Differential Localization and Operation of Distinct Mg$^{2+}$ Transporters in Apical and Basolateral Sides of Rat Liver Plasma Membrane*

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Upon activation of specific cell signaling, hepatocytes rapidly accumulate or release an amount of Mg$^{2+}$ equivalent to 10% of their total Mg$^{2+}$ content. Although it is widely accepted that Mg$^{2+}$ efflux is Na$^{+}$-dependent, little is known about transporter identity and the overall regulation. Even less is known about the mechanism of cellular Mg$^{2+}$ regulation. Even less is known about the mechanism of uptake. Using sealed and right-sided rat liver plasma membrane vesicles representing either the basolateral (bLPM) or apical (aLPM) domain, it was possible to dissect three different Mg$^{2+}$ transport mechanisms based upon specific inhibition, localization within the plasma membrane, and directionality. The bLPM possesses only one Mg$^{2+}$ transporter, which is strictly Na$^{+}$-dependent, bi-directional, and not inhibited by amiloride. The aLPM possesses two separate Mg$^{2+}$ transporters. One, similar to that in the bLPM because it strictly depends on Na$^{+}$ transport, and it can be differentiated from that of the bLPM because it is unidirectional and fully inhibited by amiloride. The second is a novel Ca$^{2+}$/Mg$^{2+}$ exchanger that is unidirectional and inhibited by amiloride and imipramine. Hence, the bLPM transporter may be responsible for the exchange of Mg$^{2+}$ between hepatocytes and plasma, and vice versa, shown in livers upon specific metabolic stimulation, whereas the aLPM transporters can only extrude Mg$^{2+}$ into the biliary tract. The dissection of these three distinct pathways and, therefore, the opportunity to study each individually will greatly facilitate further characterization of these transporters and a better understanding of Mg$^{2+}$ homeostasis.

Magnesium, the second most abundant cation within mammalian cells, is necessary for a variety of metabolic and cellular functions (1–5). Under resting conditions, cellular-free Mg$^{2+}$ concentration is held at 0.5–0.8 mM, well below its predicted electrochemical equilibrium, and approximately 5 mM Mg$^{2+}$ is complexed with ATP and other metabolites (1, 5). In several tissues such as heart and liver, specific hormonal or metabolic stimulation causes, within a few minutes, a massive mobilization of cellular Mg$^{2+}$ (6–9). Although cytosolic-free Mg$^{2+}$ undergoes minimal changes, several intracellular signals leading to a cAMP increase induce a loss of 5–10% of total cellular Mg$^{2+}$ (6–9). Conversely, signals activating protein kinase C result in an accumulation of Mg$^{2+}$ and a consequent increase of total cellular Mg$^{2+}$ by 5–10% (10).

These findings underscore the operation of a very powerful Mg$^{2+}$ transport machinery within the plasma membranes of mammalian cells. Yet, the Mg$^{2+}$ transporter(s) has not been isolated, and even the basic kinetic properties of Mg$^{2+}$ cellular transport remain confusing and contradictory. For example, the dependence of Mg$^{2+}$ release on extracellular Na$^{+}$ has been established in invertebrate and mammalian cells (11–16) but Na$^{+}$-independent pathways have also been reported (1, 8, 9, 17, 18). Equally contradictory are the findings of variable Na$^{+}$/Mg$^{2+}$ stoichiometries (1, 19), the role of trans-membrane potential, and the partial and variable effectiveness of inhibitors such as amiloride (for review see Refs. 1, 5, 18, and 20). In principle, these discrepancies could be accounted for by the co-existence of separate plasma membrane Mg$^{2+}$ transporters each operating with different mechanisms and sensitivity to inhibitors. Hence, this work was designed to dissect putatively different Mg$^{2+}$ transporters using an appropriate cellular model, the plasma membrane of rat hepatocytes.

In view of its size and secretory capacity, the liver should play a major role in Mg$^{2+}$ homeostasis. Recent evidence in the literature demonstrates that the perfused liver or isolated hepatocytes can accumulate or release very large amounts of Mg$^{2+}$ upon specific metabolic stimulation (7, 8). Recently, this laboratory was able to successfully obtain sealed liver plasma membranes (LPM)1 to study Mg$^{2+}$ transport across the hepatocyte cell membrane (21). This model provides well defined and controllable experimental conditions in which membrane transport can be quantitatively investigated in the absence of interfering intracellular signaling pathways or transport by organelles. Using sealed plasma membranes, we recently found that either extravesicular Na$^{+}$ or Ca$^{2+}$ could mobilize intravesicular Mg$^{2+}$ (21).

