Intravesicular Glucose Modulates Magnesium^{2+} Transport in Liver Plasma Membrane From Streptozotocin-Treated Rats

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Plasma membrane vesicles purified from livers of 4-week-old streptozotocin-injected diabetic rats present an increased basal and cation-stimulated magnesium (Mg)$^{2+}$ transport as compared with vesicles purified from age-matched nondiabetic animals. Furthermore, diabetic basolateral membranes are unable to accumulate extravesicular Mg^{2+} in exchange for intravesicular sodium (Na)$^{+}$. Loading diabetic vesicles with varying concentrations of D-glucose, in addition to Mg^{2+}, renormalizes basal and Na^{+}- or calcium (Ca)$^{2+}$-induced Mg^{2+} extrusion in a dose-dependent manner, but does not restore Na^{+}/Mg^{2+} exchanger reversibility. A similar effect on Mg^{2+} extrusion is observed when D-glucose is replaced with 2-deoxy-glucose, amylopectin, or glycogen. The loading with 3-methyl-O-glucose or L-glucose, instead, affects basal and Na^{+}-dependent Mg^{2+} extrusion, but not Ca^{2+}-dependent Mg^{2+} fluxes. In contrast, loading the vesicles with hexoses other than glucose or varying extravesicular glucose concentration from 5 to 20 mmol/L does not modify basal or cation-stimulated Mg^{2+} fluxes. Taken together, these data indicate that basal and cation-stimulated Mg^{2+} transport across the hepatocyte plasma membrane is altered under diabetic conditions as a result of a decrease in intravesicular (intracellular) glucose.

TOTAL MAGNESIUM (Mg)$^{2+}$ concentration ranges between 14 and 20 mmol/L in mammalian cell types and tissues[1,2] and plays a key role in regulating numerous functions and enzymes in the cytoplasm and within cellular organelles.[1,2] Although no mammalian Mg^{2+} transport mechanism has been cloned to date, experimental evidence supports the presence of large Mg^{2+} fluxes in and out of the cell after various hormonal[3-6] and nonhormonal stimuli.[7,8] The amount of Mg^{2+} transported in either direction across the cell membrane is larger than cytosolic-free [Mg^{2+}], accounting for approximately 5% to 10% of total cellular Mg^{2+} content (~1 to 2 mmol/L). A sodium (Na)$^{+}$-dependent transport mechanism, putatively identified as a Na^{+}/Mg^{2+} exchanger, appears to be the predominant transporter in the majority of mammalian cells and tissues investigated.[2,4,5,9,10] Under conditions in which Na^{+} movement is inhibited by amiloride or imipramine,[5,11] or by the removal of extracellular Na^{+},[3,4,12] Mg^{2+} extrusion occurs via a not well characterized Na^{+}-independent pathway (see Gunther[2] as a review). The operation of both these Mg^{2+} transport mechanisms has been shown in purified liver plasma membrane vesicles.[13,14] In fact, we have located a bidirectional Na^{+}/Mg^{2+} exchanger in the basolateral domain and a unidirectional Na^{+}- and calcium (Ca^{2+})-dependent Mg^{2+} extrusion mechanism in the apical domain of the hepatocyte.[14]

In intact cells, the stimulation of β-adrenergic or glucagon receptors,[3-6,15] the administration of forskolin,[3-6] or cell permeant cyclic adenosine monophosphate (cAMP) analogs[3,4,15] all activate the Na^{+}/Mg^{2+} exchanger, most likely via a phosphorylation process.[12] It is worth noting that adrenergic-mediated Mg^{2+} extrusion in liver cells is associated with glycogen breakdown and glucose output, and that inhibition of glucose output by phloretin also decreases Mg^{2+} extrusion.[15] In contrast, agents that inhibit cAMP production[16] or activate the protein kinase C pathway[3,4,17] block Mg^{2+} extrusion or revert it into a Mg^{2+} uptake. Among these agents, insulin appears to play an important role. The pretreatment of liver[18] and cardiac cells[19] with insulin prevents or reverts β–adrenergic-stimulated, cAMP-mediated Mg^{2+} extrusion in both cell types. Moreover, insulin per se can induce an accumulation of Mg^{2+} in cardiac cells through a mechanism that involves glucose transport.[19] Clinical and experimental observations further support a role of insulin in regulating Mg^{2+} homeostasis. Serum and tissue Mg^{2+} content are decreased in both insulin-dependent diabetes mellitus (IDDM) and non–insulin-dependent diabetes mellitus (NIDDM) in patients as well as in animal models[20,21] through mechanisms that are largely undefined.

In the present study, purified total liver plasma membrane (tLPM), basolateral liver plasma membrane (bLPM), and apical liver plasma membrane (aLPM) vesicles were used to test the hypothesis that the decrease in liver Mg^{2+} content observed under diabetic conditions[22] results from an altered operation of the Mg^{2+} transport mechanisms and to determine the role of extravesicular (extracellular) and intravesicular (intracellular) glucose on transmembrane Mg^{2+} fluxes.

MATERIALS AND METHODS

Materials

All chemicals were of the purest analytical grade (Sigma, St Louis, MO). Nitrex nylon mesh was from Tetko (Briarcliff Manor, NY).

Methods

Diabetes induction. Overnight starved male Sprague-Dawley (200 g body weight) were randomly divided into 2 groups. The animals of 1 group were rendered diabetic by intraperitoneal (IP) injection of 65 mg/kg streptozotocin in citrate buffer (pH 4). After the injection, the animals had free access to food (Purina Chow, St Louis, MO) and water. Diabetes insurgence was assessed by determining glucose appearance in the urine (GlucoStrip, Fisher, Pittsburgh, PA) and glycemia in a small aliquot of blood (20 μL) withdrawn from the tail of the

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animal (Gluco-meter, Gemco, Hudson, OH). No insulin coverage was provided throughout the study.

