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Parallel Stimulation of Glucose and Mg^{2+} Accumulation by Insulin in Rat Hearts and Cardiac Ventricular Myocytes

Andrea M.P. Romani, Veronica D. Matthews, Antonio Scarpa

Abstract—The stimulation of β-adrenoceptors in cardiac cells results in a rapid loss of cellular Mg^{2+}. Because insulin physiologically counteracts several of the cellular effects mediated by the activation of β-adrenoceptors and the elevation of cytosolic cAMP levels, we investigated whether insulin administration could prevent Mg^{2+} mobilization from rat hearts and ventricular myocytes. Rat hearts were perfused in a retrograde Langendorff system, and the changes in extracellular Mg^{2+} were measured by atomic absorbance spectrophotometry. Pretreatment of the hearts with 6 nmol/L insulin completely prevented the Mg^{2+} extrusion induced by the β-adrenergic agonist isoproterenol. Furthermore, the administration of insulin per se induced an accumulation of Mg^{2+} by the heart. This accumulation was small but detectable in the presence of 25 to 35 μmol/L $[Mg^{2+}]_o$ and increased in proportion to $[Mg^{2+}]_o$. Insulin-mediated Mg^{2+} accumulation was not observed in hearts perfused with a medium devoid of glucose or with a medium containing the inhibitors of glucose transport, cytochalasin B and phloretin. Insulin-stimulated [^{3}H]2-deoxyglucose accumulation was measured in collagenase-dispersed cardiac ventricular myocytes in the presence of varying levels of $[Mg^{2+}]_o$. Glucose transport was not observed below 25 μmol/L $[Mg^{2+}]_o$, and it also increased in proportion to $[Mg^{2+}]_o$. Taken together, these results indicate the presence of a major uptake of Mg^{2+} into cardiac cells that is stimulated by insulin and may require the insulin-induced operation of a glucose transporter. Hence, extracellular and/or intracellular Mg^{2+} may modulate glucose transport and/or utilization. (***Circ Res*. 2000;86:326-333.**)

Key Words: Mg^{2+} ■ cardiac myocytes ■ hearts ■ insulin ■ glucose transport

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In recent years, a large number of reports have indicated that the selective stimulation of β-adrenoceptors [1–9] results in a marked extrusion of cellular Mg^{2+} from cardiac myocytes,[1–3] hepatocytes,[4–6] and other cell types[7–9] into the extracellular compartment. In addition, the infusion of isoproterenol or catecholamine results in a 15% to 20% increase in the total serum Mg^{2+} level in the anesthetized rat.[10,11] At the cellular level, Mg^{2+} extrusion can be elicited by the administration of forskolin[2,4] or cell-permeant cAMP analogues (eg, 8-bromo-cAMP)[2,4–9] and be inhibited by the administration of Rp-cAMP,[7] a cell-permeant blocking agent specific for protein kinase A. Taken together, these results support the idea that Mg^{2+} extrusion is mediated via a cAMP-dependent process, most likely the phosphorylation of a specific Mg^{2+} transporter.[9]

Experimental evidence suggests a role for insulin in regulating cellular or tissue Mg^{2+} content. Our laboratory has recently reported that because of its ability to prevent cAMP production[12] and accelerate cAMP catabolism via phosphodiesterase,[13] insulin can effectively modulate the extrusion of Mg^{2+} induced by β-adrenergic agonists in liver cells.[6] In addition, evidence has been provided indicating that insulin increases cytosolic free $[Mg^{2+}]$ in beta pancreatic islets,[14] 3T3 fibroblasts,[15] and platelets[16] by promoting an entry of Mg^{2+} across the plasma membrane and/or a release of Mg^{2+} from an intracellular organelle(s). Last, a marked decrease in cellular Mg^{2+} content has been observed in diabetes types I and II[17–19] both in humans[17,18] and animals,[19] and this decrease has been suggested to be a possible cause of the long-term complications associated with diabetes.[20]

In the present study, the ability of insulin to modulate cellular Mg^{2+} in cardiac myocytes was investigated. The results obtained indicate that insulin can modulate cellular Mg^{2+} content by limiting the amount of Mg^{2+} extruded from cardiac cells stimulated by β-adrenergic agonists or by inducing a Mg^{2+} accumulation in the cells. Furthermore, the presence of a synergism between glucose transport and Mg^{2+} accumulation in cardiac cells suggests a key role of Mg^{2+} in controlling glucose utilization for energetic purposes within the cell.

Materials and Methods

Chemicals

Collagenase (CLS-1) was from Worthington. [^{3}H]2-Deoxyglucose was from Amersham. All other chemicals were from Sigma Chemical Co. Whatman glass fiber filters were from Fisher.

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Rat Heart Perfusion

Male Sprague-Dawley rats (250 g body weight) were anesthetized by intraperitoneal injection of sodium pentobarbital. The heart was removed and perfused in a Langendorff system at a flow rate of 7 mL/min with a medium containing (mmol/L) NaCl 120, KCl 3, KH_2PO_4 1.2, $CaCl_2$ 1.2, $MgCl_2$ 1.2, glucose 20, HEPES 10, and $NaHCO_3$ 12, pH 7.2, equilibrated at 37°C with O_2/CO_2 (95:5 [vol/vol]).[2,3] The $[Mg^{2+}]_o$ in the perfusion medium was varied from 0 to 1000 μmol/L. Where indicated in the figures, isoproterenol, 8-chloro-cAMP,[2] or insulin was added to the perfusion medium.