A major difference in liver plasma membrane vesicles is that they release intravesicular Mg$^{2+}$ in the presence of extravesicular Na$^{+}$ or Ca$^{2+}$ alone, without the need of metabolic stimulation (21) necessary in intact hepatocytes (7, 8). Under these conditions, cellular Mg$^{2+}$ transport appears “uncoupled” and maximally operating in the absence of stimulatory cellular signals. One intriguing possibility is the presence in hepatocytes of a physiological “break” on Mg$^{2+}$ transport, which could be transiently removed as the result of activation of cellular pathways. Such a break (i.e. protein phosphorylation and dephosphorylation) appears to be absent in LPM probably because they are devoid of ATP and signaling pathways.

Those studies also provided direct evidence that extracellular Na$^{+}$ and Ca$^{2+}$ elicited a larger Mg$^{2+}$ efflux than either cation alone and suggested the presence of distinct Na$^{+}$/Mg$^{2+}$ and Ca$^{2+}$/Mg$^{2+}$ transporters (21). Unfortunately, both putative

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1 The abbreviations used are: LPM, liver plasma membranes; bLPM, basolateral liver plasma membranes; aLPM, apical liver plasma membranes; AAS, atomic absorption spectrophotometry.
transporters seemed to be inhibited by the same inhibitor. Additionally, it was not clear whether the two transport mechanisms were localized within the same vesicle or in two distinct populations of vesicles.

The hepatocyte is a highly polarized cell with differential localization of enzymes or transporters in the two main membrane domains, the basolateral and apical plasma membrane. The present study has now taken advantage of the possibility of obtaining separate populations of sealed and properly oriented plasma membranes comprised mostly of either apical (aLPM) or basolateral (bLPM) domains. The data presented here demonstrate that the Na\(^+\)-dependent and Ca\(^{2+}\)-dependent Mg\(^{2+}\) transporters are distinct in terms of localization within the plasma membrane, inhibition, and overall operation. The data indicate that two distinguishable Na\(^+\)-dependent Mg\(^{2+}\) exchange mechanisms are localized to both the basolateral and apical domain, whereas a Ca\(^{2+}\)/Mg\(^{2+}\) exchanger operates only in the apical domain. Such a finding should be of primary importance to kinetically characterize individual Mg\(^{2+}\) transporters and to better identify them.

**EXPERIMENTAL PROCEDURES**

**Plasma Membrane Isolation**—Total LPM were isolated as described previously (21, 22). Briefly, male Harlan Sprague-Dawley rats (250–350 g) were anesthetized by intraperitoneal injection of 50 mg of pentobarbital/kg of body weight. The abdomen was opened and the liver was perfused by the portal vein with 50 ml of isolation medium: 250 mM sucrose, 5 mM K-Hepes, 1 mM EGTA (pH 7.4), at 37 °C. The liver was rapidly removed, finely minced, washed twice in the isolation medium, and homogenized in 50 ml of medium using 10 passes with a tight fitting pestle. The homogenate was diluted to 6% (w/v) with the same buffer, and the homogenate was diluted to 6% (w/v) with the same buffer and sedimented at 1400 g for 30 min. The resulting pellets were diluted to a concentration of 5 mg/ml and homogenized by 75 passes with a tight fitting pestle at 4 °C as described previously (21).

**Homogenate and homogenated in 50 ml of medium using 10 passes with a loose fitting pestle.**

**Percoll (Amersham Pharmacia Biotech) was added to the resuspension in the proportion of 1.4 ml of biotin at 6% (final concentration) in the isolation medium by four passes with the tight fitting pestle.**

**Total LPM were isolated as described previously (21).** All chemicals and assays were of the purest analytical grade (Sigma). Nitrex nylon mesh was obtained from Tetko, Inc. (Briarcliff Manor, NY). Percoll was from Amersham Pharmacia Biotech.

**Statistical Analysis**—Data are presented as mean ± S.E. Data were first analyzed by one-way ANOVA (analysis of variance). Multiple means were then compared by the Student-Newman-Keuls method.

