Na\(^+\)/Mg\(^{2+}\) antiport in non-erythrocyte vertebrate cells

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Abstract. Experiments, results and conclusions on Na\(^+\)/Mg\(^{2+}\) antiport in lymphocytes, HL60 cells, Ehrlich ascites tumor cells, platelets, pancreatic acinar cells, sublingual acini, hepatocytes, ruminal epithelial cells, kidney cells, smooth muscle cells, heart muscle cells and skeletal muscle cells were reviewed. Only in a few experiments was the Mg\(^{2+}\) efflux via the Na\(^+\)/Mg\(^{2+}\) antiport studied directly by measuring the alterations in [Mg\(^{2+}\)]\(_o\) and intracellular Na\(^+\) content. In most cell types, the Na\(^+\)/Mg\(^{2+}\) antiport was investigated indirectly by measuring [Mg\(^{2+}\)]\(_i\) and [Na\(^+\)]\(_i\) after changing the physiological Mg\(^{2+}\) homeostasis by effectors or by loading the cells with Mg\(^{2+}\). The effects of inhibitors and incubation in Na\(^+\)-free medium on the alterations in [Mg\(^{2+}\)]\(_i\) and [Na\(^+\)]\(_i\) were taken as evidence of the Na\(^+\)/Mg\(^{2+}\) antiport. By these methods, the Na\(^+\)/Mg\(^{2+}\) antiport was found in all investigated mammalian cell types. As far as they have been studied, the kinetic properties and regulation of Na\(^+\)/Mg\(^{2+}\) antiport are reviewed.

Key words: Na\(^+\)/Mg\(^{2+}\) antiport, Mg\(^{2+}\) efflux, furaptra, [Mg\(^{2+}\)]

In all cell types [Mg\(^{2+}\)]\(_i\) is about the same as [Mg\(^{2+}\)]\(_o\). Considering the inside negative membrane potential of cells at a potential-driven equilibrium, [Mg\(^{2+}\)]\(_o\) should be much higher than measured. Therefore there must be a permanent efflux of Mg\(^{2+}\). As Mg\(^{2+}\) membrane permeability is very low, steady-state Mg\(^{2+}\) efflux is also very low.

In erythrocytes, Mg\(^{2+}\) efflux could be drastically enhanced through artificial Mg\(^{2+}\) loading. Thus Mg\(^{2+}\) efflux mechanisms could be characterized. The main Mg\(^{2+}\) efflux in erythrocytes operates via the Na\(^+\)/Mg\(^{2+}\) antiport [1].

Meanwhile the Na\(^+\)/Mg\(^{2+}\) antiport has also been detected in other cell types. Due to methodical problems, in most investigated cell types, the Mg\(^{2+}\) efflux could not be measured directly through an increase in [Mg\(^{2+}\)]\(_o\). Therefore the Na\(^+\)/Mg\(^{2+}\) antiport was indirectly studied by measuring the alterations in [Mg\(^{2+}\)]\(_i\) and [Na\(^+\)]\(_i\), with fluorescent indicators. Usually these alterations were induced by various effectors and were prevented by inhibitors such as imipramine, quinidine or amiloride. Sometimes the involvement of Na\(^+\) in Mg\(^{2+}\) efflux was only tested by omission of extracellular Na\(^+\). However the effectors and inhibitors are not specific for the Na\(^+\)/Mg\(^{2+}\) antiport and there was no reasonable ratio \(\Delta [\text{Na}^{+}]_i / \Delta [\text{Mg}^{2+}]_i\), indicating that other ion transporters, Mg\(^{2+}\) buffering and compartmentation may be involved in the alterations of [Mg\(^{2+}\)]\(_i\) and [Na\(^+\)]\(_i\). There has been no systematic investigation of [Mg\(^{2+}\)]\(_i\) on the rate of Mg\(^{2+}\) efflux, the kinetics of Mg\(^{2+}\) efflux, its regulation and no investigation of Na\(^+\)-independent Mg\(^{2+}\) efflux in the various cell types. The following review describes the characterization, kinetic properties

Abbreviations:
[Na\(^+\)], [K\(^+\)], [Mg\(^{2+}\)], [Ca\(^{2+}\)] extracellular concentrations of Na\(^+\), K\(^+\), Mg\(^{2+}\), Ca\(^{2+}\);
[Na\(^+\)], [Mg\(^{2+}\)], [Ca\(^{2+}\)], [H\(^+\)] concentrations of intracellular free Na\(^+\), Mg\(^{2+}\), Ca\(^{2+}\), H\(^+\);
PKA protein kinase A
PKC protein kinase C
PGE prostaglandin E
and regulation of Na+/Mg2+ antiport in various vertebrate cell types as far as studied.

**Na+/Mg2+ antiport in lymphocytes**

Mg2+ efflux from Mg2+-loaded thymocytes and non-Mg2+-loaded lymphocytes was measured directly. Mg2+ loading was performed identically as with erythrocytes by means of A23187. Mg2+ efflux from Mg2+-loaded rat thymocytes in Na+ medium containing 150 mM NaCl amounted to 100 nmol/mg protein x 15 min. It was inhibited by amiloride and was almost completely prevented (96%) by incubation in choline Cl or sucrose medium. Mg2+ efflux in NaCl medium was accompanied by an uptake of Na+ at a ratio of 2 Na+/1 Mg2+ [2]. These results indicate that the Mg2+ efflux from Mg2+-loaded thymocytes is almost completely performed by the Na+/Mg2+ antiport.

Na+/Mg2+ antiport in thymocytes increased with increasing Mg2+ loading. Increased Mg2+ loading led to an elevation in the Hill coefficient of the Mg2+ efflux. This result can be explained by increased cooperativity of Mg2+ at higher [Mg2+]i [3].

The Na+/Mg2+ antiporter in thymocytes performed a 24Mg2+/28Mg2+ exchange that was inhibited by amiloride. The 28Mg2+/24Mg2+ exchange was increased in KCl medium [4]. This result indicates that extracellular Na+ inhibits 28Mg2+ uptake in exchange for intracellular 24Mg2+, as found with erythrocytes [1].

In more detailed kinetic experiments [5], isolated rat thymocytes were loaded with Mg2+ by means of A23187 in the presence of 6 and 12 mM Mg2+. The resulting [Mg2+]i s were not measured. The Na+/Mg2+ antiport was studied directly by measuring the alterations in [Mg2+]i, or total cellular Mg2+ and Na+ contents.