In the experiments performed in the absence of extracellular glucose, 5 mmol/L pyruvate and 5 mmol/L lactate were added to the perfusion medium. Alternatively, pyruvate and lactate were added to the perfusion medium in addition to glucose. Cytochalasin B or phloretin was dissolved in the perfusion medium 5 minutes before insulin administration.

Aliquots of the perfusate were collected every 30 seconds, and the Mg^{2+} content was measured by atomic absorbance spectrophotometry in a Perkin-Elmer 3100 spectrophotometer after proper dilution. Net Mg^{2+} accumulation was estimated as described previously.[21]

At the end of the experiment, the heart was homogenized in 10% HNO_3 and extracted overnight. The Mg^{2+} content in the acid supernatant was measured by atomic absorbance spectrophotometry as described previously.[3]

Isolation of Cardiac Ventricular Myocytes and Determination of Mg^{2+} Accumulation

Cardiac ventricular myocytes were isolated by collagenase digestion as described by De Young et al.[22] An aliquot of cell suspension was washed at 600*g* for 1 minute and transferred into the incubation medium described previously, in the presence of varying concentrations of extracellular Mg^{2+} or glucose. Mg^{2+} accumulation into the cells was determined as reported previously.[3]

Determination of Glucose Transport

For the experiments in perfused hearts, 0.2 mCi/mL [^{3}H]2-deoxyglucose was added to the perfusion medium. Half-milliliter aliquots of the perfusate were collected in duplicate and transferred in scintillation vials to measure the radioactivity by β-scintillation counting in a Beckman LS7000 counter. At the end of the perfusion, the heart was homogenized in 10% HNO_3 and extracted overnight. The radioactivity accumulated into the tissue was measured by β-scintillation counting in aliquots of the homogenate.

For the experiments in isolated myocytes, the cells were incubated as previously reported, in the presence of 0.2 mCi/mL [^{3}H]2-deoxyglucose. After insulin administration, glucose accumulation was determined, as reported previously,[23] as the radioactivity retained onto glass fiber filters (NF Whatman, pore size 0.25 μm).

Protein was assessed by the procedure of Lowry et al[24] with bovine serum albumin used as a standard.

Statistical Analysis

The data were reported as mean±SE. Data were first analyzed by 1-way ANOVA. Multiple means were then compared by the Tukey multiple comparison test, which was performed with a *q* value established for significance at $P<0.05$.

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Results

The administration of 1 or 10 μmol/L isoproterenol to rat hearts perfused in a Langendorff system resulted in a detectable increase in heart contractility (not shown) and in a marked extrusion of Mg^{2+} from the organ into the perfusate (Figure 1A). Mg^{2+} efflux became evident within 2 minutes after the addition of the β-adrenergic agonist to the perfusion medium and persisted for an additional 5 minutes before returning toward basal levels. The time course of these

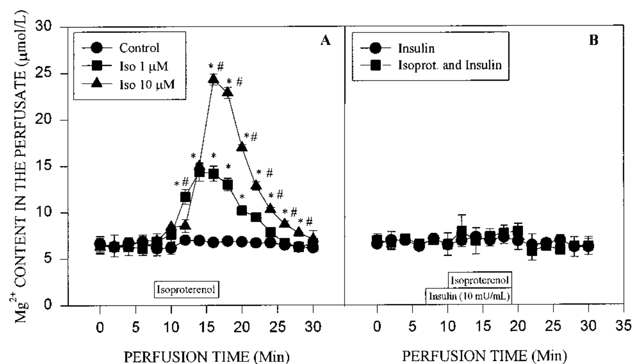

Figure 1. Efflux of Mg^{2+} from perfused rat hearts stimulated by isoproterenol (iso or isoprot.) in the absence (A) or presence (B) of insulin. Mg^{2+} efflux from rat hearts perfused in a Langendorff retrograde manner was induced by administration of 1 or 10 μmol/L isoproterenol. Insulin (10 mU/mL=6 nmol/L) was administered 5 minutes before 10 μmol/L isoproterenol. Data were determined every 30 seconds but are represented at 90-second intervals for clarity. Data are mean±SE of 5 different hearts for all experimental conditions. *$P<0.05$ vs control. #$P<0.05$ vs 1 μmol/L isoproterenol.

changes was independent of the dose and persistence of the agonist in the perfusion medium, the rate of contractility elicited by the adrenergic agonist, and $[Mg^{2+}]_o$, as already reported.[2,3] Isoproterenol-induced Mg^{2+} extrusion was also observed when the concentration of Mg^{2+} in the perfusate was increased to 100 and 250 μmol/L. Despite the high noise-to-signal ratio observed under these experimental conditions, the net amount of Mg^{2+} extruded from the heart was similar (410.6±36.1 and 413.0±32.3 nmol Mg^{2+}/g heart for 8 minutes at 100 and 250 μmol/L $[Mg^{2+}]_o$, respectively, versus 528.4±23.6 nmol Mg^{2+}/g heart for 8 minutes at 10 μmol/L $[Mg^{2+}]_o$; n=5 for all the conditions).

Pretreatment of the heart with 10 mU/mL (6 nmol/L) insulin completely prevented the Mg^{2+} extrusion induced by 1 μmol/L (not shown) or 10 μmol/L isoproterenol (Figure 1B). A similar inhibition was also observed when 250 μmol/L 8-chloro-cAMP was used instead of isoproterenol[2] to mobilize Mg^{2+} (not shown). A similar inhibitory effect on isoproterenol-induced Mg^{2+} extrusion was also observed in hearts perfused in the presence of 15 μmol/L $[Mg^{2+}]_o$ when insulin was added 3 minutes after the β-adrenergic agonist, ie, at a time point at which Mg^{2+} extrusion into the perfusate could be detected effectively, although it was not maximal (Figure 2).