**RESULTS**

**Purity and Orientation of bLPM and aLPM after Isolation**—The purity and enrichment of bLPM and aLPM were assessed using 5'-nucleotidase, cytochrome c oxidase, and glucose 6-phosphatase activities as markers for plasma membrane, mitochondria, and endoplasmic reticulum, respectively (see Table I). Alkaline phosphatase, Na\(^+/K\)-ATPase, and 5'-nucleotidase were used to assess the purity of bLPM and aLPM. In addition, Na\(^+/K\)-ATPase and 5'-nucleotidase were used to assess LPM orientation (see Table I).

**Plasma Membrane Loading**—5-ml aliquots of bLPM or aLPM vesicles were resuspended in 25 ml of incubation medium (1.5 μv) in the presence of 20 mM MgCl\(_2\) or, when specified, Na\(^+\) or Ca\(^{2+}\) and loaded. The Na\(^+\)-loaded vesicles was assessed using freshly isolated LPM or vesicles quickly frozen and stored in liquid nitrogen until used (within one week). All procedures were carried out at 4 °C.

**Plasma Membrane Purity and Orientation**—The purity of the LPM vesicles was assessed using 5'-nucleotidase, cytochrome c oxidase, and glucose 6-phosphatase activities as markers for plasma membrane, mitochondria, and endoplasmic reticulum, respectively (see Table I). Alkaline phosphatase, Na\(^+/K\)-ATPase, and 5'-nucleotidase were used to assess the purity of bLPM and aLPM. In addition, Na\(^+/K\)-ATPase and 5'-nucleotidase were used to assess LPM orientation (see Table I).

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previously, correct (inside-in) orientation of LPM is greatly
affected by the presence of Mg$^{2+}$, which has been shown to
induce correct membrane sidedness (27, 28). The sidedness of
LPM was determined as previously reported (21). Both the
Mg$^{2+}$-loaded aLPM and bLPM were approximately 80%
inside-in.

Dose Response of Na$^+$ and Ca$^{2+}$ Induced Mg$^{2+}$ Efflux
from bLPM and aLPM and the Effect of Various Inhibitors—Fig. 1A
shows that bLPM loaded with 20 mM MgCl$_2$ and suspended
in the absence of Na$^+$ and Ca$^{2+}$ released negligible Mg$^{2+}$ over
several min of incubation (control). The addition of increasing
concentrations of extravesicular NaCl induced a dose-depend-
ent efflux of Mg$^{2+}$, which was maximal at 10 mM NaCl (138.1 ±
15.1 nmol of Mg$^{2+}$/mg of protein, n = 4). Concentrations of
NaCl larger than those reported in the figure (e.g. 25 or 50 mM)
did not induce a statistically significant increase in Mg$^{2+}$ efflux
(142.9 ± 12.5 nmol of Mg$^{2+}$/mg of protein, n = 5, for 25 mM
NaCl versus 138.1 ± 15.1 nmol of Mg$^{2+}$/mg of protein, n = 4,
for 10 mM NaCl). Irrespective of the concentration of NaCl used,
Mg$^{2+}$ efflux at 37 °C was maximal within the first min after the
Na$^+$ addition (Fig. 1). In contrast to what was previously ob-
erved in the total LPM population (21), the addition of 500 μM
CaCl$_2$ to bLPM elicited a negligible release of Mg$^{2+}$ (18.4 ±
6.0 nmol of Mg$^{2+}$/mg of protein as compared with 267.7 ±
17.0 in the total LPM population (21).

Several agents have been reported to inhibit the Na$^+$/Mg$^{2+}$
transporter. Amiloride has been shown to effectively inhibit
Mg$^{2+}$ extrusion via the putative Na$^+$/Mg$^{2+}$ exchanger in sev-
eral mammalian cell types, including hepatocytes (5, 8, 14, and
18). Amiloride also has been shown to inhibit the Na$^+/H^+$
exchanger as well as Na$^+$ entry pathways in epithelia (29, 30).
In addition, Feray and Garay (13) demonstrated that tricyclic
antidepressant drugs, such as imipramine, are nonspecific in-
hibitors of Mg$^{2+}$ transport, i.e. Na$^+$/Mg$^{2+}$ exchange, in human
red cells. Interestingly, the addition of 1 mM amiloride to bLPM
did not inhibit Na$^+$-induced Mg$^{2+}$ efflux (Fig. 1B) as compared
with a 50% inhibition observed in the total LPM population
(21). In fact, 1 mM amiloride enhances the Na$^+$-induced Mg$^{2+}$
extrusion 3 min after addition of NaCl (Fig. 1B), a phenomenon
for which we have no explanation. By contrast, the addition of
200 μM imipramine effectively inhibited Na$^+$-induced Mg$^{2+}$
mobilization up to approximately 85% (Fig. 1B).