Four weeks after the induction of diabetes, diabetic and age-matched nondiabetic animals were used as liver donors. The animals were anesthetized by IP injection of sodium pentobarbital solution (50 mg/kg body weight). At the time of sacrifice, diabetic animals presented a marked decrease in body weight versus nondiabetic animals (265 ± 15 *v* 449 ± 23 g, respectively, n = 12). Glycemia was approximately 4-fold higher than in nondiabetic controls (415 ± 20 *v* 92 ± 7 mg glucose/dL, respectively, n = 12). The abdomen of the animals was opened and the liver quickly removed and rinsed in 250 mmol/L sucrose. A portion of the organ was homogenized in 10% nitric acid (HNO_3) and extracted overnight. The denaturated protein was sedimented in microfuge tubes and the cation content of the acid extract (Mg^{2+}, Na^+ and K^+) measured by atomic absorbance spectrophotometry (AAS) in a Perkin Elmer 3100 (Norwalk, CT). The remainder of the organ was used for plasma membrane isolation. Livers from diabetic rats presented a reduced tissue content of Mg^{2+} (7.07 ± 0.77 *v* 8.96 ± 0.20 μmol/g wet tissue, $P < .05$) and K^+ (46.62 ± 2.28 *v* 68.13 ± 6.37 μmol/g wet tissue, $P < .007$) and an increased Na^+ content (21.55 ± 2.96 *v* 16.67 ± 1.82 μmol/g wet tissue, $P < .05$) compared with livers from nondiabetic rats (n = 10 for both groups of animals).

Isolation of total plasma membrane vesicles. tLPM vesicles were isolated and stored as described in detail elsewhere.[13] The purity of plasma membrane vesicles was assessed by using 5′-nucleotidase, cytochrome-*c* oxidase, and glucose 6-phosphatase activities as markers for plasma membrane, mitochondria, and endoplasmic reticulum, respectively.[13] Purified tLPM from diabetic and nondiabetic livers presented negligible levels of cytochrome *c* oxidase (<0.02 nmol cytochrome oxidized/mg protein/min in both fractions) and glucose 6 phosphatase activities (~0.2 and ~0.03 μmol Pi/mg protein/min in diabetic and nondiabetic tLPM, respectively). Diabetic and nondiabetic tLPM were both enriched approximately 2-fold to 3-fold in 5′-nucleotidase activity. These results indicate that, although not extremely enriched in tLPM, the preparation from diabetic livers presented a negligible contamination by mitochondria or endoplasmic reticulum components, comparable to that of nondiabetic tLPM. Glucose 6-phosphatase activity in diabetic livers was approximately 7-fold larger than that measured in nondiabetic livers, consistent with previous reports.[23] The orientation of loaded and unloaded tLPM vesicles was determined by measuring Na^+/K^+-adenosine triphosphatase (ATPase)[24] and 5′-nucleotidase[13] activities. The comparison of these activities to those of detergent-disrupted vesicles (considered as 100%) confirmed our previous report[13] that ≥ 90% of diabetic and nondiabetic tLPM were in the inside-in configuration after loading with Mg^{2+}. Na^+/K^+-ATPase activity in diabetic tLPM was greater than 80% lower than in nondiabetic tLPM ($P < .042$).

Negligible amounts of endogenous carryover K^+ and Mg^{2+} and adenine phosphonucleotides were detected by atomic absorbance spectrophotometry and high-performance liquid chromatography (HPLC), respectively, in both diabetic and nondiabetic tLPM vesicles.

Isolation of aLPM and bLPM plasma membrane vesicles. After isolation, tLPM were further purified into aLPM and bLPM as reported previously.[3] Alkaline phosphatase (1.13 ± 0.35 *v* 1.09 ± 0.20 μmol Pi/mg protein/min in nondiabetic *v* diabetic aLPM, respectively, n = 8, $P > .05$) and 5′-nucleotidase activities (1.35 ± 0.14 *v* 0.45 ± .03 μmol Pi/mg protein/min in nondiabetic *v* diabetic aLPM, respectively, n = 8, $P < .05$) for aLPM and Na^+/K^+-ATPase activity for bLPM (0.30 ± 0.05 *v* 0.045 ± 0.01 μmol Pi/mg protein/min in nondiabetic *v* diabetic bLPM, respectively, n = 8, $P < .05$) were used to assess fraction purity and vesicle orientation (see Cefaratti et al[14] for more detail).

Na^+/K^+-ATPase activity in diabetic bLPM was also lower than in nondiabetic bLPM (~70%, $P < .05$) while 5′-nucleotidase activity was approximately 60% lower ($P < .05$) in diabetic aLPM as compared with nondiabetic vesicles.