Because atomic absorbance spectrophotometry cannot measure unidirectional Mg^{2+} fluxes, in principle it is possible that the absence of Mg^{2+} extrusion is the result of a decreased Mg^{2+} efflux and/or an increased Mg^{2+} influx into cardiac cells. The latter possibility appears to be supported by the decline in the basal Mg^{2+} level below the initial value after insulin administration (Figure 2).

Hence, the possibility that insulin induced Mg^{2+} accumulation into cardiac cells was further investigated by increasing $[Mg^{2+}]_o$ in the perfusate from the contaminant concentration present in Figure 1. Insulin administration resulted in a small but detectable Mg^{2+} accumulation when the heart was perfused with 25 μmol/L $[Mg^{2+}]_o$ (not shown). The decrease in

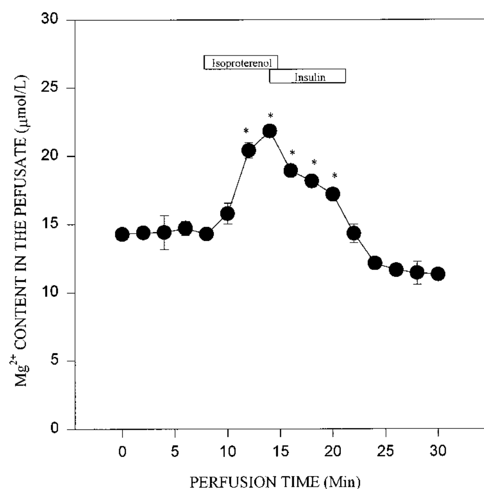

Figure 2. Mg^{2+} extrusion from perfused hearts sequentially stimulated by isoproterenol and insulin in the presence of 15 μmol/L $[Mg^{2+}]_o$. Rat hearts, perfused in the presence of 15 μmol/L $[Mg^{2+}]_o$, were stimulated by 10 μmol/L isoproterenol and, 3 minutes later, by 10 mU/mL insulin. Data were determined every 30 seconds but are represented at 90-second intervals for clarity. Data are mean±SE of 4 hearts. *$P<0.05$ vs the average of the 5 time points preceding isoproterenol administration.

Mg^{2+} content in the perfusion medium, an indication of Mg^{2+} accumulation into cardiac cells, increased progressively in hearts perfused with 35 or 50 μmol/L $[Mg^{2+}]_o$ (Figure 3A) or higher levels of $[Mg^{2+}]_o$ (not shown). In Figure 3B, the net Mg^{2+} accumulation by the perfused heart during 8 minutes of insulin administration is reported as the total amount of Mg^{2+} disappearing from the perfusate. The net Mg^{2+} accumulation was accounted for by ≈500 to 600 nmol/g heart for $[Mg^{2+}]_o$ of 100 and 200 μmol/L and ≈700 to 800 nmol/g heart for $[Mg^{2+}]_o$ of 500 or 1000 μmol/L. The Mg^{2+} determination in acid extracts of the heart at the end of the experiment indicates an increase in total tissue Mg^{2+} content from 61.97±3.24 to 71.42±3.18 and to 77.21±4.50 nmol/mg protein^{-1} in hearts perfused with 200 and 1000 μmol/L $[Mg^{2+}]_o$, respectively ($P<0.05$, n=4 for all experimental conditions).

A similar inhibitory effect of insulin on isoproterenol- or cAMP-induced Mg^{2+} extrusion has been observed in perfused liver.[6] However, insulin per se did not induce any detectable Mg^{2+} uptake into liver cells, regardless of the $[Mg^{2+}]_o$ used.[6] One notable difference between cardiac and liver cell metabolism is the different class of glucose transporter present in the plasma membrane, namely, Glut1 and Glut4 in cardiac cells[25] and Glut 2 in hepatocytes.[25] Glut4 transporters (and Glut1 to a lesser extent),[26] but not Glut2,[25] are recruited to the sarcolemma by insulin administration. Therefore, we next investigated the possibility that glucose transport is involved in mediating the accumulation of Mg^{2+} induced by insulin.

The requirement of glucose transport for Mg^{2+} accumulation is supported by the data reported in Figure 4. In the presence of 50 μmol/L $[Mg^{2+}]_o$, the absence of glucose in the perfusate (replaced with lactate and pyruvate, Figure 4B) completely prevented the insulin-mediated Mg^{2+} accumulation (Figure 4A). To exclude the possibility that the lack of