Fig. 2, A and B, illustrate the Na$^+$ and Ca$^{2+}$ dose-dependent
effect, respectively, on Mg$^{2+}$ efflux in aLPM. In Fig. 2A maxi-
mal Mg$^{2+}$ efflux is induced by much greater concentrations of
NaCl than in bLPM (e.g. 25 mM induces an efflux of 184.7 ±
36.7 nmol of Mg$^{2+}$/mg of protein). At variance from bLPM,
Ca$^{2+}$ could also induce Mg$^{2+}$ efflux in these vesicles. The maxi-
mal Mg$^{2+}$ efflux induced by 500 μM CaCl$_2$ was 227.2 ±
50.2 nmol of Mg$^{2+}$/mg of protein, n = 4 (Fig. 2B). The concentra-
tions of Na$^+$ or Ca$^{2+}$ able to elicit maximal Mg$^{2+}$ efflux from aLPM
are very similar to those effective in the total LPM population
(21). Also, in contrast to bLPM, 1 mM amiloride inhibited both
Na$^+$- and Ca$^{2+}$-induced Mg$^{2+}$ efflux in aLPM to a comparable
extent (Fig. 2C). 200 μM imipramine was equally effective at
inhibiting both Mg$^{2+}$ extrusion mechanisms (not shown).

Partial Kinetics of Mg$^{2+}$ Efflux—The Mg$^{2+}$ efflux at 37 °C
shown in the previous experiments was already maximal at the
first time observation point, after 2 min. Hence, it was difficult
to distinguish whether the Mg$^{2+}$ appearing in the supernatant
was the result of net efflux and/or the release of Mg$^{2+}$ from
surface binding sites. Some kinetic resolution can be achieved
by lowering the temperature of incubation. Fig. 3, A and B,
show the rates of Na$^+$- or Ca$^{2+}$-dependent Mg$^{2+}$ extrusion
in the supernatant from 20 mM MgCl$_2$-loaded bLPM (Fig. 3A)
or aLPM (Fig. 3B) incubated in a thermostated vessel at 10 °C.

Under these conditions, the efflux of Mg$^{2+}$ is comparable, but
the rates of release are much slower and kinetically
distinguishable.

Specificity of Transport—Na$^+$-dependent Mg$^{2+}$ extrusion in

FIG. 1. Na$^+$, but not Ca$^{2+}$, induces Mg$^{2+}$ efflux from 20 mM MgCl$_2$-
loaded basolateral LPM (A). Imipramine but not amiloride inhibits
Mg$^{2+}$ efflux (B). Mg$^{2+}$ efflux is reported as the net change in Mg$^{2+}$
content in the supernatant with respect to that before the addition of
extravesicular Na$^+$ or Ca$^{2+}$ (Fig. 1A). Dose response for NaCl induced
Mg$^{2+}$ efflux from 20 mM MgCl$_2$-loaded bLPM vesicles incubated in the
absence of extravesicular Na$^+$ or Ca$^{2+}$ (Fig. 1A). The Mg$^{2+}$ content in the
supernatant was measured by AAS. After withdrawal, the concentrations of
NaCl or CaCl$_2$ indicated in the figure were added, and an aliquot corre-
sponding to 500 μl of incubation medium was withdrawn in duplicate
and rapidly sedimented in microfuge tubes. Mg$^{2+}$ content in the super-
natant was measured by AAS. After withdrawal, the concentrations of
NaCl or CaCl$_2$ indicated in the figure were added, and an aliquot of the
incubation mixture was withdrawn in duplicate at the reported time
points and processed as above. The figure represents the net change in Mg$^{2+}$
content in the supernatant with respect to that present before ion
addition. In Fig. 1B, 1 mM amiloride or 200 μM imipramine was added
2 min before Na$^+$ addition. Data are mean ± S.E. of six and eight
different preparations for Na$^+$ for Ca$^{2+}$, respectively. ANOVA and or
Student-Newman-Keuls method was performed at all time points.
*p < 0.05 versus control and imipramine.
bLPM is specific for Na\(^+\). Addition of 25 mM KCl did not elicit a significant Mg\(^{2+}\) extrusion, and the addition of 25 mM LiCl elicited an efflux of 55.7 ± 29.1 nmol of Mg\(^{2+}\)/mg of protein. The efflux induced by Li\(^+\) was not statistically significant from the control but was statistically significant from 10 mM NaCl (Fig. 4A). Similarly, the addition of 25 mM LiCl or 25 mM KCl to aLPM also elicited negligible Mg\(^{2+}\) efflux (not shown), indicating that the Mg\(^{2+}\) extrusion observed in aLPM strictly depends on extracellular Na\(^+\). Compared with Na\(^+\), the Ca\(^{2+}\) requirement for Mg\(^{2+}\) release from aLPM is far less specific, because Mg\(^{2+}\) efflux could also be induced by the addition of equimolar concentrations of other divalent cations (Fig. 4B).