Loading of LPM. Aliquots of tLPM, aLPM, or bLPM were loaded with 20 mmol/L Mg^{2+}, Na^+ or Ca^{2+} as reported previously.[13,14] We have observed that the indicated Mg^{2+} concentration is optimal to study Mg^{2+} transport in this experimental model.[13] After removal of excess extravesicular cations by centrifugation,[13] the Mg^{2+}-loaded vesicles were resuspended in 5 mL of 250 mmol/L sucrose, 50 mmol/L Tris-Hepes, (pH 7.4) devoid of Mg^{2+} (*Mg^{2+}-free medium*) and stored on ice until used. Loading efficiency was assessed by treating the vesicles with ionophore (A23187) or detergent (Triton X-100), and measuring the amount of Mg^{2+} extruded in the extravesicular space or retained into the vesicle pellet by AAS.[13]

In the experiments in which plasma membrane vesicles were loaded with 20 mmol/L Mg^{2+} plus glucose, glucose derivatives, glycogen, amylopectin, or hexoses other than glucose, the reported concentration of any of these agents was added to the loading volume, and the mixture processed according to the protocol used for vesicles loaded with Mg^{2+} alone.

Measurement of Mg^{2+} fluxes. Mg^{2+} fluxes were measured by AAS. An aliquot of Mg^{2+}-loaded vesicles (tLPM, bLPM, or aLPM) was incubated in the Mg^{2+}-free medium mentioned above at 37°C under continuous stirring at a final concentration of approximately 300 μg protein/mL. After 2 minutes of equilibration, aliquots of the incubation mixture were withdrawn in duplicate at 2-minute intervals and the vesicles sedimented in microfuge tubes at 7,000 *g* for 45 seconds. Total Mg^{2+} content in the supernatants was measured by AAS. The pellet was digested overnight in 500 μL of 10% HNO_3. The following day the denaturated protein was sedimented in microfuge tubes and the Mg^{2+} content of the acid extract measured by AAS. The first time point after the equilibration period (t = 0) was used to establish a baseline. After withdrawing the sample, the concentrations of Na^+ or Ca^{2+} reported in the figures were added to the incubation mixture and the incubation continued for an additional 6 minutes. Because Mg^{2+} content in the supernatant varied considerably among preparations as a result of the loading carryover, the data are reported as the net variation in extravesicular Mg^{2+} content, normalized per milligram of protein, for simplicity. To calculate net Mg^{2+} extrusion, Mg^{2+} content in the supernatant at t = 0 minute was calculated and subtracted from the values of the subsequent time points of incubation. Similar experimental procedures were used for Na^+- or Ca^{2+}-loaded vesicles.

Glucose transport. To determine whether entrapped intravesicular glucose was cotransported with Mg^{2+}, a NADH-coupled hexokinase trap was used (enzymatic kit from Sigma). Plasma membrane vesicles were incubated as reported above for measuring Mg^{2+} fluxes. After sedimentation of the vesicles, aliquots of the supernatant were added to the NADH-coupled hexokinase mixture, and the disappearance of NADH was followed at 340 nm in a double-beam Varian spectrophotometer (Walnut Creek, CA). Alternatively, 1 μCi/mL ^{14}C-glucose was added as radioisotope label to cold glucose at the time of loading, and the loading procedure performed as described previously. Plasma membrane vesicles were incubated and sedimented as reported above in the presence of the NADH-coupled hexokinase trap to prevent glucose re-entry into the vesicles. Aliquots of the supernatants were withdrawn, and the radioactivity was measured in a Beckman LS7000 beta-counter.

Protein determination. Protein was measured according to the procedure of Lowry et al[25] using bovine serum albumin as a standard.

Statistical analysis. Data are presented as means ± SE. Data were first analyzed by 1-way analysis of variance (ANOVA). Multiple means were then compared by Tukey's multiple comparison test or by the Student-Newman-Keuls method performed with a level of statistical significance designated as $P \leq .05$

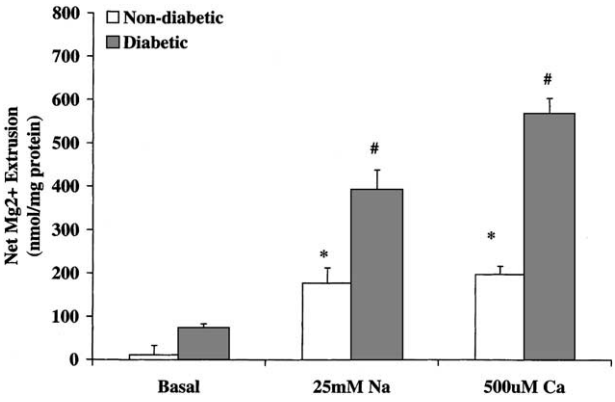

Fig 1. Mg^{2+} efflux from tLPM isolated from nondiabetic and diabetic rats and loaded with 20 mmol/L $MgCl_2$. TLPM vesicles were isolated and incubated as reported in Materials and Methods. Samples were withdrawn in duplicate at t = 0 (not reported in the figure) to establish a baseline. After withdrawal of the sample, 25 mmol/L NaCl or 500 μmol/L $CaCl_2$ was added to the incubation mixture and samples withdrawn in duplicate every 2 minutes. The baseline value was subtracted from the subsequent time points to determine net Mg^{2+} extrusion. As Mg^{2+} extrusion was already maximal at t = 2 minutes, only this point is reported in the figure. Data are means ± SE of 8 different preparations each incubated in triplicate. *Statistically significant *v* basal value; #statistically significant *v* the corresponding value in nondiabetic tLPM.