In Mg2+-loaded thymocytes, the Mg2+ efflux was coupled with Na+ uptake, whereby its rate depended on [Na+]. Mg2+ efflux in choline medium amounted only to 3.6% of Mg2+ efflux in Na+ medium, as found in other experiments [2].

The double reciprocal (Lineweaver-Burk) plot of the [Na+], dependency of Mg2+ efflux yielded a curved plot, characteristic of allosteric enzymes. This kinetic property was incorrectly interpreted by two Michaelis-Menten relationships showing low and high Na+ affinity of Mg2+ efflux (see the insets in figures 1 and 2 in reference [5]). In contrast to these plots, the figure 5 in reference [5] shows Michaelis-Menten kinetics for [Na+], dependency of the Mg2+ efflux.

Further kinetic analysis of the [Na+]i dependency of the Na+ influx was carried out by assuming that the Na+ influx can be described by two terms. One term at low [Na+]i represents hyperbolic Michaelis-Menten kinetics, the other at high [Na+]i, represents sigmoidal Hill kinetics with a Hill coefficient of 2. From this kinetic analysis it was concluded that the Na+ influx at low [Na+]i, is performed by a high Na+ affinity 1 Na+/1 Mg2+ antiporter and, at high [Na+]i, additionally by a low Na+ affinity 2 Na+/1 Mg2+ antiporter. Both antiporters are activated by Mg2+ loading and are inhibited by amiloride. The low Na+ affinity Na+/Mg2+ antiporter is inhibited by extracellular Mg2+.

Mg2+ efflux from non-Mg2+-loaded rat spleen lymphocytes in NaCl medium amounted to 0.2 nmol/mg protein x 1 min and was 33 times lower than from thymocytes loaded in the presence of 12 mM Mg2+ (see above). Mg2+ efflux from non-Mg2+-loaded lymphocytes in NaCl medium was inhibited by amiloride and imipramine [6]. Na+-independent Mg2+ efflux from non-Mg2+-loaded lymphocytes in choline Cl medium amounted to 56% of Mg2+ efflux in NaCl medium [6]. A similar result was obtained with Ehrlich ascites tumor cells (see below).

This high fraction of Na+-independent Mg2+ efflux compared to the low fraction of Na+-independent Mg2+ efflux in Mg2+-loaded cells results from the drastic activation of the Na+/Mg2+ antiport by Mg2+ loading, whereas the Na+-independent Mg2+ efflux is only increased in parallel to the Mg2+ gradient by Mg2+ loading [1]. No experiments were done to characterize the mechanism of Mg2+ efflux from lymphocytes in choline medium, which is performed via the choline/Mg2+ antiport in erythrocytes [1].

The Na+/Mg2+ antiport in thymocytes and lymphocytes was activated by dbcAMP and β-adrenergics [3, 6, 7]. The activation by dbcAMP was reduced with increasing Mg2+ loading [3]. After reaching a maximum activity of the Na+/Mg2+ antiport by Mg2+ loading, there may be no further activation by dbcAMP, similarly as with erythrocytes [1].

The activation of Mg2+ efflux by 0.1 mM dbcAMP in non-Mg2+-loaded and slightly Mg2+-loaded thymocytes was accompanied by a small increase in [Mg2+], (0.03 and 0.05 mM) [3] which had not caused activation of the Na+/Mg2+ antiport. These results can be explained as follows: dbcAMP may activate PKA, followed by phosphorylation of the Na+/Mg2+ antiporter. The phosphorylated Na+/Mg2+ antiporter may have an increased affinity for intracellular Mg2+ and thus increase Mg2+ efflux activity. During the increased Mg2+ efflux period, [Mg2+]i remained constant [3], indicating that the excreted Mg2+ must
Na+/Mg2+ antiport in HL60 cells

Mg2+ efflux from Mg2+-loaded HL60 cells (a human promyelocytic leukemia cell line) in NaCl medium was measured directly. It amounted to 60 nmol/mg protein x 15 min. It was inhibited by amiloride [2] and imipramine [9]. Mg2+ efflux was almost completely suppressed by incubation in cholineCl or sucrose medium [2]. The same was found with thymocytes [2].

Mg2+ efflux from non-Mg2+-loaded HL60 cells amounted to 15.4 nmol/mg protein x 30 min [9] and was 8 times lower than in Mg2+-loaded HL60 cells [2].

Mg2+ efflux was activated by dbcAMP and inhibited when extracellular Na+ was substituted by choline. Interferon-α and PGE1, which stimulated Mg2+ efflux from lymphocytes [3] and Ehrlich ascites tumor cells (see below) were ineffective in HL60 cells [9].

The Na+/Mg2+ antiport from HL60 cells is reversed when the cells are loaded with Na+ by means of nystatin and reincubated in Mg2+-containing medium with low [Na+]o [10]. Driving forces are the [Na+]i/[Na+]o, and the [Mg2+]o/[Mg2+]i, gradients. Mg2+ uptake by reversed Na+/Mg2+ antiport was inhibited by amiloride, quinidine and imipramine as Mg2+ efflux via the Na+/Mg2+ antiport. Extracellular Na+ inhibited Mg2+ uptake by reducing the reversed Na+ gradient and by competition with extracellular Mg2+ [10]. The same results were found with erythrocytes [1].

Na+/Mg2+ antiport in Ehrlich ascites tumor cells

Mg2+ efflux in NaCl medium from non-Mg2+-loaded ascites tumor cells was measured directly. It amounted to 10.9 - 16.3 nmol/mg protein x 30 min [11, 12]. Substitution of NaCl in the incubation medium by choline Cl reduced the Mg2+ efflux by about 50% [11]. Substitution of NaCl with KCl decreased Mg2+ efflux by 75% [12]. The remaining Na+-independent Mg2+ efflux was not characterized. Amiloride, imipramine and quinidine inhibited the Mg2+ efflux [12]. The Mg2+ efflux was ATP-dependent [12].

The Mg2+ efflux was stimulated by SBcAMP as well as by arachidonic acid, PGE1, and PGE2 which stimulate cAMP synthesis [11, 13]. The activation of the Mg2+ efflux by SBcAMP and PGE2 was completely prevented by protein kinase inhibitors. It was concluded that cAMP induces phosphorylation of the Na+/Mg2+ antiporter [13].