Bidirectionality of the Mg\(^{2+}\) Exchange—Fig. 5A shows the result of a set of experiments in which bLPM loaded with 20

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FIG. 2. NaCl-induced (A) or CaCl\(_2\)-induced (B) Mg\(^{2+}\) efflux from 20 mM MgCl\(_2\)-loaded aLPM and inhibition by amiloride (C). Amiloride (1 mM) was added 2 min before the Ca\(^{2+}\) or Na\(^+\) addition. All experiments were performed as described in the legend to Fig. 1. Data are mean ± S.E. of three preparations. ANOVA and the Student-Newman-Keuls method for multiple comparisons were performed at all time points. *, p < 0.05 versus control and amiloride.
mM NaCl were stimulated by the addition of varying concentrations of extravesicular Mg$^{2+}$. The observation that extravesicular Mg$^{2+}$ can induce a Na$^{+}$ efflux from Na$^{+}$-loaded bLPM demonstrates that the Na$^{+}$/Mg$^{2+}$ extrusion mechanism is bi-directional. On the other hand, when aLPM were loaded with 20 mM Na$^{+}$ and stimulated with Mg$^{2+}$, no Na$^{+}$ efflux or Mg$^{2+}$ influx was observed, indicating that the Na$^{+}$/Mg$^{2+}$ transport mechanism in these vesicles is unidirectional (Fig. 5B). When similar experiments were performed in aLPM loaded with CaCl$_2$, the addition of external MgCl$_2$ did not induce Ca$^{2+}$ extrusion or, consequently, Mg$^{2+}$ uptake. This indicates that the Ca$^{2+}$/Mg$^{2+}$ transporter in aLPM is also unidirectional, i.e. Mg$^{2+}$ extrusion for Ca$^{2+}$ uptake (Fig. 5C).

Table II compares the initial loading content under each experimental loading condition as well as the percentage of cation releasable upon the addition of Triton X-100. Table II also reconfirms that when no movement is observed such as in Fig. 5, B or C, the absence of efflux is not due to mismatched loading conditions.

**DISCUSSION**

Properties of Plasma Membrane Mg$^{2+}$ Transport—As indicated in the Introduction, the regulation of Mg$^{2+}$ homeostasis in liver and other tissues is poorly understood. In response to activation of specific cell signaling, it has been shown that massive amounts of total Mg$^{2+}$ can be translocated within minutes in or out of hepatocytes (7, 8, 10). This indicated a very powerful and rapid transport machinery of Mg$^{2+}$ transport within the plasma membrane.

Several groups have demonstrated that the plasma membrane of different cell types possesses very active Mg$^{2+}$ transport mechanisms (6–14). Data in the literature support the
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operation of at least two distinct Mg$^{2+}$ transport mechanisms in mammalian cells tentatively identified as a Na$^+$-dependent and a Na$^+$-independent exchange pathway, respectively [8, 9, 12–17]. Experiments performed using a variety of perfused organs or isolated cells indicate that Mg$^{2+}$ transporters operate with varying stoichiometric ratios and transport properties (for review see Ref. 1, 3, 5, 18, 19, or 20) under different experimental conditions. This information makes it difficult to reconcile the available data in a comprehensive scheme.