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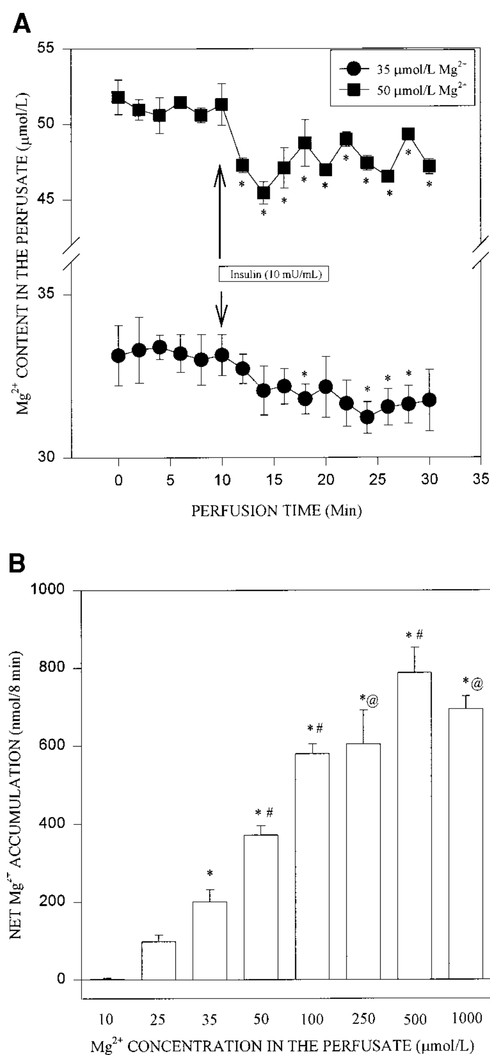

Figure 3. Mg^{2+} accumulation by perfused hearts stimulated by insulin in the presence of varying levels of $[Mg^{2+}]_o$. A, Rat hearts were perfused in the presence of 35 or 50 μmol/L $[Mg^{2+}]_o$ and stimulated by insulin. Data were determined every 30 seconds but are represented at 90-second intervals for clarity. Data are mean±SE of 5 and 7 hearts for 35 and 50 μmol/L $[Mg^{2+}]_o$, respectively. *$P<0.05$ vs the average of the 5 time points preceding insulin administration. B, Net Mg^{2+} accumulation in perfused hearts stimulated by insulin in the presence of varying levels of $[Mg^{2+}]_o$ is reported. Net Mg^{2+} accumulation during 8 minutes of stimulation by insulin was estimated as the disappearance of Mg^{2+} from the perfusate. Data are mean±SE of 7 hearts for 50 μmol/L $[Mg^{2+}]_o$ and 5 hearts for all other levels of $[Mg^{2+}]_o$. *$P<0.05$ vs 10 μmol/L $[Mg^{2+}]_o$. #$P<0.05$ vs all other levels of $[Mg^{2+}]_o$. @P=NS vs 100 or 500 μmol/L.

Mg^{2+} accumulation observed under these experimental conditions could be attributable to the sudden change in metabolic substrate, in a separate set of experiments, 5 mmol/L pyruvate and 5 mmol/L lactate were introduced into the perfusion medium at the start, in addition to glucose. Glucose was removed at the time of insulin administration, to be reintroduced after hormone removal, but pyruvate and lactate were maintained throughout the experimental protocol. Also, under these experimental conditions, insulin administration did not result in an accumulation of Mg^{2+} in the heart (total tissue Mg^{2+} content was 64.0±6.4 versus 61.7±5.4 nmol

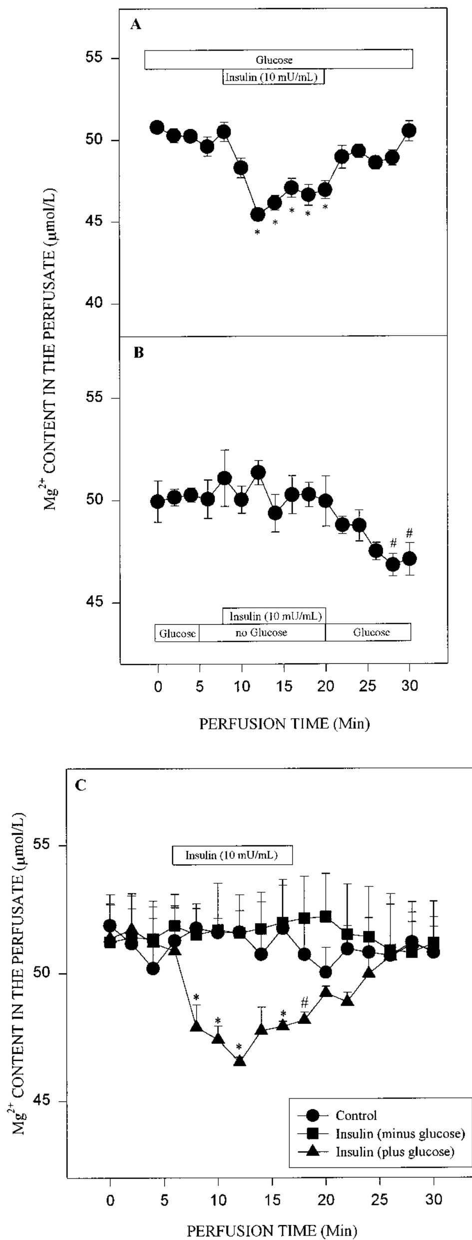

Figure 4. Mg^{2+} accumulation in hearts perfused in the absence of extracellular glucose. A and B, Rat hearts were perfused in the presence of 50 μmol/L $[Mg^{2+}]_o$ in the presence (A) or in the absence (B) of 15 mmol/L glucose (replaced with 5 mmol/L lactate and 5 mmol/L pyruvate) and stimulated by insulin for 8 minutes. C, Effect of insulin on rat hearts perfused in the presence of 5 mmol/L lactate and 5 mmol/L pyruvate plus 15 mmol/L glucose is shown. The infusion of glucose-free medium was initiated 5 minutes before insulin administration and limited to the time of insulin infusion. Data were determined every 30 seconds but are represented at 90-second intervals for clarity. Data are mean±SE of 5 different hearts for all experimental conditions. Values of P are as follows for panels A through C: A, *$P<0.05$ vs glucose-free medium. B, #$P<0.05$ vs glucose medium. C, *$P<0.05$ vs control and insulin minus glucose; #$P<0.05$ vs insulin minus glucose only.

