in frog skeletal muscle to be 5.2 mM by electron probe microanalysis. It is calculated that most of the diffusable magnesium is bound to ATP, creatine phosphate, and parvalbumin and that only 0.2 mM is free ionized Mg^{2+}.

Some of this variability could be physiologically important. $[Mg^{2+}]_i$ increases when muscle cells are incubated in elevated $[Mg^{2+}]_o$ or decreased $[Na^+]_o$ [11, 15]. Thus, the large range of values that have been reported for $[Mg^{2+}]_i$ could reflect differences in the incubation conditions among other factors.

Magnesium permeability and transport

Under physiological conditions (1.0 mM $[Mg^{2+}]_i$, 1.8 mM $[Mg^{2+}]_o$), E_{Mg} = 7.6 mV. Therefore, Mg^{2+} is not in thermodynamic equilibrium across the sarcolemma at a resting membrane potential of −80 mV. Thus, either Mg^{2+} influx must be negligibly slow or muscle cells must possess a means of extruding Mg^{2+} across the sarcolemma. There is good evidence, however, that Mg^{2+} enters cardiac cells at a slow, but significant rate. Page and Polimeni [8] determined the time course of accumulation and efflux of $^{28}Mg^{2+}$ in rat myocardium after i.v. injection. The exchangeable fraction of Mg^{2+} was 98% of the total cellular Mg^{2+}, and the rate of exchange between plasma $^{28}Mg^{2+}$ and cellular $^{28}Mg^{2+}$ had a $T_{\frac{1}{2}}$ of 182 min and a flux of 0.21 pmol/(cm^2·sec), which we estimate to be approximately 1.2×10^3 molecules/sec/μm^2. Thus, the magnitude of Mg^{2+} flux is slightly smaller than the 0.295 pmol/(cm^2·sec) reported for Ca^{2+} in guinea pig atria [16].

Magnesium influx. Although it is clear that cardiac muscle cells are permeable to Mg^{2+}, it is not known by what pathways magnesium ions enter cardiac cells. The available evidence suggests that Mg^{2+} does not enter via voltage-gated channels. Studies using whole-cell or single channel recordings have failed to detect inward Mg^{2+} current through Ca^{2+} channels in several preparations of cardiac tissue ([17–19]; Hartzell and White, unpublished results). In contrast, Kohlhardt *et al.* [20] suggested that Mg^{2+} could pass through the slow inward channel in cat papillary muscle because the slow inward current was increased in the presence of Mg^{2+}. This current was blocked by D600, a calcium channel antagonist. This result is in distinct contrast to most studies showing that external Mg^{2+} blocks Ca^{2+} channels. The reason for this discrepancy could be due to species differences but is more likely due to an indirect effect of Mg^{2+} on some other ionic current contaminating I_{Ca}, because other currents were not blocked in these old studies.

Mg^{2+}, however, may permeate voltage-gated Ca^{2+} channels in other cell types. For example, Mg^{2+} can carry current through Ca^{2+} channels in frog skeletal muscle [21], and Mg^{2+} is about 25% as permeable as Ca^{2+} through the light-sensitive channel in photoreceptors [22]. Interestingly, it has been reported that $[Mg^{2+}]_i$ in rod photoreceptors is regulated by light [23].

Spah and Fleckenstein [24] described a Mg^{2+}-dependent action potential which they proposed was due to the activity of a Mg^{2+}-selective channel. In this study, however, the $[Na^+]_o$ was 140 mM, and the "Mg^{2+} current" was inhibited by tetrodotoxin or lidocaine, agents which block Na^+ channels. Therefore, these findings probably reflect stimulation of I_{Na} by high $[Mg^{2+}]_o$ [2]. This explanation is supported by the finding that raising $[Mg^{2+}]_o$ increases I_{Na} in frog atria, and that no inward current occurs in the absence of Na^+ and Ca^{2+} [18]. Likewise, Fukushima and Hagiwara [25] found that a small inward current exists in mouse lymphocytes under conditions of high $[Mg^{2+}]_o$ and low $[Ca^{2+}]_o$; however, this small current disappears entirely when external Na^+ is replaced with tetraethylammonium (TEA).