RESULTS

Under basal conditions, Mg^{2+}-loaded diabetic tLPM vesicles released spontaneously approximately 90 nmol Mg^{2+}/mg protein as compared with approximately 20 nmol/mg protein released from nondiabetic tLPM over a similar period of time (Fig 1). The addition of 25 mmol/L Na^+ or 500 μmol/L Ca^{2+} to the extravesicular milieu elicited an extrusion of Mg^{2+} from diabetic tLPM that was 3-fold to 4-fold larger than the extrusion elicited from nondiabetic vesicles under similar experimental conditions (Fig 1). These concentrations of Na^+ or Ca^{2+} were selected as they elicit maximal Mg^{2+} extrusion in nondiabetic tLPM.[13] In both diabetic and nondiabetic LPM, the Na^+ or Ca^{2+}-induced Mg^{2+} extrusion was already maximal at t = 2 minutes, the first time point we could experimentally detect, not changing significantly at later time points. Hence, all values are reported as net Mg^{2+} extrusion at t = 2 minutes for simplicity (see Materials and Methods).

As previously reported in nondiabetic LPM,[13] the Mg^{2+} extrusion elicited by Na^+ in diabetic LPM was specific and did not result from an osmotic mismatch (it could not be elicited by addition of 50 mmol/L KCl or LiCl, or 100 mmol/L sucrose) and was prevented by the Na^+-transport inhibitors, amiloride, imipramine, and quinidine (not shown). Furthermore, it occurred in the presence of identical concentrations of Mg^{2+} across the plasma membrane (eg, 20 mmol/L, data not shown).

The movement of Mg^{2+} across the plasma membrane of cardiac[19] and liver cells[15] is associated with glucose transport. Hence, the possibility that the enhanced Mg^{2+} transport in diabetic tLPM resulted from a diabetes-induced alteration in glucose homeostasis was investigated.

Varying extravesicular glucose concentration from 5 (~100 mg/dL) to 20 mmol/L (>400 mg/dL) to mimic the variation in glycemia from normal to severe diabetic conditions did not modify the amplitude of Mg^{2+} extrusion in control or cation-stimulated tLPM vesicles isolated from diabetic and nondiabetic Sprague-Dawley (Table 1). In contrast, the loading of diabetic tLPM vesicles with varying concentrations of D-glucose (from 5 to 50 mmol/L) in addition to 20 mmol/L Mg^{2+} resulted in a decreased extrusion of Mg^{2+} under basal and cation-stimulated conditions (Table 2). The effect occurred in a dose-dependent fashion and was maximal for tLPM loaded with 20 mmol/L glucose. The amplitude of Mg^{2+} fluxes from tLPM loaded with 50 mmol/L glucose, in fact, was not significantly different from that observed in vesicles loaded with 20 mmol/L glucose (Table 2). Diabetic tLPM loaded with 20 mmol/L D-glucose extruded an amount of Mg^{2+} comparable to that mobilized by nondiabetic tLPM vesicles loaded with Mg^{2+} alone under basal conditions (21.03 ± 11.85 *v* 23.50 ± 8.18 nmol/mg protein, respectively, n = 8, $P > .05$, Table 2) and following addition of extravesicular Na^+- or Ca^{2+} (Table 3). In contrast, Mg^{2+} fluxes from nondiabetic tLPM were unaffected by glucose loading (Table 3), irrespective of the concentration used (not shown). The effect of glucose could not be attributed to a reduced Mg^{2+} loading of the vesicles as the amount of Mg^{2+} entrapped within diabetic tLPM vesicles loaded with 20 mmol/L Mg^{2+} plus 20 mmol/L glucose was quantitatively similar to that of vesicles loaded with 20 mmol/L Mg^{2+} alone (~1,200 nmol Mg^{2+}/mg protein). Glucose loading resulted in a comparable carryover of extracellular glucose for diabetic and nondiabetic tLPM (~36 to 40 μmol/mL incubation, n =

Table 1. Net Mg^{2+} Fluxes From Nondiabetic and Diabetic tLPM Vesicles Loaded With 20 mmol/L Mg^{2+} and Incubated in the Presence of Varying Extravesicular Glucose Concentrations

	Nondiabetic tLPM			Diabetic tLPM		
$[Glucose]_0$	Control	Na^+	Ca^{2+}	Control	Na^+	Ca^{2+}
0 mmol/L	23.50 ± 8.18	142.17 ± 25.42	216.83 ± 28.36	90.89 ± 25.26	384.70 ± 58.35	500.53 ± 49.41
5 mmol/L	24.60 ± 4.52	149.73 ± 10.92	226.44 ± 17.28	83.38 ± 28.08	335.61 ± 92.57	453.53 ± 49.20
20 mmol/L	25.42 ± 6.61	130.04 ± 17.97	196.91 ± 21.52	106.74 ± 14.40	365.84 ± 55.40	445.76 ± 23.48

NOTE. Data represent net Mg^{2+} extrusion from tLPM, expressed as nmol Mg^{2+}/mg protein, in the extravesicular compartment at t = 2 minutes from the beginning of the incubation for control tLPM vesicles and the corresponding time for tLPM vesicles stimulated by the addition of extravesicular 25 mmol/L Na^+ or 500 μmol/L Ca^{2+}. Data are means ± SE of 8 preparations for both diabetic and nondiabetic tLPM. The experimental conditions were performed in quadruplicate and the data points withdrawn in duplicate for each preparation. All values in diabetic tLPM were statistically significant *v* the corresponding nondiabetic samples. Asterisks were omitted for simplicity.