Mg^{2+}/mg protein^{-1} in control hearts versus insulin-treated hearts, n=4 for both experimental conditions, $P>0.05$). By contrast, when glucose was maintained throughout the experimental protocol in addition to pyruvate and lactate, the administration of insulin resulted in a disappearance of Mg^{2+}

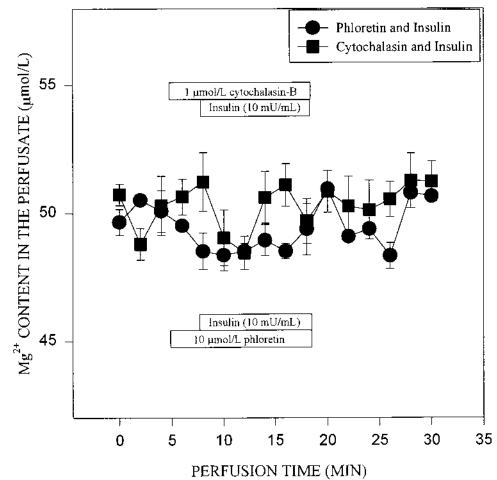

Figure 5. Effect of glucose transport inhibitors on insulin-induced Mg^{2+} accumulation in perfused hearts. Rat hearts were perfused in the presence of 50 μmol/L $[Mg^{2+}]_o$ and 15 mmol/L glucose and stimulated by insulin. Where indicated, cytochalasin B (1 μmol/L) and phloretin (10 μmol/L) were added to the perfusion medium. Data were determined every 30 seconds but are represented at 90-second intervals for clarity. Data are mean±SE of 5 different hearts for all experimental conditions.

from the perfusate (Figure 4C) and an accumulation in the heart (total tissue Mg^{2+} content was 70.8±2.5 versus 60.8±3.4 nmol Mg^{2+}/mg protein^{-1} in the presence or in the absence of insulin, respectively).

In a separate set of experiments, cytochalasin B and phloretin were used as glucose transport inhibitors. Whereas cytochalasin B blocks the translocation of glucose transporters to the plasma membrane by disrupting cytoskeleton integrity, phloretin inhibits glucose transport operation at the plasma membrane by interacting at the extracellular site of the transporter.[23,25] When 1 μmol/L cytochalasin B or 10 μmol/L phloretin was added to the perfusate in the presence of glucose, the insulin-induced Mg^{2+} accumulation in the heart was almost completely inhibited (Figure 5). Finally, when insulin-induced Mg^{2+} accumulation was measured at varying extracellular glucose concentrations, a minimal glucose concentration of 2 mmol/L appeared to be required for the Mg^{2+} accumulation to occur (Figure 6). Net Mg^{2+} accumulation accounted for 1.53±0.35 (n=4), 2.92±0.98 (n=4), and 17.80±2.32 nmol Mg^{2+}/mg protein^{-1} (n=5) for insulin-stimulated hearts perfused in the presence of 2, 5, and 10 mmol/L glucose, respectively. The last 3 time points under the curve of uptake with 10 mmol/L glucose are significantly different ($P<0.05$) compared with the corresponding time points reported in Figure 4A. Presently, we have no explanation for this discrepancy.

The presence of a synergism between glucose and Mg^{2+} accumulation is further corroborated by the results reported in Figure 7. Because nonphosphorylated glucose can cross the sarcolemma in either direction, $[^3H]$2-deoxyglucose, which remains trapped in the cytosol after the phosphorylation by hexokinase,[25] was used to quantify the amount of glucose accumulated by cardiac ventricular myocytes after 5 minutes of stimulation by 10 mU/mL (6 nmol/L) insulin. Cardiac ventricular myocytes rather than perfused hearts were used to

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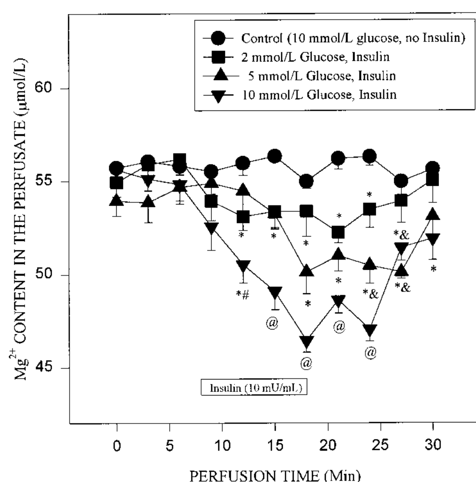

Figure 6. Mg^{2+} accumulation in perfused hearts stimulated by insulin in the presence of varying extracellular glucose concentrations. Rat hearts were perfused in the presence of 2, 5, and 10 mmol/L glucose and stimulated by insulin for 10 minutes. Data were determined every 30 seconds but are represented at 90-second intervals for simplicity. Data are mean±SE of 4 different hearts for all experimental conditions. $*P<0.05$ vs control. $\&P<0.05$ vs 2 mmol/L glucose. $\#P<0.05$ vs 5 mmol/L glucose. $@P<0.05$ vs all other conditions.