The inability of Mg^{2+} to permeate slow Ca^{2+} channels in cardiac muscle has been attributed to the slow rate of interaction between Mg^{2+} and the ion channel [26]. The Ca^{2+} channel is believed to be a single-file pore containing at least two ion binding sites [27, 28], and the permeability of an ion depends upon its affinity for these sites. According to this model, an ion which possesses high affinity for channel binding sites (e.g. Cd^{2+}, La^{3+}) will have the tendency to block the channel and, thus, will be poorly permeant. Conversely, an ion with low binding affinity (e.g.

Li^+, Na^+) will move through the channel rapidly and exhibit little blocking tendency. Ions with intermediate affinity (e.g. Ca^{2+}, Ba^{2+}, Sr^{2+}) can produce large fluxes, but will also inhibit passage of lower affinity ions. Mg^{2+}, however, appears to occupy a rather unique position among cations in that it exhibits characteristics of both a weak blocker and a poor permeator. These qualities may reflect the slow rate of dehydration of Mg^{2+} (exchange of water molecules on its inner shell of hydration for polar groups in the channel wall), which is three or four orders of magnitude slower than for other permeant ions [29]. Since dehydration at a narrow region of the channel is probably the rate-limiting step in the passage of ions it is not surprising that Mg^{2+} should exhibit low permeability (see discussions in Refs. 19, 26 and 29).

Active Mg^{2+} uptake systems have been demonstrated in bacteria, but there is no direct evidence that they exist in the heart. For example, the bacteria *Salmonella typhimurium* and *Escherichia coli* possess two distinct Mg^{2+} uptake systems [30, 31]. In *S. typhimurium*, one system transports Co^{2+} as well as Mg^{2+}, and exhibits similar kinetic profiles for both ions (for Mg^{2+}: $K_m = 15\ \mu M$. $V_{max} = 0.25$ nmol/min/10^8 cells). The other system is induced by growth of cells in a medium with low $[Mg^{2+}]$. This mechanism exhibits a more rapid Mg^{2+} uptake and higher affinity ($K_m = 3.0\ \mu M$, $V_{max} = 0.4$ nmol/min/10^8 cells) [31].

In at least one system, Mg^{2+} influx is hormonally-regulated. Maguire and co-workers [32–34] have described a highly selective Mg^{2+} uptake mechanism in S49 lymphoma cells which is under hormonal control. In these cells, Mg^{2+} influx is inhibited by stimulation of β-adrenoceptors, whereas Mg^{2+} efflux is unaffected. This inhibitory effect is mediated through the same population of β-adrenoceptors that stimulate adenylate cyclase. However, cAMP does not mediate the inhibitory effect of β-agonists on Mg^{2+} influx, because the *kinase*$^-$ mutant of S49 cells, which lacks cAMP-dependent protein kinase activity, exhibits isoproterenol-induced depression of Mg^{2+} uptake. Only a small compartment of internal $[Mg^{2+}]_i$, approximately 2%, is under hormonal control. This finding is quite exciting because it suggests that $[Mg^{2+}]_i$ may not be uniform, but may vary regionally within the cell. This raises the possibility that even processes having K_m values for Mg^{2+} smaller than 0.5 mM may be regulated by changes in local $[Mg^{2+}]_i$. Additional evidence that Mg^{2+} flux can be regulated hormonally comes from Cunden and Singh [35], who report that acetylcholine (ACh) induces a large reversible increase in Mg^{2+} efflux from isolated rat lacrimal glands. We are unaware of effects of hormones on Mg^{2+} flux in cardiac cells, but such systems might exist and should be searched for. Such a hormonally-controlled system could affect the function of ion channels and second messenger systems in cardiac cells.