The activation of Mg2+ efflux by dbcAMP occurred at constant [Mg2+]o [11]. It was therefore concluded that phosphorylation of the Na+/Mg2+ antiporter increased its affinity for intracellular Mg2+ [13], as found in thymocytes [4] and hepatocytes (see below). As [Mg2+]o behaviour was constant during the increased Mg2+ efflux period, the source of the excreted Mg2+ remains an open question (see also [8]).

Na+/Mg2+ antiport in platelets

The presence of Na+/Mg2+ antiport in platelets was concluded indirectly by measuring [Mg2+]i and [Na+]i, by changing their homeostasis.

When human platelets were incubated in a physiological salt medium containing 0.1 mM ouabain for 60 min, [Na+]i increased from 8.0 mM to 25.8 mM. At the same time, [Mg2+]i increased from 0.389 mM to 0.523 mM. This result was explained by a reduction in the [Na+]i/[Na+]o gradient, which should have reduced the Na+/Mg2+ antiport and thus increased [Mg2+]i [14]. Inhibition of the Na+/Mg2+ antiport was not investigated. However, [Ca2+]i increased simultaneously from 27.9 to 37.2 nM, so that besides an alteration of the Na+/Mg2+ antiport an interaction of the Na+/Ca2+ antiport may be involved.

In other experiments, [Mg2+]i in platelets of control rats amounted to 0.69 mM. After treating rats with angiotensin II for 3 weeks, [Mg2+]i was reduced to 0.57 mM. Correspondingly, [Na+]i in the platelets of control rats amounted to 20.4 mM and in the platelets of angiotensin II-treated rats to 27.8 mM. When angiotensin II-treated rats were simultaneously treated with imipramine or quinidine, the alterations in [Mg2+]i and [Na+]i were almost prevented. These results were taken as evidence for the existence of Na+/Mg2+ antiport in platelets [15].

A reasonable stoichiometric ratio of the Na+/Mg2+ antiport cannot be deduced from the alterations in [Mg2+]i (-0.12 mM) and [Na+]i (+7.4 mM). This can be explained by the additional activation of other Na+ transporters through angiotensin II [16] and by buffering of intracellular Mg2+.

Remarkably, [Mg2+]i and [Na+]i in platelets from rats treated only with imipramine or quinidine were not significantly altered [15].
**Na\(^+\)/Mg\(^{2+}\) antiport in pancreatic acinar cells**

Mg\(^{2+}\) efflux from Mg\(^{2+}\)-loaded, collagenase-dissociated rat pancreatic acinar cells was investigated directly [17]. The isolated cells were loaded with Mg\(^{2+}\) by means of A23187 in the presence of 12 mM Mg\(^{2+}\). By this method, the total Mg\(^{2+}\) content was increased from 260.7 \(\mu\)mol/100 mg protein to 805.1 \(\mu\)mol/100 mg protein.

Mg\(^{2+}\) efflux from Mg\(^{2+}\)-loaded cells in Krebs-Ringer-Hepes medium was inhibited by lidocaine, dinitrophenol, quinidine and amiloride. The Mg\(^{2+}\) efflux of the Mg\(^{2+}\)-loaded cells was almost completely abolished when Na\(^+\) of the incubation medium was replaced by Tris, choline or N-methyl-D-glucamine. After reaching the original Mg\(^{2+}\) content, there was no further significant Mg\(^{2+}\) efflux. Thus Mg\(^{2+}\) efflux from pancreatic acinar cells showed similar properties to the Na\(^+\)/Mg\(^{2+}\) antiport from Mg\(^{2+}\)-loaded erythrocytes [1].

**Na\(^+\)/Mg\(^{2+}\) antiport in rat sublingual acini**

Isolated cells were loaded with Mg\(^{2+}\) by means of ionomycin in the presence of 5 mM Mg\(^{2+}\) [18]. By this method, [Mg\(^{2+}\)]\(_{i}\) was increased from 0.35 to 0.66 mM, as measured with mag-flura-2. Superfusion of the Mg\(^{2+}\)-loaded cells with Mg\(^{2+}\)-free medium induced a rapid decrease in [Mg\(^{2+}\)]\(_{i}\). The decrease in [Mg\(^{2+}\)]\(_{i}\), was dependent on [Na\(^+\)], Sucrose and N-methyl-D-glucamine medium inhibited 70% and 82% of the [Mg\(^{2+}\)]\(_{i}\). decrease. [Mg\(^{2+}\)]\(_{i}\) decrease was electroneutral and inhibited by quinidine. In Mg\(^{2+}\)-loaded cells, the decrease in [Mg\(^{2+}\)]\(_{i}\), was not stimulated by dbcAMP.

Incubation of non-Mg\(^{2+}\)-loaded cells in Mg\(^{2+}\)-free NaCl medium yielded only a small decrease in [Mg\(^{2+}\)]\(_{i}\), indicating a drastic activation of the Na\(^+\)/Mg\(^{2+}\) antiport by intracellular Mg\(^{2+}\) [18]. These properties correspond to the properties of the Na\(^+\)/Mg\(^{2+}\) antiport in erythrocytes [1].