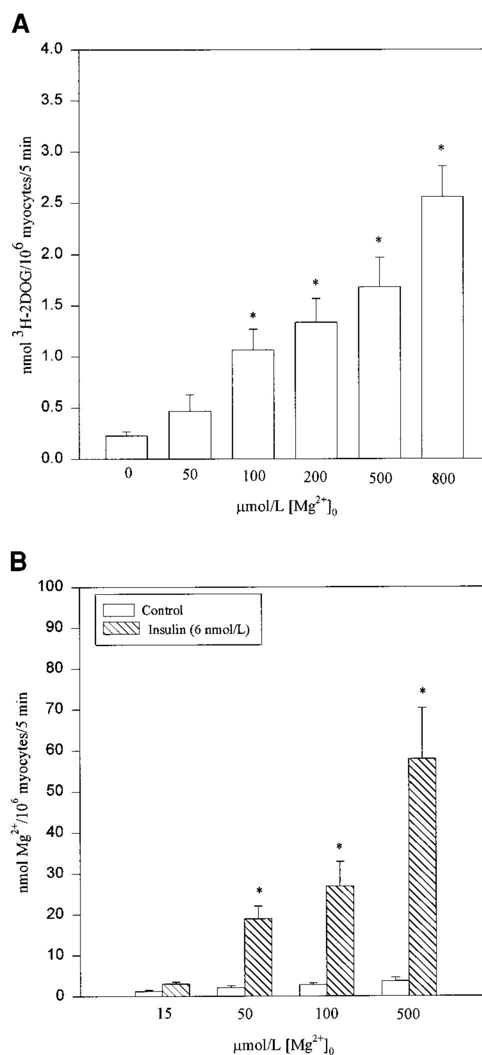

Figure 7. Net glucose (A) and Mg^{2+} (B) accumulation in collagenase-dispersed cardiac ventricular myocytes. Rat cardiac ventricular myocytes were incubated in the presence of 15 mmol/L glucose labeled with [^{3}H]2-deoxyglucose (^{3}H-2DOG) and varying levels of $[Mg^{2+}]_o$ and stimulated by insulin for 5 minutes. See Materials and Methods for detail. Data are mean±SE of 5 different myocytes preparations, each of them performed in triplicate, for both glucose and Mg^{2+} determinations. Values of P are as follows for panels A and B: A, $*P<0.05$ vs 0 mmol/L $[Mg^{2+}]_o$. B, $*P<0.05$ vs respective control.

exclude possible artifacts related to perfusion flow rate and to cell heterogeneity. The data, reported in Figure 7A, indicate that $[Mg^{2+}]_o$ is required to observe an accumulation of glucose into cardiac cells. This accumulation accounted for 0.47±0.16 nmol glucose/10^6 cells at 50 μmol/L $[Mg^{2+}]_o$ and increased to 1.07±0.20, 1.68±0.29, and 2.56±0.30 nmol/10^6 cells when $[Mg^{2+}]_o$ was 100, 500, and 800 μmol/L, respectively. Under these experimental conditions, Mg^{2+} accumulation was 26.9±6.1 nmol/10^6 cells for 5 minutes at 100 μmol/L $[Mg^{2+}]_o$ and 58.0±12.3 nmol/10^6 cells for 5 minutes at 500 μmol/L $[Mg^{2+}]_o$ (Figure 7B). Based on the total cellular Mg^{2+} content of cardiac ventricular myocytes, these values account for increases of 10% and 22% in total Mg^{2+} content, respectively.

After it had been determined that the presence of extracellular glucose or the operation of glucose transporter is necessary to observe insulin-induced Mg^{2+} accumulation, rat hearts were perfused in the presence of pyruvate and lactate but in the absence of glucose and stimulated by 10 μmol/L isoproterenol and 10 mU/mL insulin to determine whether the effect of insulin on the β-adrenoceptor–mediated Mg^{2+} extrusion observed in Figures 1 and 2 could be ascribed to an inhibitory effect on β-adrenergic signaling[12,13] and/or to a stimulated accumulation of Mg^{2+} into the heart. As Figure 8A shows, in the presence of 50 μmol/L $[Mg^{2+}]_o$ but in the absence of extracellular glucose, insulin was still able to block the extrusion of Mg^{2+} elicited by isoproterenol infusion. By contrast, in the presence of glucose, the administration of insulin before adrenergic agonist infusion resulted in an accumulation of Mg^{2+} that could not be reverted by the subsequent infusion of isoproterenol (Figure 8B).

Discussion

The administration of β-adrenergic agonists to cardiac cells elicits a marked extrusion of cellular Mg^{2+} in the extracellular compartment[1–3] via an increase in cAMP and the activation of a specific Na^+-Mg^{2+} exchanger.[27,28] Recently, we have reported that insulin can modulate Mg^{2+} content in liver cells by preventing the β-adrenoceptor–mediated Mg^{2+} mobilization from the cell.[6]

The present study, undertaken to investigate whether insulin has a similar modulatory role in cardiac cells, provides several novel observations. First, it provides evidence for a role of insulin in preserving Mg^{2+} content in cardiac cells by preventing the Mg^{2+} mobilization induced by β-adrenoceptor stimulation. Second, it indicates that insulin induces an accumulation of Mg^{2+} into cardiac cells through a transport mechanism that is linked to the operation of glucose transporter in the cardiac sarcolemma. Third and most important, it suggests that Mg^{2+} is indispensable for the accumulation of