Magnesium efflux. Because cardiac cells are permeable to Mg^{2+}, albeit at a very low rate, mechanisms must exist for Mg^{2+} efflux in order to maintain internal $[Mg^{2+}]_i$ in the range of 1 mM. Numerous studies have established the existence of Mg^{2+} transport systems in various tissues; however, such a mechanism has not yet been characterized in cardiac cells (for a more extensive treatise on Mg^{2+} transport, see Ref 6). Baker and Crawford [36] described a Mg^{2+} transport system in squid axons that is dependent upon extracellular Na^+. A similar Mg^{2+}-Na^+ cotransport system has also been described in vascular smooth muscle [37, 38]. Although definitive evidence for a Mg^{2+}-Na^+ exchange mechanism is lacking for cardiac muscle, there is some suggestive evidence that such a system exists. For example, Fry [15] has shown that $[Mg^{2+}]_i$ in guinea pig and ferret ventricular muscle can be regulated by changes in extracellular $[Mg^{2+}]$ and $[Na^+]$ in a manner that implies a Na^+-Mg^{2+} exchange mechanism. This same system could be responsible for Mg^{2+} influx, depending on the ion gradients, the coupling ratio and membrane potential.

$[Mg^{2+}]_i$ may also be regulated by intracellular transport systems [15]. Isolated heart mitochondria can both take up and extrude Mg^{2+} by a respiration-dependent, uncoupler-sensitive process [39, 40].

Regulation and synthesis of cyclic AMP

The adenylate cyclase-cyclic adenosine monophosphate (cAMP) second messenger system exerts a major regulatory influence on cardiac activity. Activation of this system by β-adrenergic agonists results in phosphorylation of a variety of regulatory proteins, including the Ca^{2+} channel [41–43], troponin-C [44], C-protein [45], and phospholamban [46], all of which probably play important roles in cardiac contractility.

Hormonal activation of adenylate cyclase activity involves hormone–receptor activation of a guanine nucleotide regulatory protein ("G-protein") [47, 48]. Activation of G-proteins is thought to occur as a result of the hormone-occupied receptor stimulating exchange of bound GDP for GTP on the G-protein α-subunit. When GTP binds to α, the trimeric G-protein dissociates into αGTP and $\beta\gamma$ subunits. The αGTP subunit then activates the catalytic subunit of the adenylate cyclase.

The interactions between the G-protein subunits, hormone receptors, and guanine nucleotides are influenced in complex ways by Mg^{2+} [49]. There appear to be two sites for Mg^{2+} to act on G-proteins [47]. The first site has a high affinity for Mg^{2+}, in the nanomolar range. Interaction of Mg^{2+} with this site enables nucleotide hydrolysis (GTPase activity) by the G-protein, stabilizes the interaction of GTPγS with the α subunit by greatly reducing the rate of nucleotide dissociation, and enhances "activation" of the G-protein as measured by intrinsic fluorescence. The second Mg^{2+} site has a lower affinity for Mg^{2+}, in the range of 1 to 100 mM. Apparently, interaction of Mg^{2+} at this site is required for hormonal activation of the G-protein. Thus, occupation of this Mg^{2+} site is required for hormonally-stimulated GTP or GTPγS binding, for hormonally-stimulated or $\beta\gamma$-stimulated GTP dissociation from α, and for GTPγS-induced dissociation of the G-protein subunits [47, 50–52]. At physiological $[Mg^{2+}]$ (approximately 0.5 mM), activation of G_s by Gpp(NH)p is slow and minimal, but as the level of Mg^{2+} is increased, both the rate and extent of G_s activation are enhanced [53]. This requirement for Mg^{2+} varies depending upon the type of G-protein. In general, dissociation

of GDP from oligomeric G_s and its activation require higher Mg^{2+} concentrations than does activation of G_i or G_o [47, 53, 54].

It has been shown recently that G-proteins can directly activate certain kinds of ion channels: cardiac calcium channels and atrial potassium channels [55–58]. These α-subunit G-protein effects are also Mg^{2+} dependent.