Table 2. Net Mg^{2+} Fluxes From Diabetic tLPM Vesicles Loaded With 20 mmol/L Mg^{2+} Plus Varying Intravescular Glucose Concentrations

	Diabetic tLPM		
$[Glucose]_i$	Control	Na^+	Ca^{2+}
0 mmol/L	90.89 ± 25.26	384.7 ± 58.35	500.49 ± 49.41
5 mmol/L	89.07 ± 38.39	309.15 ± 59.61	476.33 ± 42.01
10 mmol/L	78.99 ± 13.03	297.99 ± 57.95	421.68 ± 23.58
20 mmol/L	21.03 ± 11.85*	150.11 ± 27.76*	246.22 ± 18.05*
50 mmol/L	33.50 ± 5.27*	134.32 ± 15.66*	262.59 ± 24.06*

NOTE. Data represent net Mg^{2+} extrusion from tLPM, expressed as nmol Mg^{2+}/mg protein, in the extravesicular compartment at t = 2 minutes from the beginning of the incubation for control tLPM vesicles, and the corresponding time for tLPM vesicles loaded with 20 mmol/L Mg^{2+} and the reported glucose concentrations, and stimulated by the addition of extravesicular 25 mmol/L Na^+ or 500 μmol/L Ca^{2+}. Data are means ± SE of 5 preparations. The experimental conditions were performed in quadruplicate and the data points withdrawn in duplicate for each preparation.

*Statistically significant *v* the corresponding sample incubated in the absence of extravesicular glucose.

15, for both vesicles) and did not change during the time of incubation irrespective of the experimental conditions.

To determine whether the effect of glucose was selective, diabetic tLPM vesicles were loaded with a variety of glucose-isomers or derivatives or with hexoses other than glucose. The results, reported in Table 3, indicate that 20 mmol/L 2-deoxy-glucose, amylopectin, glycogen, galactose, or maltose all exerted an effect comparable to that of D-glucose. The loading with 20 mmol/L L-glucose or 3-methyl-glucose decreased basal and Na^+-induced Mg^{2+} extrusion, but not Ca^{2+}-dependent Mg^{2+} mobilization. In contrast, the loading with other hexoses, such as mannose, fructose, or inositol did not affect basal and cation-stimulated Mg^{2+} fluxes.

Distinct Mg^{2+} transport mechanisms operate in different domains of hepatocyte plasma membrane.[14] A reversible Na^+/Mg^{2+} exchanger operates in the basolateral portion, whereas unidirectional Na^+- and Ca^{2+}-dependent Mg^{2+} extrusion mechanisms are located in the apical domain of the cell.[14] To determine whether the increased Na^+-dependent Mg^{2+} extrusion in diabetic LPM depended on the enhanced activity of both Na^+-activated transport mechanisms, bLPM and aLPM plasma membrane vesicles were purified. The distribution of marker enzymes indicates that diabetic and nondiabetic bLPM were approximately 10-fold enriched in Na^+/K^+-ATPase activity over their respective homogenates, although the activity in diabetic vesicles remained consistently lower than in nondiabetic vesicles. The enrichment in 5′-nucleotidase activity for diabetic and nondiabetic aLPM was approximately 2-fold and 10-fold, respectively, over their respective homogenates. The yield of diabetic bLPM was approximately 65% lower as compared with nondiabetic bLPM (~13% *v* 36.4%, respectively, $P < .05$), whereas the yield for diabetic aLPM (35%) was similar to that obtained for nondiabetic aLPM (38%).

Figure 2 show the amplitude of Mg^{2+} extrusion from diabetic and nondiabetic aLPM after the addition of maximal concentrations of extracellular Na^+ or Ca^{2+}.[13,14] Also, in aLPM and bLPM, Mg^{2+} extrusion was maximal at t = 2 minutes after Na^+ or Ca^{2+} administration. Hence, only this

Table 3. Mg^{2+} Fluxes From Nondiabetic and Diabetic Plasma Membrane Vesicles Loaded With 20 mmol/L Mg^{2+} Plus 20 mmol/L Glucose, Glucose Derivatives, Hexoses Other Than Glucose, Hexa-Carbon Moieties, or ATP

	Nondiabetic tLPM			Diabetic tLPM		
Loading	Control	Na^+	Ca^{2+}	Control	Na^+	Ca^{2+}
Mg^{2+} only	23.50 ± 8.18	142.17 ± 25.42	216.83 ± 28.36	90.89 ± 25.26	384.70 ± 58.35*	500.53 ± 49.41*
D-glucose	22.47 ± 3.98	147.24 ± 11.38	209.42 ± 24.16	21.03 ± 11.85†	150.11 ± 27.76†	246.22 ± 18.05†
L-glucose	30.07 ± 9.94	147.52 ± 17.47	201.27 ± 25.06	21.07 ± 12.15†	153.60 ± 20.92†	474.48 ± 81.23
2-deoxy-glucose	30.81 ± 7.31	89.15 ± 28.37	157.25 ± 23.21	22.82 ± 14.27†	79.02 ± 16.65†	208.91 ± 20.99†
3-deoxy-glucose	ND	ND	ND	20.59 ± 12.93†	159.02 ± 32.47†	253.49 ± 29.79†
3-methyl-D-glucose	30.77 ± 6.17	103.28 ± 20.89	99.29 ± 18.61	25.41 ± 11.11†	149.12 ± 16.37†	529.67 ± 88.29
Amylopectin	ND	ND	ND	20.71 ± 13.15†	167.92 ± 32.57†	383.17 ± 51.60
Glycogen	24.38 ± 6.15	171.49 ± 25.15	206.96 ± 18.10	27.64 ± 7.13†	141.13 ± 19.77†	145.50 ± 6.67†
Galactose	ND	ND	ND	19.35 ± 13.11†	168.34 ± 15.96†	213.18 ± 20.82†
Maltose	ND	ND	ND	25.44 ± 10.44†	121.36 ± 18.76†	256.25 ± 43.53†
Fructose	33.65 ± 6.09	185.50 ± 28.92	228.01 ± 69.92	32.37 ± 9.04†	225.39 ± 27.71	482.74 ± 70.53
Mannose	ND	ND	ND	63.82 ± 18.66	322.51 ± 38.38	484.09 ± 64.83
Inositol	28.23 ± 9.21	189.00 ± 28.56	248.28 ± 20.20	81.03 ± 13.67	280.05 ± 21.04	491.13 ± 64.93