**Na\(^+\)/Mg\(^{2+}\) antiport in liver cells**

When rat livers were perfused with Mg\(^{2+}\)-free NaCl medium, the addition of 10 M noradrenaline induced a transient Mg\(^{2+}\) efflux lasting 10 min [19] or 15 min [20]. Mg\(^{2+}\) efflux amounted to 0.14 mmol/kg wet weight or 2% of total liver Mg\(^{2+}\) [20]. Other authors reported a loss of 15% of total Mg\(^{2+}\) from isolated hepatocytes by \(\beta\)-adrenergic agonists [21]. In C\(^{2+}\)-free perfusion, Mg\(^{2+}\) efflux was dependent on extracellular Na\(^+\) and was completely absent in the perfusion with KCl medium [20]. Noradrenaline-induced Mg\(^{2+}\) efflux was completely inhibited by 1 mM amiloride or 0.5 mM imipramine [20]. These results indicate that a noradrenaline-induced Mg\(^{2+}\) efflux operates via the Na\(^+\)/Mg\(^{2+}\) antiport. Due to the small amount of Mg\(^{2+}\) efflux, the Na\(^+\)/Mg\(^{2+}\) ratio could not be determined. To characterize the mechanisms that induced the Mg\(^{2+}\) efflux, [Mg\(^{2+}\)]\(_{i}\) was measured in isolated hepatocytes. Noradrenaline, isoproterenol and dbcAMP induced a small (0.05 mM) increase in [Mg\(^{2+}\)]\(_{i}\), for 30 sec. Thereafter during Mg\(^{2+}\) efflux, [Mg\(^{2+}\)]\(_{i}\), remained constant at its normal level [20]. These results were explained by phosphorylation through the Na\(^+\)/Mg\(^{2+}\) antiporter by PKA. The phosphorylated Na\(^+\)/Mg\(^{2+}\) antiporter may have an increased affinity for intracellular Mg\(^{2+}\) and thus increase Mg\(^{2+}\) efflux, which is normalized by dephosphorylation after 10-15 min. cAMP-activated phosphorylation by PKA is essential for the activity of the Na\(^+\)/Mg\(^{2+}\) antiport, as shown with liver plasma membrane vesicles (LPMV). Mg\(^{2+}\)-loaded LPMV expressed Mg\(^{2+}\) efflux that was decreased by 90% after treatment with alkaline phosphatase and was restored when phosphatase-treated LPMV were loaded with PKA and stimulated with cAMP [21]. These results indicate that the Mg\(^{2+}\) efflux depends on cAMP-activated phosphorylation of the Na\(^+\)/Mg\(^{2+}\) antiport.

In more detailed experiments, isolated liver plasma membranes were separated in basolateral and apical plasma membranes by discontinuous sucrose gradient centrifugation. After Mg\(^{2+}\) loading, the basolateral membrane fraction expressed Na\(^+\)-specific Na\(^+\)/Mg\(^{2+}\) antiport inhibited by imipramine but not inhibited by amiloride. The apical membrane fraction contained a Na\(^+\)-specific Na\(^+\)/Mg\(^{2+}\) antiport and an unspecific electroneutral Ca\(^{2+}\)/Mg\(^{2+}\) antiport operating besides Ca\(^{2+}\) also with Co\(^{2+}\), Mn\(^{2+}\), Sr\(^{2+}\) and Ba\(^{2+}\) [22]. Both antiports in apical membranes were inhibited by amiloride, imipramine and quinidine [23].

The released Mg\(^{2+}\) may originate in part (30-50%) from mitochondria that may have lost 20-25% of their total Mg\(^{2+}\) [24]. However the Mg\(^{2+}\) release from mitochondria was not confirmed [25]. Therefore the source of the released intracellular Mg\(^{2+}\) is unknown. For discussion see [8]. It is probable that some bound Mg\(^{2+}\) may be released, although the average [Mg\(^{2+}\)]\(_{i}\) remains constant. As [Mg\(^{2+}\)]\(_{i}\), in cytosol is compartmentalized [26], the behaviour of [Mg\(^{2+}\)]\(_{i}\), in the various compartments during Mg\(^{2+}\) efflux is not known.

The perfusion experiments have shown that the Na\(^+\)/Mg\(^{2+}\) antiport is activated by \(\beta\)-adrenergic agonists via cAMP. In other perfusion experiments, \(\alpha\),...
adrenergic agonists e.g. phenylephrine were found to induce a 15 min lasting transient Mg\textsuperscript{2+} efflux mediated by the Ca\textsuperscript{2+}/Mg\textsuperscript{2+} antiport [27] and the Na\textsuperscript{+}/Mg\textsuperscript{2+} antiport [28]. Phenylephrine activates phospholipase C and the formation of diacylglycerol (DAG) and inositol trisphosphate (IP\textsubscript{3}). IP\textsubscript{3} induces the release of stored Ca\textsuperscript{2+} from the endoplasmic reticulum followed by [Ca\textsuperscript{2+}] \textsubscript{i}-induced Ca\textsuperscript{2+} influx and increased formation of Ca\textsuperscript{2+}-calmodulin. Ca\textsuperscript{2+}-calmodulin may activate Mg\textsuperscript{2+} efflux [28]. The mechanism of the Ca\textsuperscript{2+}-calmodulin-activated Mg\textsuperscript{2+} efflux was not investigated.

The \(\beta\)-agonists induce Mg\textsuperscript{2+} efflux and also glyco-
genolysis and glucose extrusion from hepatocytes. These effects may be associated. In livers of overnight-starved rats in which the total Mg\textsuperscript{2+} content was reduced by 9%, isoproterenol and phenylephrine did not induce Mg\textsuperscript{2+} efflux [29].

The isoproterenol- and 8BrcAMP-induced Mg\textsuperscript{2+} efflux from perfused rat liver was almost completely inhibited by simultaneous addition of insulin to the perfusate, while insulin had no significant effect on phenylephrine-induced Mg\textsuperscript{2+} efflux. It was discussed whether insulin activates a calmodulin-activated phosphodiesterase which degrades cAMP [30].

\textbf{Na\textsuperscript{+}/Mg\textsuperscript{2+} antiport in the gastrointestinal tract}

Mg\textsuperscript{2+} transport in the gastrointestinal tract has been extensively reviewed elsewhere [31]. It is performed by para- and transcellular Mg\textsuperscript{2+} transport. The Na\textsuperscript{+}/Mg\textsuperscript{2+} antiport is involved in transcellular Mg\textsuperscript{2+} transport.

The Na\textsuperscript{+}/Mg\textsuperscript{2+} antiport has been indirectly studied in sheep ruminal epithelial cells (REC) by measuring [Mg\textsuperscript{2+}]\textsubscript{i} and [Na\textsuperscript{+}]\textsubscript{i} with fluorescent probes [32]. During incubation in Na\textsuperscript{+}-free Mg\textsuperscript{2+}-containing medium, [Na\textsuperscript{+}] was reduced and [Mg\textsuperscript{2+}] was increased. The increase in [Mg\textsuperscript{2+}] was inhibited by imipramine and quinine [32]. This result may indicate an Mg\textsuperscript{2+} uptake via the Na\textsuperscript{+}/Mg\textsuperscript{2+} antiporter due to reversed Na\textsuperscript{+} and Mg\textsuperscript{2+} gradients.