The affinity of the receptor–G-protein–adenylate cyclase complex for Mg^{2+} is modulated by hormones [32, 59–61]. In S49 cells in the absence of hormone, the K_a of the complex for free magnesium is ~2.0 mM [62]. Since the normal $[Mg^{2+}]_i$ in these cells is less than 0.3 mM, adenylate cyclase activity under basal conditions is low. Upon hormonal stimulation, however, the affinity of the adenylate cyclase system for Mg^{2+} increases ~20-fold, which places the $[Mg^{2+}]$ required for enzyme activation within the range of $[Mg^{2+}]$ present in the cytoplasm. Thus, it appears that Mg^{2+} may serve as the key activator of adenylate cyclase, and that the function of excitatory hormones may be to decrease the requirement for Mg^{2+} to a level close to that which exists within the cytosol. Since hormone–receptor interaction lowers the apparent K_m of Mg^{2+} for G-protein-mediated activation of adenylate cyclase, hormone receptors may be considered as "magnesium-switches" [63].

The catalytic subunit of adenylate cyclase itself requires magnesium in complex with ATP as a substrate for synthesis of cAMP. In addition, the catalytic subunit of adenylate cyclase apparently has a divalent cation binding site, but this site probably does not play a major physiological role. In the *cyc*$^-$ variant of S49 lymphoma cells, which lacks a functional G_s protein, Mg^{2+} has no effect on adenylate cyclase activity. Mn^{2+}, however, does increase catalytic activity, presumably through interaction with this binding site on the catalytic subunit of adenylate cyclase [32, 62, 64, 65]. In contrast, when purified G_s-protein is added to *cyc*$^-$ cell membranes [66], Mg^{2+} increases adenylate cyclase activity and also increases the binding affinity of the receptor for agonists by at least an order of magnitude [32], as one would expect from the effect of Mg^{2+} on the G-protein system as described above. In light of these and other findings it appears that at least three metal ion binding sites exist in the receptor–G-protein–adenylate cyclase complex, but that the primary regulatory role of Mg^{2+} is mediated through the low affinity binding site associated with the G-protein.

Effects of Mg^{2+} *on ion channels*

Genesis of the cardiac action potential and, hence, beat-to-beat regulation of cardiac activity, depend upon the flux of ions through hydrophilic channels in the sarcolemma [67, 68]. Recently, it has been discovered that Mg^{2+} has important effects upon both potassium and calcium ion channels in heart cells. To date, six distinct cardiac K^+ channels have been identified by single-channel current recordings. Several of these K^+ channels exhibit inward rectification, i.e. they allow K^+ to pass more readily in the inward direction than in the outward direction [69]. It appears that this inward rectification may be caused by internal Mg^{2+} blocking the outward movement of K^+ through these channels. This was shown first by studies with excised patches of membrane containing I_{K1} channels associated with the "anomalous" or inward rectifier. When the solution bathing the cytoplasmic side of the membrane contains Mg^{2+}, these channels rectify inwardly, as they normally do in the cell. When Mg^{2+} is removed, the channel no longer rectifies, but carries current equally well in both directions [70–73]. This suggests that internal Mg^{2+} functions as a naturally occurring K^+ channel blocking agent which plugs the open channel in a voltage-dependent manner from the inner surface of the sarcolemma, thereby preventing outward passage of K^+. The exact function of the I_{K1} channel has been a matter of some dispute, but it may play a role in cardiac pacemaking, because block of this channel converts a quiescent cell into a spontaneously-beating cell and pacemaker cells tend to lack this channel [74]. I_{K1}, which is very strong in non-pacemaking tissue like ventricle, may tend to keep this tissue hyperpolarized near E_K and quiescent. The inward rectification of this channel would be important in assuring that, once the tissue is excited by pacemaker cells, the action potential would not be shunted by a large K^+ conductance. Thus, internal Mg^{2+} could play a role in rectification of this channel and, hence, depolarization and repolarization.