These conflicting results could be consistent with the operation of several Mg$^{2+}$ transporters within the same cell, each operating under different conditions and sensitivity to inhibitors. In this work, we were successful in obtaining effective separation of LPM into two fractions enriched in aLPM or bLPM, which maintained low permeability to cations and proper sidedness. Using these preparations to study Mg$^{2+}$ transporters, some conflicting results could be consistent with the operation of at least two distinct Mg$^{2+}$ transport mechanisms in mammalian cells tentatively identified as a Na$^+$-dependent and a Na$^+$-independent exchange pathway, respectively (8, 9, 12–17). Experiments performed using a variety of perfused organs or isolated cells indicate that Mg$^{2+}$ transporters operate with varying stoichiometric ratios and transport properties (for review see Ref. 1, 3, 5, 18, 19, or 20) under different experimental conditions. This information makes it difficult to reconcile the available data in a comprehensive scheme.

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The overall conclusion is that the apical LPM possesses two distinct Mg$^{2+}$ transporters, one Ca$^{2+}$- and one Na$^+$-dependent. In contrast, the basolateral LPM contains only one Mg$^{2+}$ transporter dependent upon Na$^+$. Partial evidence indicates that the Mg$^{2+}$ transporters activated by Na$^+$ in the apical and basolateral membranes may be distinct. The kinetic properties of these transporters are outlined in Table III.

We conducted several controls as previously shown (21) to conclusively demonstrate that the Mg$^{2+}$ extruded in the supernatant is the result of net translocation across the membrane and that Mg$^{2+}$ efflux is not the result of a passive leakage from Mg$^{2+}$-loaded vesicles or a release from surface binding sites after the addition of a cation or osmotic mismatch. Briefly, these experiments include the following: (a) unstimulated LPM do not release entrapped Mg$^{2+}$ over several min of incubation even in the presence of a gradient across the plasma membrane (Figs. 1 and 2); (b) both Na$^+$- and Ca$^{2+}$-induced Mg$^{2+}$ effluxes are completely prevented in the presence of inhibitors; (c) 25 mM LiCl or 25 mM KCl (Fig. 4A) fail to replace Na$^+$ in mobilizing Mg$^{2+}$; (d) at lower temperatures Mg$^{2+}$ release induced by Ca$^{2+}$ or Na$^+$ is slow and kinetically resolvable; and (e) most of the Mg$^{2+}$ releasable by Ca$^{2+}$ or Na$^+$ can be the same Mg$^{2+}$ pool released by detergent or cation ionophore (21).

**Properties of Mg$^{2+}$ Transport in the Basolateral Membrane—**

Only Na$^+$ can induce the release of intravesicular Mg$^{2+}$ from bLPM. The Na$^+$ requirement is specific, because no other monovalent or divalent cation can induce Mg$^{2+}$ efflux. The process is fully reversible, as extravascular Mg$^{2+}$ effectively mobilizes intravesicular Na$^+$. It is noteworthy that imipramine, but not amiloride, is effective at inhibiting Na$^+$-mobilization Mg$^{2+}$ extrusion in bLPM (Fig. 1B). On the contrary, amiloride inhibits the Na$^+$/Mg$^{2+}$ mechanism in aLPM by >85% (Fig. 2C). As the ratio of aLPM to bLPM is 1, on a protein basis, the differential effectiveness of amiloride to inhibit Mg$^{2+}$ transport in bLPM versus aLPM could explain our previous observation that amiloride inhibits Mg$^{2+}$ transport in total LPM by approximately 50% (21).