In other experiments, sheep REC were loaded with Mg\textsuperscript{2+} by means of A23187 in the presence of 6 mM Mg\textsuperscript{2+} [33]. In this method, [Mg\textsuperscript{2+}] was increased from 0.37 to 0.72 mM. The Mg\textsuperscript{2+} efflux was measured directly through the increase in [Mg\textsuperscript{2+}] in the incubation medium and indirectly through the alteration in [Mg\textsuperscript{2+}], [Mg\textsuperscript{2+}] efflux was dependent on [Na\textsuperscript{+}], and obeyed Michaelis-Menten kinetics. \(K_m\) amounted to 24 mM Na\textsuperscript{+}, Mg\textsuperscript{2+} efflux was inhibited by imipramine and activated by dbcAMP and PGE\textsubscript{2}. In Mg\textsuperscript{2+}-loaded REC, 97% of Mg\textsuperscript{2+} efflux was dependent on extracellular Na\textsuperscript{+}, similar to Mg\textsuperscript{2+}-loaded erythrocytes [1] and thymocytes [2, 5].

A monoclonal antibody against the Na\textsuperscript{+}/Mg\textsuperscript{2+} antiporter inhibited Mg\textsuperscript{2+} influx at high [Na\textsuperscript{+}] \textsubscript{i}, [Na\textsuperscript{+}] \textsubscript{i} > [Na\textsuperscript{+}] \textsubscript{o}, (reversed) gradient (incubation in KCl medium) and inhibited Mg\textsuperscript{2+} efflux at a low [Na\textsuperscript{+}] \textsubscript{i}, [Na\textsuperscript{+}] \textsubscript{i} < [Na\textsuperscript{+}] \textsubscript{o} gradient (incubation in NaCl medium), as measured by the alterations in [Mg\textsuperscript{2+}]\textsubscript{i}. In immunoplots the antibody labeled a 70 kDa protein [33].

The Na\textsuperscript{+}/Mg\textsuperscript{2+} antiport plays a role in Mg\textsuperscript{2+} resorption and in the maintenance of cellular Mg\textsuperscript{2+} homeostasis [33].

\textbf{Na\textsuperscript{+}/Mg\textsuperscript{2+} antiport in kidney cells}

Mg\textsuperscript{2+} transport in the nephron operates via a paracellular and a transcellular pathway. The latter includes the Na\textsuperscript{+}/Mg\textsuperscript{2+} antiport [34].

The Na\textsuperscript{+}/Mg\textsuperscript{2+} antiport was investigated indirectly with renal cell lines by means of fluorescent probes. The homeostasis of intracellular Mg\textsuperscript{2+} in Madin-Darby canine kidney (MDCK) cells was changed through incubation with angiotensin II, affecting kidney function in that the renin-angiotensin II-aldosterone system increases Na\textsuperscript{+} resorption. Angiotensin II (10^{-12} to 10^{-6} \text{M}) reduced [Mg\textsuperscript{2+}]\textsubscript{i} from 0.51 mM to 0.38 mM and increased [Na\textsuperscript{+}]\textsubscript{i} from 17.6 mM to 53 mM [35]. The effects were mediated via AT\textsubscript{1} receptors and were inhibited by imipramine and quinine and by incubation in Na\textsuperscript{+}-free medium. There was no reasonable Na\textsuperscript{+} : Mg\textsuperscript{2+} ratio. The alteration in [Na\textsuperscript{+}]\textsubscript{i}, by angiotensin II was additionally mediated via the Na\textsuperscript{+}/Ca\textsuperscript{2+} antiporter [35].

The regulation of Na\textsuperscript{+}/Mg\textsuperscript{2+} antiport in kidney was studied with the rat renal epithelial cell line NRK-52E [36]. Short-term incubation of the cells in Na\textsuperscript{+}-free medium or in medium with 5mM Mg\textsuperscript{2+} did not change [Mg\textsuperscript{2+}], as measured by mag-fura-2. [Mg\textsuperscript{2+}]\textsubscript{i} was increased from 0.32 mM to 1.0 mM by culturing the cells in medium with 5mM Mg\textsuperscript{2+} for 1-2 days. A subsequent incubation in Mg\textsuperscript{2+}-free medium decreased [Mg\textsuperscript{2+}]\textsubscript{i}. The decrease in [Mg\textsuperscript{2+}]\textsubscript{i} and total Mg\textsuperscript{2+} in NaCl medium was coupled with the uptake of extracellular Na\textsuperscript{+} and inhibited by amiloride, indicating the presence of Na\textsuperscript{+}/Mg\textsuperscript{2+} antiport in these cells. However a reasonable ratio in the alterations of total Na\textsuperscript{+} and Mg\textsuperscript{2+} was not obtained.

The Na\textsuperscript{+}/Mg\textsuperscript{2+} antiport in NRK-52E cells was inhibited by the PKC inhibitor calphostin and the tyrosine kinase inhibitor genistein. The PKC activator phorbol 12,13 dibutyrate enhanced the decrease in [Mg\textsuperscript{2+}], PKA inhibition had no effect [36].
These results indicate activation of \( \text{Na}^+ / \text{Mg}^{2+} \) antiport in these cells by PKC.

Moreover, the \( \text{Na}^+ / \text{Mg}^{2+} \) antiport in NRK-52E cells was activated by NO and 8BrcGMP [36] and by arachidonic acid (probably via PKC) [37]. The \( \text{Na}^+ / \text{Mg}^{2+} \) antiport in NRK-52E cells was inhibited by 0.1 mM imipramine but not by 0.1 mM quinidine [37]. Thus the \( \text{Na}^+ / \text{Mg}^{2+} \) antiport in NRK-52E cells seems to have different properties compared with the \( \text{Na}^+ / \text{Mg}^{2+} \) antiport in other nucleated mammalian cells, probably due to the existence of an isoform of the \( \text{Na}^+ / \text{Mg}^{2+} \) antiporter in this cell type.

In NRK-52E cells, there seems to be a non-characterized \( \text{Na}^- \)-independent \( \text{Mg}^{2+} \) efflux stimulated by the Ca\(^{2+}\)-sensing receptor [38].