Similar mechanisms may be responsible for rectification of other K^+ channels. Horie *et al.* [71] found that removal of Mg^{2+} from the cytoplasmic surface of guinea pig ventricular cell membrane diminished the inward rectification of the ATP-sensitive K^+ channel, and they concluded that physiological concentrations of internal Mg^{2+} could block outward K^+ current through these channels. These channels are activated when intracellular ATP is depleted, for example, during ischemia or hypoxia [71]. Since ATP depletion would reduce complexation with Mg^{2+}, a rise in $[Mg^{2+}]_i$ would promote rectification of this channel.

Intracellular Mg^{2+} also regulates ACh-activated K^+ channels. These channels are opened by ACh released from parasympathetic nerve terminals and are at least partly responsible for the slowing of beat frequency produced by ACh in some species [75]. These channels are similar to I_{K1}, but have different kinetics and rectification. These channels are activated by stimulation of muscarinic cholinergic receptors via a G-protein [55, 57], whose activation requires Mg^{2+}. Not surprisingly, Mg^{2+} is essential for activation of this K^+ channel by ACh, GTP, or GTPγS [58, 76]. Furthermore, the inward rectification of $I_{K(ACh)}$ (like that of I_{K1}) appears to be due to voltage-dependent block by internal Mg^{2+} [72]. Thus, it is becoming increasingly apparent that $[Mg^{2+}]_i$ is a general blocker of cardiac K^+ channels, and it is this blocking effect which causes inward rectification.

Recent studies from our laboratory (Lagrutta and Gourdon, unpublished results*) have indicated an effect of $[Mg^{2+}]_i$ on the delayed rectifier K^+ channel that is responsible for membrane repolarization after an action potential. This channel opens slowly during an action potential, causing membrane repolar-

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ization [77]. Decreasing the $[Mg^{2+}]_i$ of frog atrial cells from 1.0 to 0.3 mM by internal perfusion augments the magnitude of this current by ~1.5-fold. The decrease in I_K amplitude caused by $[Mg^{2+}]_i$, however, is produced by a different mechanism than the Mg^{2+}-induced decrease in I_{K1}. $[Mg^{2+}]_i$ has no effect on the rectification of I_K; rather, it affects the total available current that can be activated.

Finally, some cardiac cells contain Ca^{2+}-activated K^+ channels that play a role in repolarization [78]. A very similar channel in salivary gland acinar cells is activated by Mg^{2+} in a Ca^{2+}-dependent manner. It has been suggested that Mg^{2+} increases the affinity of the channel for Ca^{2+}, which is its normal activator [79]. It is not yet known whether these channels are regulated in this same way in cardiac muscle.

$[Mg^{2+}]_i$ not only affects K^+ channels, but also affects Ca^{2+} channels. In our recent report [80], we demonstrated an inhibitory influence of $[Mg^{2+}]_i$ on influx of Ca^{2+} through sarcolemmal channels which carry the slow inward (dihydropyridine-sensitive) Ca^{2+} current. The effect of $[Mg^{2+}]_i$ on I_{Ca} appears to be more complex than its effects on K^+ currents. Increasing $[Mg^{2+}]_i$ from 0.3 to 3 mM has only a relatively small inhibitory effect under basal conditions (26% decrease in I_{Ca}). In contrast, a dramatic inhibitory effect of $[Mg^{2+}]_i$ on I_{Ca} (63% decrease) occurs when I_{Ca} is stimulated by cAMP (10 μM). We conclude that this effect is due to an action of $[Mg^{2+}]_i$ beyond Ca^{2+} channel phosphorylation, because Ca^{2+} currents elevated by various procedures including internal perfusion with non-hydrolyzable analogs of cAMP or the catalytic subunit of cAMP-dependent protein kinase are all affected similarly by $[Mg^{2+}]_i$. These results suggest that the effect of $[Mg^{2+}]$ cannot be explained simply by changes in cAMP levels. We propose that $[Mg^{2+}]_i$ either acts directly on the Ca^{2+} channel or stimulates protein phosphatases that dephosphorylate Ca^{2+} channels.