**Properties of Mg$^{2+}$ Transport in the Apical Membrane—**

The data from aLPM demonstrate that the apical domain of hepatocytes has both a Na$^+$/Mg$^{2+}$ and a Ca$^{2+}$/Mg$^{2+}$ transport mechanism. The Na$^+$/Mg$^{2+}$ exchange in aLPM operates in a manner similar to the Na$^+$/Mg$^{2+}$ exchanger described in bLPM in terms of specificity for Na$^+$. However, three main differences are evident: (a) the dose response for extravascular Na$^+$ in aLPM is different than in bLPM in that the concentration for

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**Fig. 4. Cation specificity in promoting Mg$^{2+}$ efflux.** Mg$^{2+}$ efflux from bLPM following stimulation by 10 mM NaCl, 25 mM KCl, or 25 mM LiCl (A). In B, Mg$^{2+}$ efflux from loaded aLPM can be stimulated by Ca$^{2+}$ or various other divalent cations. All experiments were performed as described in the legend to Fig. 1. Data are mean ± S.E. of four preparations. ANOVA and the Student-Newman-Keuls method for multiple comparisons were performed at all time points. *, p < 0.05 versus control for A. In B all time points were significant (p < 0.05) versus control. Asterisks were omitted for clarity.
maximal activity is left-shifted in bLPM (Fig. 1A), (b) the transport is almost completely inhibited by amiloride (Fig. 2C), and (c) the process is not reversible because extravesicular Mg\(^{2+}\) cannot elicit efflux of intravesicular Na\(^{+}\) (Fig. 5B). These differences can be accounted for by the presence of two separate Na\(^{+}/Mg^{2+}\) antiporters in the apical and basolateral sides of

**TABLE II**

Comparison of total cation content under various loading conditions and percent mobilized after stimulation by 1.0% Triton X-100

Initial loading values are reported as nmol of cation/mg of protein. All other data are expressed as percent of total releasable cation mobilized after the addition of Triton X-100. The initial cation content was measured in the pellet as described under “Experimental Procedures.” All data are reported as mean ± S.E.; n = 3 for all loading conditions tested. N.A., not applicable.

|                  | Mg\(^{2+}\) loading | Ca\(^{2+}\) loading | Na\(^{+}\) loading |
|------------------|----------------------|---------------------|-------------------|
|                  | Initial loading value | % Released       | Initial loading value | % Released       | Initial loading value | % Released       |
| bLPM             | 460.3 ± 8.9          | 66.1% ± 4.7%       | N.A.               | N.A.              | 590.3 ± 77.5        | 81.0% ± 11.8%  |
| aLPM             | 377.5 ± 40.5         | 72.2% ± 5.3%       | 224.7 ± 46.5       | 69.5% ± 6.7%      | 535.9 ± 39.4        | 70.6% ± 8.6%   |

Fig. 5. Reversibility of the Na\(^{+}\)-induced, but not the Ca\(^{2+}\)-induced, Mg\(^{2+}\) transport. Net Na\(^{+}\) or Ca\(^{2+}\) efflux in bLPM (A) or aLPM (B) loaded with 20 mM NaCl and resuspended in the absence of extravesicular NaCl or aLPM loaded with 20 mM CaCl\(_2\) and resuspended in the absence of external CaCl\(_2\). The Na\(^{+}\) or Ca\(^{2+}\) content in the supernatant was assayed by AAS as described for Mg\(^{2+}\) in the legend to Fig. 1. Data presented are mean ± S.E. of four different preparations. ANOVA and the Student-Newman-Keuls method for multiple comparisons were performed at all time points. *, p < 0.05 versus control.
identified for the first time in mammalian cells. The data from novel finding of this work is that the basolateral $\text{Na}^{+}/\text{Ca}^{2+}/\text{Mg}^{2+}$ either plasma membrane domain.

The fact that this transporter is unidirectional suggests that its large mass, blood flow, and active secretory capacity, regulating $\text{Na}^{+}$ exchange, amiloride sensitivity, and reversibility will be preferred against the different mechanisms of $\text{Na}^{+}$ influx in either plasma membrane domain.

The $\text{Mg}^{2+}$ release mechanism dependent upon extracellular $\text{Ca}^{2+}$ and found in the apical membrane is a novel observation identified for the first time in mammalian cells. The data from aLFM demonstrate that the $\text{Ca}^{2+}/\text{Mg}^{2+}$ transporter is strictly localized in this domain and that the transporter is nonselective as it can be activated by several divalent cations (Fig. 4B). The fact that this transporter is unidirectional suggests that in $\text{vivo}$ it may operate only in terms of $\text{Mg}^{2+}$ efflux and $\text{Ca}^{2+}$ uptake.