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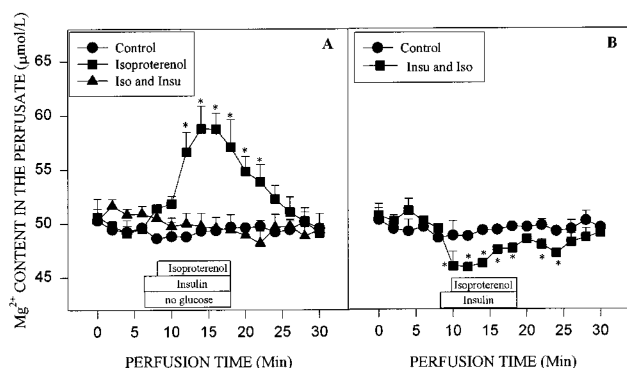

Figure 8. Inhibitory effect of insulin (Insu) on isoproterenol (Iso)-induced Mg^{2+} extrusion in the absence (A) or presence (B) of extracellular glucose. Mg^{2+} efflux from rat hearts perfused in a Langendorff retrograde manner was elicited by administration of 10 μmol/L Iso. Insu (10 mU/mL=6 nmol/L) was administered 3 minutes before Iso infusion. Perfusion medium contained 50 μmol/L $[Mg^{2+}]_o$, 5 mmol/L lactate, 5 mmol/L pyruvate, and 15 mmol/L glucose. In panel A, glucose was removed from the incubation medium during Insu and Iso infusion. In panel B, extracellular glucose was maintained throughout the experimental protocol. Data were determined every 30 seconds but are represented at 90-second intervals for clarity. Data are mean±SE of 4 different hearts for all the experimental conditions. Values of P are as follows for panels A and B: A, $^*P<0.05$ vs control and insulin-treated hearts. B, $^*P<0.05$ vs control.

glucose within cardiac myocytes. Although the physiological significance of the concomitant accumulation of glucose and Mg^{2+} needs further clarification, we can hypothesize that changes in cellular Mg^{2+} content are required for both proper glucose utilization and insulin signaling. These considerations may have particular importance in diabetes, a condition in which glucose transport and insulin signaling, as well as Mg^{2+} homeostasis, are markedly impaired.

Effect of Insulin on Cellular Mg^{2+} Homeostasis

The administration of insulin before isoproterenol (Figure 1) or cAMP addition (not shown) or after β-agonist administration (Figure 2) can completely prevent the extrusion of Mg^{2+} elicited via activation of the β-adrenergic signaling pathway. These effects of insulin can be explained by the ability of insulin to desensitize β-adrenoceptors [12,29] at the cell membrane and stimulate calmodulin-dependent phosphodiesterase,[13] thereby limiting the production and inducing a more rapid degradation of cellular cAMP. Overall, these results are consistent with the inhibitory effect observed previously in the perfused liver[6] and would indicate a more general and physiological role of insulin at modulating β-adrenoceptor–mediated Mg^{2+} extrusion in various tissues.

At variance with the observations involving liver cells,[6] insulin induces an entry of Mg^{2+} into cardiac cells. This accumulation can be reliably detected when $[Mg^{2+}]_o$ is progressively increased from 25 to 1000 μmol/L Mg^{2+}. Interestingly, under conditions in which $[Mg^{2+}]_o$ is >5 μmol/L, insulin-induced Mg^{2+} accumulation appears to prevail over isoproterenol-stimulated Mg^{2+} extrusion (Figures 2 and 8B). Consistent with the observations in liver cells[6] and the modality of action on β-adrenoceptors[12,29] and phosphodiesterase,[13] insulin still exerts a regulatory role on cellular Mg^{2+} homeostasis under conditions in which Mg^{2+} accumulation is prevented (ie, absence of extracellular glucose; Figure 8A). This may suggest that insulin and β-adrenergic agonists regulate cellular Mg^{2+} homeostasis by activating distinct Mg^{2+} transport mechanisms and that insulin can inhibit the Mg^{2+} extrusion mechanism as well as activate the Mg^{2+} entry pathway. Because of the novelty of this observation, the physiological conditions that determine the modality of insulin action on cardiac Mg^{2+} homeostasis require further investigation.

Because insulin does not stimulate Mg^{2+} accumulation in the perfused liver[6] regardless of $[Mg^{2+}]_o$ in the perfusion medium, cardiac but not liver cells must possess a specific entry mechanism activated by insulin. One of the main differences between cardiac and liver cells is the different class of glucose transporter present in the plasma membrane of these 2 cell types. In cardiac myocytes, insulin induces glucose accumulation by recruiting Glut4 (10- to 20-fold increase) and Glut1 transporters (2-fold increase) from a preconstituted intracellular pool to the sarcolemma.[26] The consequence of this recruitment is that glucose accumulation into cardiac myocytes increases severalfold and in a manner that is not simply proportional to the number of new transporters expressed in the sarcolemma.[30] By contrast, liver cells possess a distinct glucose transporter (Glut2) that is not affected in number and operation by insulin.[25]