Although $[Mg^{2+}]_i$ has relatively little effect on the amplitude of the basal I_{Ca}, $[Mg^{2+}]_i$ does have significant effects on the inactivation of Ca^{2+} channels that are not phosphorylated (Hartzell and White, unpublished results). In response to a depolarization, Ca^{2+} channels open and then spontaneously inactivate during the depolarization. This inactivation is thought to be due to two processes, one which is dependent only upon the voltage and the other which is mediated by the entry of Ca^{2+} through the channel [81, 82]. The Ca^{2+}-dependent process may involve activation of Ca^{2+}-dependent phosphoprotein phosphatases [83]. One can study the voltage-dependent inactivation by using Ba^{2+} instead of Ca^{2+} as charge carrier, because Ba^{2+} does not activate the Ca^{2+}-dependent process appreciably. Under these conditions, we find that inactivation of Ca^{2+} channels depends strongly upon the transmembrane Mg^{2+} gradient. When the electrochemical driving force for $[Mg^{2+}]$ is in the outward direction, the Ba^{2+} current inactivates more rapidly than when the driving force is inward. This suggests to us that voltage-dependent inactivation of Ca^{2+} channels may be due to the voltage- and time-dependent block of Ca^{2+} channels by internal magnesium ions.

Since $[Mg^{2+}]_i$ regulates the transsarcolemmal flux of ions in cardiac cells, it is expected that $[Mg^{2+}]_i$ will alter the shape of the cardiac action potential. The effects of $[Mg^{2+}]_i$ on K^+ currents would tend to promote the development and prolong the duration of the action potential by reducing the amount of repolarization afforded by the K^+ channels, but the effect of $[Mg^{2+}]_i$ on Ca^{2+} currents would tend to decrease action potential duration by increasing calcium channel inactivation. The exact interplay between these effects requires further attention.

Regulation of $[Ca^{2+}]_i$ *by* Mg^{2+}

As we have indicated, Ca^{2+} influx may be modulated by both $[Mg^{2+}]_i$ and $[Mg^{2+}]_o$, and it has been proposed that Mg^{2+} is a naturally-occurring Ca^{2+} antagonist [84, 85]. Thus, one might expect that systems which buffer internal Ca^{2+} may be Mg^{2+}-regulated. These systems would include: the sarcolemmal Ca^{2+}ATPase that extrudes Ca^{2+} from the cell, the sarcolemmal Na^+–Ca^{2+} exchange, the sarcoplasmic reticulum (SR) Ca^{2+}ATPase, and mitochondria. The available evidence does not support a major regulatory effect of Mg^{2+} on sarcolemmal Ca^{2+} buffering systems; however, the Ca^{2+} sequestering ability of the SR is sensitive to $[Mg^{2+}]$ within the "normal" physiological range.

Although the sarcolemmal Ca^{2+}ATPase requires Mg^{2+} for activity, the affinity of the Ca^{2+}ATPase for Mg^{2+} is about 53 μM in the presence of 1.0 mM ATP [86]. Since physiological $[Mg^{2+}]_i$ is usually ten times this value, the activity of the Ca^{2+} pump is unlikely to be regulated significantly by fluctuations in $[Mg^{2+}]_i$. Similarly, Mg^{2+} seems to have little effect on Na^+–Ca^{2+} exchange. Trosper and Philipson [87] reported that most divalent and trivalent cations inhibited Ca^{2+} uptake via this exchange mechanism in cardiac sarcolemma vesicles by >90%. In contrast, Mg^{2+} failed to have a significant effect upon Ca^{2+} uptake or efflux through Na^+–Ca^{2+} exchange.