In a mouse cortical tubular epithelial cell line, the activity of the \( \text{Na}^+ / \text{Mg}^{2+} \) antiport (measured by the decrease in \([\text{Mg}^{2+}]_i\)) with furaptra [39] was increased by culturing the surviving cells in media using increasing concentrations of \( \text{Mg}^{2+} \) up to 101 mM. In the high (101) \( \text{Mg}^{2+} \)-tolerant cells, a steeper decrease in \([\text{Mg}^{2+}]_i\), dependent on \([\text{Na}^+]_o\), was found. This suggests a Hill kinetics with a Hill coefficient of about 2. The \([\text{Na}^+]_o\) dependency of \( \text{Mg}^{2+} \) efflux contrasts with the corresponding result in erythrocytes [1] and thymocytes [5], where the \([\text{Na}^+]_o\) dependency of \( \text{Mg}^{2+} \) efflux showed Michaelis-Menten kinetics. The Hill coefficient of about 2 was taken as evidence for electrogenic \( \text{Na}^+ / \text{Mg}^{2+} \) antiport in the Mg\(^{2+}\)-tolerant cells was almost completely inhibited by 0.2 mM imipramine [39].

The development of the increased \( \text{Na}^+ / \text{Mg}^{2+} \) antiport activity in the Mg\(^{2+}\)-tolerant cells is not known. Culturing in high \( \text{Mg}^{2+} \) medium may have selected a spontaneous mutation with increased \( \text{Na}^+ / \text{Mg}^{2+} \) antiport activity \((V_{\text{max}})\), or during selection, more \( \text{Na}^+ / \text{Mg}^{2+} \) antiporter molecules may have been synthesized.

The action of the \( \text{Na}^+ / \text{Mg}^{2+} \) antiport in fish kidneys is questionable [40]. \( \text{Na}^+ / \text{Mg}^{2+} \) antiport was not detected in fish kidney plasma membrane vesicles [41].

\( \text{Na}^+ / \text{Mg}^{2+} \) antiport in the placenta

\( \text{Mg}^{2+} \) concentration in fetal serum is much higher than in maternal serum, dependent on fetal age. This indicates active \( \text{Mg}^{2+} \) transport across the placenta [42].

Initial experiments were done with \(^{28}\text{Mg}^{2+}\) [43]. In pregnant rats injected with \(^{28}\text{Mg}^{2+}\), transport of \(^{28}\text{Mg}^{2+}\) to the fetuses was inhibited by simultaneous injection of amiloride, indicating the involvement of \( \text{Na}^+ / \text{Mg}^{2+} \) antiport [43].

In other experiments, rat placenta was perfused in situ on the fetal side with \( \text{Mg}^{2+}\)-free Krebs-Ringer solution and the unidirectional transfer of \( \text{Mg}^{2+} \) from maternal plasma to the Ringer solution was measured by means of atomic absorption spectrophotometry. Adding 0.5 mM amiloride or replacing \( \text{Na}^+ \) in the perfusate with choline decreased placental \( \text{Mg}^{2+} \) transport. Non-specific effects were excluded. It was therefore concluded that placental \( \text{Mg}^{2+} \) transport is mediated by \( \text{Na}^+ / \text{Mg}^{2+} \) antiport localized on the fetal side of the placenta [44].

As a model system for placental \( \text{Mg}^{2+} \) transport, \([\text{Mg}^{2+}]_i\), was measured in cultured human choriocarcinoma JEC-3 cells with mag-fura-2 [45]. Superfusion of the cells with \([\text{Na}^+]_o\)-reduced \(([\text{Na}^+]_o = 100 \text{ mM}) \) \( \text{Mg}^{2+}\)-containing or \( \text{Na}^+\)-free \( \text{Mg}^{2+}\)-containing medium increased \([\text{Mg}^{2+}]_i\), transiently. After a few minutes, \([\text{Mg}^{2+}]_i\), returned to the basal level. The increase in \([\text{Mg}^{2+}]_i\) was caused by \( \text{Mg}^{2+} \) uptake and not inhibited by 50 mM imipramine. \([\text{Mg}^{2+}]_i\) recovery occurred in choline medium. It was independent of extracellular \( \text{Na}^+ \) but was inhibited by 50 mM imipramine [45]. These results are no evidence that the alterations of \([\text{Mg}^{2+}]_i\) are mediated via \( \text{Na}^+ / \text{Mg}^{2+} \) antiport, as was suggested by the authors.

**\( \text{Na}^+ / \text{Mg}^{2+} \) antiport in smooth muscle cells**

The \( \text{Na}^+ / \text{Mg}^{2+} \) antiport in smooth muscle strips of guinea pig tenia cecum was shown indirectly by measuring \([\text{Mg}^{2+}]_o\), with furaptra [46]. The application of a reversed \([\text{Na}^+]_o > [\text{Na}^+]_i\), gradient by superfusion with \( \text{Na}^+\)-free medium containing 10 mM \( \text{Mg}^{2+} \) increased \([\text{Mg}^{2+}]_i\). Restauration of \([\text{Na}^+]_i\), and \([\text{Mg}^{2+}]_i\), to control levels decreased \([\text{Mg}^{2+}]_i\) towards the basal level. These results may be evidence for \( \text{Mg}^{2+} \) uptake via reversed \( \text{Na}^+ / \text{Mg}^{2+} \) antiport and for \( \text{Mg}^{2+} \) efflux via \( \text{Na}^+ / \text{Mg}^{2+} \) antiport. When the cell membrane was depolarized by elevated \([\text{K}^+]_o\), \( \text{Mg}^{2+} \) uptake occurred at a lower \([\text{Na}^+]_o/[\text{Na}^+]_i\), gradient. From this result it was assumed that \( \text{Na}^+ / \text{Mg}^{2+} \) antiport is voltage-sensitive and electrogenic. The decrease in \([\text{Mg}^{2+}]_i\), with increasing \([\text{Na}^+]_i\), showed Hill kinetics with a Hill coefficient of about 3 and a half maximal rate at about 30 mM \( \text{Na}^+\). A 3 \( \text{Na}^+ / \text{Mg}^{2+} \) antiport was assumed [46].

In similar experiments, \([\text{Mg}^{2+}]_i\), was measured by \(^{31}\text{P} \text{NMR}\) [47]. After substitution of extracellular \( \text{Na}^+ \) with N-methyl-D-glucamine, \([\text{Mg}^{2+}]_i\), was reversibly increased. This increase was prevented in \( \text{Na}^+\)-free
medium and enhanced in excess Mg\(^{2+}\) solutions. Extracellular Ca\(^{2+}\) competitively inhibited Mg\(^{2+}\) entry.