NOTE. Data represent net Mg^{2+} extrusion from tLPM, expressed as nmol Mg^{2+}/mg protein, in the extravesicular compartment at t = 2 minutes from the beginning of the incubation for control tLPM vesicles, and the corresponding time for tLPM vesicles stimulated by the addition of extravesicular 25 mmol/L Na^+ or 500 μmol/L Ca^{2+}. Data are means ± SE of 8 preparations for both diabetic and nondiabetic tLPM. The experimental conditions were performed in quadruplicate and the data points withdrawn in duplicate for each preparation.

Abbreviation: ND, nondetermined.

*Statistically significant *v* the corresponding nondiabetic values.

†Statistically significant *v* the corresponding diabetic vesicles loaded with Mg^{2+} alone.

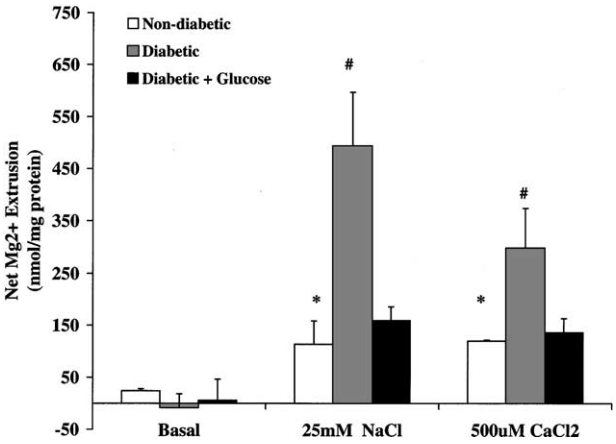

Fig 2. Mg^{2+} efflux from aLPM isolated from nondiabetic and diabetic rats and loaded with 20 mmol/L $MgCl_2$ in the absence or in the presence of 20 mmol/L D-glucose. ALPM vesicles were isolated and incubated as reported in Materials and Methods. Samples were withdrawn in duplicate at t = 0 (not reported in the figure) to establish a baseline. After withdrawal of the sample, 25 mmol/L NaCl or 500 μmol/L $CaCl_2$ were added to the incubation mixture and samples withdrawn in duplicate every 2 minutes. The baseline value was subtracted from the subsequent time points to determine net Mg^{2+} extrusion. For simplicity, only the value at t = 2 minutes after the addition of extravesicular Na^+ or Ca^{2+} is reported. Data are means ± SE of 8 different preparations each incubated in triplicate. *Statistically significant *v* basal value; #statistically significant *v* the corresponding value in nondiabetic and diabetic aLPM loaded with Mg^{2+} plus glucose.

time point is presented in this and the following figures for simplicity. As expected based on the results reported in Fig 1, the addition of 500 μmol/L Ca^{2+} elicited a 2-fold larger extrusion of Mg^{2+} in diabetic versus nondiabetic aLPM (~300 *v* ~150 nmol/mg protein/2 min, respectively, $P < .05$, Fig 2). The Na^+-dependent Mg^{2+} extrusion from these vesicles was approximately 4-fold larger than in nondiabetic aLPM (~500 *v* 120 nmol Mg^{2+}/mg protein/2 min, respectively). When aLPM were loaded with 20 mmol/L Mg^{2+} and 20 mmol/L D-glucose, the extrusion of Mg^{2+} elicited by 500 μmol/L Ca^{2+} was reduced by approximately 40%, decreasing from 298.99 ± 36.70 nmol Mg^{2+}/mg protein/2 min in the absence of glucose to 136.23 ± 26.60 nmol Mg^{2+}/mg protein/2 min in its presence (n = 6, $P < .05$, Fig 2). The Mg^{2+} extrusion elicited by 25 mmol/L Na^+ was also markedly reduced (from 493.39 ± 103.13 nmol Mg^{2+}/mg protein/2 min in the absence of intravesicular glucose to 158.90 ± 74.60 nmol Mg^{2+}/mg protein/2 min in its presence, or minus 67%, n = 6, $P < .05$).

The Na^+-dependent Mg^{2+} extrusion in diabetic bLPM, instead, was 2-fold larger than in nondiabetic bLPM (Fig 3) at all the Na^+ concentrations tested. When stimulated by the addition of 1 mmol/L NaCl, diabetic bLPM released 63.58 ± 12.34 versus 33.35 ± 10.56 nmol/mg protein/2 min mobilized from nondiabetic bLPM (n = 6, $P < .05$). In both diabetic and nondiabetic bLPM, maximal Mg^{2+} extrusion was elicited by addition of 10 mmol/L extravesicular Na^+ (Fig 3). No Ca^{2+}-dependent Mg^{2+} extrusion was observed in diabetic bLPM (Fig 3). In glucose-loaded diabetic bLPM, the Mg^{2+} extrusion prompted by 10 mmol/L extravesicular Na^+ was reduced to 89.70 ± 17.74 from 184.77 ± 25.02 nmol Mg^{2+}/mg protein in the absence of intravesicular glucose, n = 8, $P < .05$ (Fig 3). In both aLPM and bLPM vesicles, glucose derivatives affected the amplitude of Na^+- and Ca^{2+}-induced Mg^{2+} extrusion to an extent similar to that reported in Table 3 for tLPM vesicles (not shown).