On the other hand, Ca^{2+} handling by the SR does appear to be modulated by Mg^{2+}. Ca^{2+}ATPase activity of rabbit skeletal muscle, as reflected by uptake of $^{45}Ca^{2+}$ into SR vesicles, is stimulated by ~800% by low ($\leq$1.0 mM) concentrations of Mg^{2+} [88]. The dependence of Ca^{2+}ATPase activity on $[Mg^{2+}]$ in the range of 0.1 to 1 mM is extremely steep. Above 10 mM Mg^{2+}, Ca^{2+}ATPase activity declines. This effect of Mg^{2+} on Ca^{2+}ATPase activity apparently reflects the ability of Mg^{2+} to stimulate hydrolytic cleavage of the phosphoenzyme [89], which is the rate-limiting step in the reaction sequence. Raising $[Mg^{2+}]_i$ from 2.0 to 10.0 mM in intact permeabilized cardiac cells inhibits SR Ca^{2+} transport [90]. Thus, the ability of SR to accumulate Ca^{2+} is optimal when $[Mg^{2+}]_i$ is ~1.0 mM, which is well within the "normal" physiological range (see above section). $[Mg^{2+}]$ above or below this range is apparently inhibitory. Since movement of Ca^{2+} across the SR membrane is a primary mechanism of regulating $[Ca^{2+}]_i$ in mammalian cardiac cells, it is attractive to propose that minor fluctuations in $[Mg^{2+}]_i$ have major effects on internal $[Ca^{2+}]$ and, therefore, cardiac activity.

It is generally agreed that mitochondria do not play a major role in reducing the Ca^{2+} transient after a contraction, but they probably do serve to adjust

the tonic level of $[Ca^{2+}]_i$ [91]. Mitochondria from cardiac muscle sequester Ca^{2+} in a respiration-dependent manner [92]. This Ca^{2+} uptake is inhibited by Mg^{2+} with an apparent K_i of 3.1 mM. Thus, the ability of mitochondria to buffer $[Ca^{2+}]_i$ may be affected significantly by $[Mg^{2+}]_i$ in the physiological range.

Clinical implications

Alterations in $[Mg^{2+}]$ also have important clinical implications. Patients with Mg^{2+} deficiency exhibit an increased heart rate, a tendency toward paroxysmal tachycardia, and a shortening of the absolute refractory period [3]. A better understanding of the influence of Mg^{2+} on cardiac function is important since patients undergoing diuretic therapy may increase urinary loss of Mg^{2+} by as much as 50%. If prolonged, this enhanced excretion can lead to tissue Mg^{2+} deficiency. It is interesting to note that ischemic heart disease and cardiac death are less prevalent in regions with hard water, i.e. water that contains greater amounts of calcium and magnesium [93, 94]. Pathological studies indicate that magnesium is greater in cardiac tissue from patients from areas with hard water compared to tissue from patients from soft-water regions, and that the higher cardiac death rate in the soft-water areas is related to the relative lack of Mg^{2+} content in the water supply [94]. Additionally, patients who died of ischemic heart disease had a lower Mg^{2+} content in cardiac muscle than those who died from other causes [95]. However, these pathological studies measured total tissue magnesium concentration and gave no estimate of the free ionized $[Mg^{2+}]_i$.

Loss of cellular Mg^{2+} is one of the earliest signs of myocardial injury, with a 2-fold increase in Mg^{2+} efflux occurring during hypoxia [96]. Furthermore, in 675 clinical cases of sudden death due to ischemic heart disease, myocardial magnesium content was reduced by 12–38%, with a concomitant increase in calcium content [97]. Likewise, a significant drop in the magnesium content of cardiac tissue is noted after periods of ischemic injury in humans and dogs [4]. Associated with the loss of cellular Mg^{2+} is an increased efflux of K^+ and depletion of tissue K^+ [2, 5].