**Physiological Significance of Hepatocyte $\text{Mg}^{2+}$ Transporters**—The distribution of the three $\text{Mg}^{2+}$ transport mechanisms within the hepatocyte is diagrammatically illustrated in Fig. 6. Based upon the limited share of knowledge of intra- and extracellular $\text{Mg}^{2+}$ homeostasis in liver, or for that matter, in any other tissue, any discussion of the physiological role of these different transporters in hepatocytes remains speculative. Owning to its large mass, blood flow, and active secretory capacity, the liver is positioned to play a key role in the homeostasis of metabolites and ions such as $\text{Mg}^{2+}$. Hepatocytes are polarized cells in which transport between hepatocytes and plasma is regulated by the basolateral membrane, whereas that between hepatocyte and biliary tract is regulated by the apical membrane.

The basolateral membrane accounts for approximately 90% of the surface area of the hepatocyte plasma membrane. Hence, the basolateral $\text{Na}^{+}$-dependent $\text{Mg}^{2+}$ transport, described by several groups, should play the primary role in hepatocyte $\text{Mg}^{2+}$ homeostasis as well as in plasma $\text{Mg}^{2+}$ homeostasis. The novel finding of this work is that the basolateral $\text{Na}^{+}/\text{Mg}^{2+}$ overall exchange is reversible, whereas the $\text{Na}^{+}$-dependent and $\text{Ca}^{2+}$-dependent $\text{Mg}^{2+}$ transport mechanisms in the apical membrane are not. This implies that the basolateral $\text{Na}^{+}$-dependent $\text{Mg}^{2+}$ transporter is most likely the effector for the observed large and rapid changes in plasma (31) and tissue (6–10) $\text{Mg}^{2+}$, observed in either direction after the addition of $\alpha$- or $\beta$-adrenergic agonists (7–9, 32), protein kinase $\text{C}$ activation (10), insulin (33), etc.

In contrast, the apical membrane $\text{Mg}^{2+}$ transporter is unidirectional and can only transport $\text{Mg}^{2+}$ from the hepatocytes to the bile in exchange for $\text{Ca}^{2+}$ or $\text{Na}^{+}$. Data in the literature indicate that the concentration of total $\text{Ca}^{2+}$ in the bile ranges from 2 to 16 mM (34). The unidirectional $\text{Ca}^{2+}/\text{Mg}^{2+}$ exchange could be responsible for decreasing biliary $\text{Ca}^{2+}$ thereby preventing the formation of bile stones or be an additional mechanism contributing to the overall reabsorption of $\text{Ca}^{2+}$ (34–36). The physiological significance of the unidirectional $\text{Na}^{+}$-dependent $\text{Mg}^{2+}$ efflux from hepatocytes through the apical membrane is equally conjectural and may operate in tandem with the described $\text{Na}^{+}$/bile acid uptake or for direct $\text{Mg}^{2+}$ extrusion (37). It will be interesting to observe whether these transporters are up-regulated in conditions of chronic alcoholism or diabetes when the tissue concentrations of $\text{Mg}^{2+}$ in liver and other tissues dramatically decrease (38, 39).

Although the present data may provide more questions than answers as to the physiological and metabolic implications of liver $\text{Mg}^{2+}$ transport, they dissect an overall complex $\text{Mg}^{2+}$ cellular transport in individual components. Identifying three individual transporters on the basis of localization, inhibition, reversibility, and cation specificity will permit the design of experimental approaches to further investigate the distribution of these $\text{Mg}^{2+}$ transporters in other tissues, the more detailed mechanism of transport and the physiological significance in terms of $\text{Mg}^{2+}$, and other ion and metabolic homeostasis.

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**TABLE III**

| Cation required | Inhibition by amiloride | Inhibition by imipramine | Bidirectionality |
|-----------------|------------------------|-------------------------|-----------------|
| Basolateral Na$^+$ | Na$^+$ | No | Yes | Yes |
| Apical Na$^+$ | Na$^+$ | Yes | Yes | No |
| Apical Ca$^{2+}$ = Co$^{2+}$ > Mn$^{2+}$ > Sr$^{2+}$ = Ba$^{2+}$ | Ca$^{2+}$ | Yes | Yes | No |

**FIG. 6. **Multiple Mg$^{2+}$ transport in the hepatocyte. See “Discussion” for details.
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