In nondiabetic bLPM the Na^+/Mg^{2+} exchanger can operate in either direction based on the Na^+ and Mg^{2+} concentrations across the vesicle membrane (Fig 4,).[14] This capability appears to be lost in diabetic bLPM (Fig 4) irrespective of the concentration of Na^+ used during the loading procedure, the concentration of Mg^{2+} added in the extravesicular compartment (not shown), or the presence of D-glucose or glucose derivatives within the vesicles (Fig 4).

DISCUSSION

In the last decade, several laboratories have provided evidence that Mg^{2+} can cross the plasma membrane of mammalian cells in either direction after specific hormonal or nonhormonal stimulation. Data obtained in purified tLPM,[13] bLPM,[14] and aLPM vesicles[14] indicate the operation of a reversible Na^+/Mg^{2+} exchanger in the basolateral domain and 2 unidirectional Mg^{2+} extrusion mechanisms activated by Na^+ and Ca^{2+}, respectively, in the apical portion of the hepatocytes cell membrane.[14]

Clinical and experimental evidence[20,21] indicate that both type 1 and type 2 diabetes are characterized by a decrease in Mg^{2+} content in the blood or within tissues. A study in progress in our laboratory[22] indicates that the decrease in total

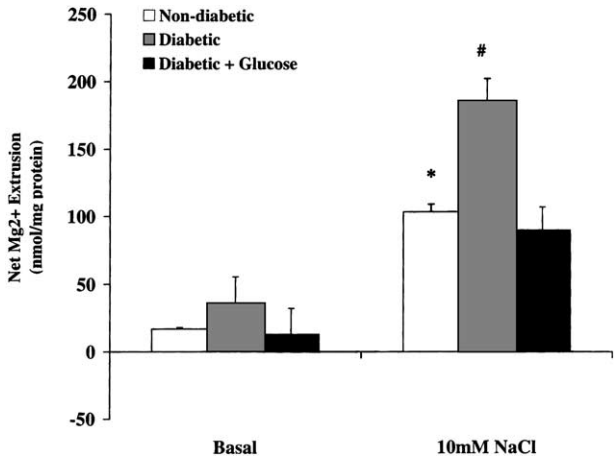

Fig 3. Mg^{2+} efflux from bLPM isolated from nondiabetic and diabetic rats and loaded with 20 mmol/L $MgCl_2$ in the absence or in the presence of 20 mmol/L D-glucose. BLPM vesicles were isolated and incubated as reported in Materials and Methods. Samples were withdrawn in duplicate at t = 0 (not reported in the figure) to establish a baseline. After withdrawal of the sample, 25 mmol/L NaCl was added to the incubation mixture and samples withdrawn in duplicate every 2 minutes. The baseline value was subtracted from the subsequent time points to determine net Mg^{2+} extrusion. For simplicity, only the value at t = 2 minutes after the addition of extravesicular Na^+ or Ca^{2+} is reported. Data are means ± SE of 8 different preparations each incubated in triplicate. *Statistically significant *v* basal value; #statistically significant *v* the corresponding value in nondiabetic and diabetic bLPM loaded with Mg^{2+} plus glucose.

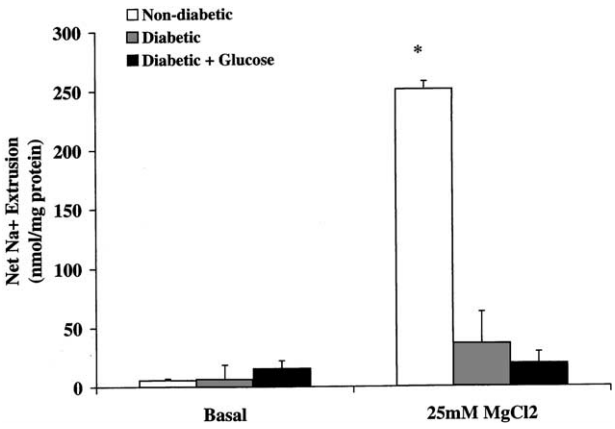

Fig 4. Na^+ efflux from bLPM isolated from nondiabetic and diabetic rats. BLPM vesicles were isolated, loaded with 20 mmol/L NaCl, and incubated as reported in Materials and Methods. Samples were withdrawn in duplicate at t = 0 minute (not reported in the figure) to establish a baseline. After withdrawal of the sample, 25 mmol/L $MgCl_2$ was added to the incubation mixture and samples were withdrawn in duplicate every 2 minutes. The baseline value was subtracted from the subsequent time points to determine net Mg^{2+} extrusion. For simplicity, only the value at t = 2 minutes after the addition of extravesicular Mg^{2+} is reported. Data are means ± SE of 8 different preparations each incubated in triplicate. *Statistically significant *v* basal value.

Mg^{2+} content detected in skeletal muscles and in the liver of streptozotocin-injected rats is associated with an increased Na^+ content. Yet, the mechanisms responsible for such an altered ion pattern are still undefined.

In the present study, highly purified tLPM, bLPM, and aLPM vesicles were used to test the hypothesis that the changes in total Mg^{2+} and Na^+ content observed in diabetic liver resulted from an altered operation of specific Mg^{2+} transport mechanisms and to evaluate the role of intra- and extracellular glucose on the amplitude of Mg^{2+} fluxes.