A plausible explanation for these findings is that, during ischemia and hypoxia, ATP production decreases and total tissue [ATP] declines. Associated with the reduction in [ATP] is an initial increase in free $[Mg^{2+}]_i$ due to a reduction in the amount of Mg^{2+} bound to ATP. This increase in $[Mg^{2+}]_i$ may be compensated by an increased efflux, which eventually results in depletion of intracellular $[Mg^{2+}]$. If Mg^{2+} efflux occurs by a Na^+–Mg^{2+} counter-transport, the increased efflux of Mg^{2+} would be coupled to an increase in Na^+ influx. If $[Na^+]_i$ rises significantly, this could be a contributing factor in producing calcium overload, which is a major contributing factor in myocardial injury [98, 99], due to reversal of the Na^+–Ca^{2+} exchanger [100, 101]. Ca^{2+} overload may also result from a decreased ability of the SR to accumulate Ca^{2+} because of changes in $[Mg^{2+}]_i$. The lowering of cellular ATP levels results in the opening of ATP-depletion K^+ channels [102]. Outward movement of K^+ through these and other kinds of K^+ channels reduces internal K^+ and subsequently depolarizes the cells. Outward movement of K^+ through these channels could be increased by depletion of internal $[Mg^{2+}]_i$ that normally renders these channels inwardly rectifying. Efflux of Mg^{2+} is aggravated by stimulation of β-adrenoceptors [4, 103, 104], which increases utilization of ATP. Under these conditions, calcium overload might be worsened by increased influx of Ca^{2+} through voltage-gated Ca^{2+} channels [80].

The importance of magnesium in cardiac function is not limited to cardiac cells proper. Recent studies have indicated that extracellular Mg^{2+} has profound effects on the contractile state of coronary arteries. Ku and Ann [105] have postulated recently that Mg^{2+} exerts an inhibitory influence upon release of endothelium derived relaxing factor (EDRF) from the canine coronary endothelium. On the other hand, Altura and Altura [106] demonstrated that Mg^{2+} was essential for acetylcholine-induced relaxation of canine coronary arteries. Both studies, however, reported an enhanced contractile state of coronary arteries with damaged endothelium under conditions of reduced extracellular Mg^{2+}. Thus, the importance of Mg^{2+} in EDRF-mediated relaxation of coronary arteries requires additional clarification. It is apparent from these and other studies, however, that magnesium deficiency may be an important factor in the initiation of coronary vasospasm and may potentiate the effects of other vasoconstrictor agents. Therefore, hypomagnesemia may be implicated in the etiology of ischemic heart disease, coronary vasospasm, sudden death, and/or hypertension, particularly where significant damage to the vascular endothelium has occurred.

Conclusion

In considering the recent studies on Mg^{2+} in cardiac cells, we propose that $[Mg^{2+}]_i$ is carefully regulated, and its alteration can have profound effects upon cardiac physiology. This proposal is based upon the evidence that internal free $[Mg^{2+}]_i$ is normally in the range of 1 mM and that several systems are profoundly affected by $[Mg^{2+}]$ in this range. Autonomic control of the heart is dependent upon Mg^{2+} in numerous ways: binding of neurotransmitter to their receptors, coupling of receptors to adenylate cyclase, activation of G-proteins and adenylate cyclase, activation of proteins by Mg^{2+}-dependent phosphotransferases, rectification of various types of K^+ channels, activity of phosphorylated Ca^{2+} channels, voltage-dependent inactivation of Ca^{2+} channels, and optimal activity of mechanisms which maintain $[Ca^{2+}]_i$ at resting levels.

To substantiate this proposal, however, considerable work remains to be done. We know very little about the mechanisms that regulate $[Mg^{2+}]_i$ in cells: the routes of influx, the routes of efflux, and the cytoplasmic systems that buffer internal Mg^{2+}. Precedents exist for hormonally-regulated Mg^{2+} transport systems in several cell types, but similar systems have yet to be found in the heart. Future research on the importance of Mg^{2+} in cardiac function should involve: (a) development of more accurate means of determining $[Mg^{2+}]_i$, (b) investigation into the nature of Mg^{2+} transport systems and Mg^{2+}-

buffering systems, (c) definition of how Mg^{2+} induces inward rectification of K^+ channels, and (d) exploration of the effects of internal Mg^{2+} on Ca^{2+} channel activation and inactivation. Such investigations hopefully will bring Mg^{2+} some of the respect that it has long deserved.

Note added in proof: Two excellent reviews on magnesium in cells have recently appeared [108, 109].

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