Involvement of Glucose Transport in Insulin-Mediated Mg^{2+} Accumulation

The results obtained in the absence of external glucose or in the presence of the inhibitors of glucose transport, cytochalasin B and phloretin, are consistent with the idea that insulin-induced Mg^{2+} accumulation requires operation and/or internalization of glucose transporters. Because insulin administration increases the expression of Glut4 to a great degree and Glut1 only marginally in the sarcolemma, it is conceivable that Glut4 is the main class of glucose transporters involved in Mg^{2+} accumulation. Support for this hypothesis is provided by the effect of glucose transport inhibitors. Whereas phloretin blocks both Glut1 and Glut4 in the sarcolemma by interacting at the extracellular site of the transporter, cytochalasin B (by disrupting cytoskeletal integrity) mainly affects Glut4, by preventing the recruitment of this transporter to the sarcolemma after insulin administration. In addition, Mg^{2+} accumulation requires a minimal extracellular glucose concentration of 2 mmol/L to occur, which falls well within the K_m of the Glut4 transporter.[25] However, because Mg^{2+} accumulation increases proportionally with the extracellular glucose concentration, the additional involvement of the Glut1 transporter (with a higher K_m[25]) cannot be altogether excluded. Last, it is interesting to note that insulin and isoproterenol stimulate an accumulation and an extrusion of Mg^{2+}, respectively, whereas they both induce a glucose accumulation in cardiac cells. Because insulin primarily activates Glut4 and isoproterenol activates Glut 1,[25] further indirect evidence is provided for the role of Glut4 in Mg^{2+} accumulation.

Our data do not clearly indicate whether Mg^{2+} is cotransported with glucose or whether the cation enters the cell through a transport pathway distinct from the glucose trans-

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porters. However, the inhibitory effect of cytochalasin B or phloretin suggests that the Mg^{2+} entry mechanism is activated by insulin indirectly via glucose transporter operation. Moreover, it appears that glucose reintroduction can induce Mg^{2+} accumulation even after insulin is removed from the system (Figure 4A). Most likely, this phenomenon is due to the persistence of an activated Glut4 transporter in the sarcolemma that is able to transport glucose after its reintroduction. In view of the fact that a glucose-triggered Mg^{2+} accumulation has been observed in pancreatic beta cells,[31] the possibility that Mg^{2+} accumulation is generally associated with glucose transporter operation is a suggestive hypothesis that requires further investigation. Mg^{2+} uptake in cardiac myocytes appears to be 1 order of magnitude larger than glucose accumulation. Based on an estimated cell volume (Reference 3 and references therein), the amount of Mg^{2+} accumulated into cardiac myocytes after insulin administration in the presence of physiological $[Mg^{2+}]_o$ would result in a potential severalfold increase in cytosolic free $[Mg^{2+}]$. Yet, only minor changes in cytosolic free $[Mg^{2+}]$ were measured by fluorescent indicators in cells stimulated by insulin,[15,16] suggesting that accumulated Mg^{2+} is rapidly redistributed among intracellular organelles. At the present time, the absence of Mg^{2+} accumulation under conditions in which glucose is replaced with pyruvate and lactate would reasonably exclude the possibility that Mg^{2+} accumulation is associated with, or dependent on, energy production.

Evidence for Role of Cellular Mg^{2+} in Mediating Effects of Insulin

A dependence of insulin-induced glucose transport in rat cardiac myocytes on intracellular Mg^{2+} has been reported by Eckel et al.[32] The authors observed that insulin-stimulated glucose entry was completely abolished when EDTA buffer was used on A23187-treated myocytes[32] and proposed an involvement of Mg^{2+} in insulin signaling. Whether this involvement is at the level of Mg^{2+}-dependent hexokinase, cytoskeletal elements, or other intracellular enzymes involved in the translocation of glucose transporter to and from the sarcolemma is presently undefined. Evidence has been provided for a reduced autophosphorylation of insulin receptors and a reduced phosphorylation of insulin receptor–related kinases in Mg^{2+}-deficient animals[33] and for significant alterations in glycemia and glucose utilization in rats after a long-term Mg^{2+}-deficient diet.[34] Altogether, these observations strongly support a role of Mg^{2+} in modulating insulin response and cellular glucose utilization.

Conclusions

At the present time, we can only speculate about the physiological implication of Mg^{2+} accumulation in the heart. It is conceivable that Mg^{2+} plays 2 distinct though not mutually exclusive roles at the level of glucose entry and glucose utilization. As for glucose entry, Mg^{2+} may modulate the activity of cytoskeleton and kinases involved in the translocation of glucose transporters[35] or regulate allosterically glucose transport operation by changing the V_{max} of the transporter. A similar effect of extracellular Mg^{2+} on the inositol transporter in intestinal cells has been reported.[36] If corroborated by experimental evidence, either of these possibilities may explain why, after insulin stimulation, the rate of glucose transport increases by ≈20-fold versus the expected 10-fold increase calculated on the basis of the number of Glut4 recruited to the sarcolemma.[30] Alternatively, Mg^{2+} entry may be required to favor glucose utilization in cardiac cells, because many of the glycolytic enzymes, including hexokinase, are Mg^{2+} dependent to varying degrees. Our recent observation[37] that Mg^{2+} plays a regulatory role in the activity of several mitochondrial dehydrogenases supports the hypothesis that Mg^{2+} accumulated into the cell may be rapidly redistributed from the cytosol into the mitochondria or other organelles and regulate rates of respiration or concentrations of substrates necessary for specific metabolic pathways.

Together with previous evidence in the literature, the data reported in the present study indicate a close link between glucose transport and Mg^{2+} accumulation in cardiac ventricular myocytes. Although this link may be present in other cell types, it may be predominant in the heart, in which insulin modulates the operation of glucose transporters. Furthermore, indirect support for this link is provided by the observation that cellular Mg^{2+} levels and glucose utilization are markedly reduced in diabetic humans and animals. The relevance of this relation under both physiological and pathological conditions remains to be elucidated.

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