The Na\(^{+}/\text{Mg}^{2+}\) antiport in vascular smooth muscle cells (VSMC) was studied indirectly in primary cultures of isolated VSMC by measuring [Mg\(^{2+}\)], and [Na\(^{+}\)], with fluorescent probes and changing Mg\(^{2+}\) homeostasis of the cells by angiotensin II. Incubation of the cells with angiotensin II decreased [Mg\(^{2+}\)], and increased [Na\(^{+}\)], dose-dependently. The angiotensin II-induced decrease in [Mg\(^{2+}\)], was inhibited in Na\(^{-}\)-free medium and by quinidine and imipramine [15, 48], indicating the existence of Na\(^{+}/\text{Mg}^{2+}\) antiport.

Mg\(^{2+}\)-free, Na\(^{-}\)- and Ca\(^{2+}\)-containing solution, the ions are very actively transported in cardiomyocytes. Mg\(^{2+}\) ratio for the angiotensin II effect.

[Mg\(^{2+}\)]\,/[Na\(^{+}\)] gradient as a driving force) and buffer-

The increase in [Mg\(^{2+}\)] \((\text{line 2,6})\) cannot be caused by Mg\(^{2+}\) uptake via reversed Na\(^{+}/\text{Mg}^{2+}\) antiport. It was concluded that at 1 mM or zero [Na\(^{+}\)], in Ca\(^{2+}\)-containing medium (line 2,6), there is Ca\(^{2+}\) uptake via reversed Na\(^{+}/\text{Ca}^{2+}\) antiport followed by an increase in [Ca\(^{2+}\)]. Probably, in Na\(^{-}\)-free medium (line 2,4,6) there is

Na\(^{+}/\text{Mg}^{2+}\) antiport in heart muscle cells

There are controversial conclusions regarding the existence of Na\(^{+}/\text{Mg}^{2+}\) antiport in cardiomyocytes. These were caused by indirect experiments, by the low activity of Na\(^{+}/\text{Mg}^{2+}\) antiport and by the interactions of Mg\(^{2+}\) with intracellular Ca\(^{2+}\) and Na\(^{+}\). Both ions are very actively transported in cardiomyocytes.

When isolated rat hearts were perfused with a Mg\(^{2+}\)-free, Na\(^{-}\)- and Ca\(^{2+}\)-containing solution, the addition of 0.3 \mu M isoproterenol resulted in a rapid transient Mg\(^{2+}\) efflux lasting 20 min. Thereafter, Mg\(^{2+}\) concentration in the perfusate reached control values. The isoproterenol-induced Mg\(^{2+}\) efflux could be prevented with 1 mM amiloride [50]. The isoproterenol-induced Mg\(^{2+}\) loss amounted to 7% of total Mg\(^{2+}\) content in the heart.

In similar experiments, 10 \mu M noradrenaline also induced a transient Mg\(^{2+}\) efflux that was completely blocked by propranolol [51]. In control experiments there was no increase in LDH and K\(^{+}\) in the perfusate, thus excluding cell damage [51]. The transiency of Mg\(^{2+}\) efflux is further evidence against cell damage.

In isolated ventricular myocytes, forskolin and permeable cAMP derivatives had the same effect on Mg\(^{2+}\) efflux as noradrenaline [51].

There may be two mechanisms for the \(\beta\)-agonist-induced Mg\(^{2+}\) efflux:

1) As measured by \(^{31}\)P NMR in isolated perfused hearts, \(\beta\)-agonists can decrease the ATP concentration and can increase [Mg\(^{2+}\)], and [H\(^{+}\)], [52]. The decrease in [ATP] and increase in [H\(^{+}\)], can liberate bound Mg\(^{2+}\). The increased [Mg\(^{2+}\)], may activate the Na\(^{+}/\text{Mg}^{2+}\) antiport.

2) \(\beta\)-Agonists can activate PKA \(\text{via cAMP}\). The PKA-phosphorylated Na\(^{+}/\text{Mg}^{2+}\) antipporter may have a higher affinity for intracellular Mg\(^{2+}\).

In other experiments [53] [H\(^{+}\)], was also increased but [Mg\(^{2+}\)], was reduced by 100 \mu M isoprenaline. The simultaneous addition of 100 \mu U/ml insulin to 100 \mu M isoprenaline prevented the decrease in [Mg\(^{2+}\)], in the perfused rat hearts. A similar interaction of \(\beta\)-agonists and insulin was found in liver cells (see above).

Other investigators measured [Mg\(^{2+}\)], with Mg\(^{2+}\)-
sensitive microelectrodes and found that 20 \mu M noradrenaline or 1.6 \mu M isoprenaline had no effect on [Mg\(^{2+}\)], [54].

The controversial effects of isoprenaline on [Mg\(^{2+}\)], were not explained. Noradrenaline-induced Mg\(^{2+}\) efflux from isolated cardiomyocytes amounted to 10% of total Mg\(^{2+}\) content and was dependent on [Na\(^{+}\)]. The Mg\(^{2+}\) efflux disappeared when Na\(^{+}\) from the incubation medium was substituted by choline or N-methyl-D-glucamine. These experiments were done with Ca\(^{2+}\)-containing media. When extracellular Ca\(^{2+}\) was reduced or omitted, the Mg\(^{2+}\) efflux was reduced or prevented. A similar result was obtained when the Ca\(^{2+}\) channel blockers verapamil or nifedipine were added to the Ca\(^{2+}\)-containing medium [55]. These results may indicate that \(\beta\)-agonist increased Ca\(^{2+}\) influx and the increase in [Ca\(^{2+}\)], followed by liberation of bound Mg\(^{2+}\) may be essential. The increased [Mg\(^{2+}\)] \text{via the Na}/\text{Mg} antiport.

Based on the effects of Ca\(^{2+}\) on intracellular Mg\(^{2+}\), some investigators concluded that there is no Na\(^{+}/\text{Mg}^{2+}\) antiport in cardiomyocytes. In these experiments, [Mg\(^{2+}\)], was measured in isolated cultured embryonic chick hearts by \(^{31}\)P NMR spectroscopy [56] or furaptra [57] in media containing Na\(^{+}\), K\(^{+}\), Ca\(^{2+}\) and Mg\(^{2+}\). The omission of Na\(^{+}\) or of Ca\(^{2+}\) or of Na\(^{+}\) plus Ca\(^{2+}\), of Mg\(^{2+}\) or of Mg\(^{2+}\) plus Na\(^{+}\) in the incubation medium affected [Mg\(^{2+}\)], as shown in table 1.