Plasma Membrane Purification

The isolation of tLPM from diabetic livers and their subsequent fractionation in aLPM and bLPM indicate that all these vesicles have an orientation (> 90% inside-in) similar to that of nondiabetic plasma membranes and a negligible contamination by mitochondria or endoplasmic reticulum components. The yield of diabetic aLPM versus nondiabetic vesicles, evaluated by 5′-nucleotidase or alkaline phosphatase assay is markedly reduced, while the amount of protein recovered is essentially similar for diabetic and nondiabetic vesicles (35% and 38%, respectively). The yield of diabetic bLPM, instead, is lower than that of nondiabetic vesicles based on both protein recovery (12% *v* 36%, respectively) and Na^+/K^+-ATPase activity (>80% reduced in diabetic vesicles). Despite the reduced yield, bLPM vesicles, like aLPM and tLPM vesicles, are greater than 90% in inside-in configuration.

Mg^{2+} Transport

Mg^{2+} transport across liver plasma membrane is markedly altered under diabetic conditions. In diabetic LPM, the addition of Na^+ or Ca^{2+} elicits fluxes of Mg^{2+} that are 2-fold to 3-fold larger than those observed in LPM from nondiabetic animals, thus suggesting that the reduced Mg^{2+} content in the liver of diabetic rats results from an increased operation of the Mg^{2+} transport mechanisms located in both apical and basolateral domains of the hepatocytes cell membrane. As Mg^{2+} loading is essentially the same for both experimental groups, the larger fluxes of Mg^{2+} in diabetic vesicles are likely to depend on an increased activity rate of the Mg^{2+} transporters, and/or an enhanced number of copies of these transporters. The limited information in terms of regulation and structure of the Mg^{2+} transporters prevents us from discriminating between these possibilities. In addition, our results indicate that diabetic bLPM are unable to accumulate Mg^{2+} in exchange for intravesicular Na^+ (Fig 4) via reverse operation of the Na^+/Mg^{2+} antiporter. Whether this defect is consequent to structural/functional modifications of the transporter or loss of a subpopulation of bLPM in which this transporter operates (the yield of diabetic bLPM is only one third of that of nondiabetic vesicles) is presently undefined and is a topic for future investigation. However, as hepatocytes isolated from diabetic rats are also unable to accumulate Mg^{2+} after in vitro stimulation by vasopressin, or phorbol-myristate acetate,[22] it can be excluded that the defect in Mg^{2+} uptake is an artifact of bLPM purification.

Glucose Effect on Mg^{2+} Fluxes

Changes in extra and intravesicular glucose concentration in an order of magnitude similar to those observed under diabetic conditions have a different effect on Mg^{2+} transport in LPM vesicles. Varying extravesicular glucose concentration does not significantly modify Mg^{2+} fluxes. In contrast, loading tLPM, bLPM, or aLPM with glucose decreases basal and cation-stimulated Mg^{2+} extrusion in a dose-dependent fashion, returning the amplitude of Mg^{2+} fluxes to values comparable to those observed in nondiabetic LPM. This effect is specific for glucose, as it can be mimicked to a comparable extent by glucose derivatives, but not by other hexoses (Table 3). Mg^{2+} loading is unaffected by the presence of intravesicular glucose, thus excluding that the hexose effect depends on a reduced amount of Mg^{2+} entrapped within the vesicles. The observation that the loading with 3-methyl-O-glucose or L-glucose only affects basal and Na^+-dependent Mg^{2+} extrusion, but not the Ca^{2+}-activated pathway and the amplitude of Mg^{2+} extrusion from vesicles loaded with hexoses other than glucose, also argue against a reduced Mg^{2+} loading. The possibility that glucose and glucose derivatives exert a chelating effect on intravesicular Mg^{2+} is not fully supported by the relative low-binding affinity of glucose and glucose derivatives for Mg^{2+}.[26]. Furthermore, these affinity values are not different from those of the other hexoses tested in the present study,[26] and therefore cannot entirely explain (1) the specificity of glucose effect; (2) the presence of such an effect only in vesicles from diabetic livers, and (3) the differential response to extravesicular Na^+ or Ca^{2+} observed under certain loading conditions (Table 3). No difference in glucose cycling across the plasma membrane is observed between diabetic and nondiabetic LPM under basal or cation-stimu-

lated conditions, thus excluding that glucose transport across the plasma membrane is a limiting factor for Mg^{2+} transport. In addition, some of the glucose derivatives that reduce Mg^{2+} fluxes (eg, 2-deoxy-glucose) do not cycle effectively across the plasma membrane. Presently, we do not have a clear explanation at hand for this phenomenon. Yet, the ability of glucose and only certain glucose derivatives to affect specifically the Na^+/Mg^{2+} antiporter would suggest an allosteric interaction of these molecules at a specific intravesicular site that is modified under diabetic conditions. Glucose loading, however, does not restore the reversibility of the bidirectional Na^+/Mg^{2+} exchanger in diabetic bLPM, implying that either glucose is not involved in regulating the modus operandi of the Mg^{2+} entry mechanism or it cannot revert the diabetes-induced modifications of the Na^+/Mg^{2+} exchanger.

Conclusions

The data reported here indicate that plasma membranes from diabetic livers are unable to accumulate Mg^{2+}, but extrude large amounts of Mg^{2+} under basal and cation-stimulated conditions. Our data also indicate that intravesicular glucose contributes significantly to renormalize the amplitude of Mg^{2+} extrusion. How glucose modulates Mg^{2+} transport and why this effect is lacking on the Na^+/Mg^{2+} exchanger reversibility are questions in need of further investigation.

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