The increase in [Mg\(^{2+}\)], in Mg\(^{2+}\)-free plus Na\(^{-}\)-free medium (line 6) cannot be caused by Mg\(^{2+}\) uptake \(\text{via reversed Na}/\text{Mg} antiport). It was concluded that at 1 mM or zero [Na\(^{+}\)], in Ca\(^{2+}\)-containing medium (line 2,6), there is Ca\(^{2+}\) uptake \(\text{via reversed Na}/\text{Ca} antiport\) followed by an increase in [Ca\(^{2+}\)].
also a reversed Na+/H+ antiport and an increase in [H+]i, as found in other experiments [58]. At the increased [Ca2+]i and [H+]i, bound Mg2+ is removed from its binding sites by competition with Ca2+ and H+ [56-58]. The small reduction in [Mg2+]i in Ca2+-free Na+-containing medium (line 3) may indicate a low activity of Na+/Mg2+ antiport or increased intracellular Mg2+ binding. Additional proofs such as the behaviour of [Na+]i, [Ca2+]i and the effect of inhibitors were not studied.

In similar experiments with ferret and guinea pig papillary muscle cells, [Mg2+]i was measured with Mg2+-sensitive microelectrodes and similar results were obtained. It was also concluded that there is no Na+/Mg2+ antiport in ferret and guinea pig myocardium [54, 59].

In other experiments [60], isolated rat ventricular myocytes were loaded with Mg2+ by means of 10 μM ionomycin in Ca2+-free Tyrode solution in the presence of 10 mM Mg2+. Following removal of ionomycin and reincubation in Ca2+-free Tyrode solution, [Mg2+]i was measured by furaptra decreased, indicating Mg2+ influx. The decrease in [Mg2+]i was dependent on extracellular Na+ and was inhibited by imipramine. Na+ dependency was analyzed by substitution of extracellular Na+ with N-methyl-D-glucamine and showed Hill kinetics for the [Mg2+]i decrease with a Hill coefficient of 2 and half maximal activation at [Na+]o of 82 mM. Mg2+ efflux was dependent on membrane potential. In Na+ medium with 55.9 mM K+, a lower [Na+]o/[Na+]i gradient was needed as a driving force [60]. No final explanation was given for the Hill coefficient of 2 and dependency on membrane potential or the effect of high [K+]o, respectively. Following experiments with Mg2+-loaded cardiomyocytes also showed Hill kinetics for extracellular Na+ with a Hill coefficient of 1.7 and half maximal activation at [Na+]o of 55 mM [61]. Moreover it was found that increased [Na+]i and increased [Mg2+]i inhibited Mg2+ efflux via Na+/Mg2+ antiport as in erythrocytes [1]. When the Na+ gradient was reversed, there was only a small Mg2+ influx via reversed Na+/Mg2+ antiport [61].

In later experiments [63], isolated rat ventricular myocytes were loaded with Mg2+ by superfusing the cells with Ca2+-free and Na+-free solution containing 30 mM Mg2+. The re-addition of extracellular Na+ (without Ca2+) caused [Mg2+]i to fall to the basal level, its rate increasing linearly with [Na+]o/[Mg2+]i. Recovery was completely inhibited by 0.2 mM imipramine. The Na+/Mg2+ antiport in rat ventricular myocytes was activated by intracellular Mg2+ [64]. Since the possibility has been raised that Mg2+ efflux from heart myocytes may occur through the Na+/Ca2+ antiporter, the effect of two inhibitors of the Na+/Ca2+ antiport, KB-R7943 and SEA 0400, was tested. Neither inhibitor had any significant effect on [Mg2+]i recovery [63]. Also, in recent experiments Mg2+ efflux via the Na+/Ca2+ antiport and via Na+, K+, Cl-/Mg2+ antiport were excluded [65].

Membrane depolarization by increasing superfusate [K+]o to 70 mM increased the [Mg2+]i recovery rate in Na+-containing solution more than three fold. It was concluded that [Mg2+]i recovery is mediated by Mg2+ efflux via 1 Na’/1 Mg2+ antiport [63]. The controversial results with respect to the Hill kinetics of Na+ dependency of Na+/Mg2+ antiport in cardiomyocytes [60] remain an open question.

As a consequence, Na+/Mg2+ antiport in cardiomyocytes can only be studied when the cells are loaded with Mg2+ and [Ca2+]i, is taken into account.

### Table 1. [Mg2+]i in cultured embryonic chick cardiomyocytes incubated in solutions with various cations. [Mg2+]i was measured by 31P NMR spectroscopy [56] and furaptra [57]. The original values of [Mg2+]i were calculated as a percentage of the controls (100%).

<table>
<thead>
<tr>
<th>Medium</th>
<th>[Mg2+]i (%)</th>
<th>[Mg2+]i (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Control (Na+, K+, Ca2+, Mg2+)</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>2 [Na+]o: 1 or 0 mM</td>
<td>211.1</td>
<td>264.6</td>
</tr>
<tr>
<td>3 Ca2+-free</td>
<td>88.8</td>
<td>93.8</td>
</tr>
<tr>
<td>4 Ca2+-free; [Na+]o: 1 or 0 mM</td>
<td>164.4</td>
<td>106.3</td>
</tr>
<tr>
<td>5 Mg2+-free</td>
<td>110.2</td>
<td>106.3</td>
</tr>
<tr>
<td>6 Mg2+-free; [Na+]o: 1 or 0 mM</td>
<td>162.0</td>
<td>333.3</td>
</tr>
</tbody>
</table>
Na\(^+\)/Mg\(^{2+}\) antiport in skeletal muscle

In skeletal muscle cells, the Na\(^+\)/Mg\(^{2+}\) antiport was studied indirectly \[66, 67\]. In frog sartorius muscle fibers, [Mg\(^{2+}\)]\(_i\) was measured with Mg\(^{2+}\)-sensitive microelectrodes filled with ETH 1117. Exposure of muscle fibers for up to 2 h in Na\(^+\)-free Ringer solution by replacement of Na\(^+\) by Li\(^+\) produced no change in [Mg\(^{2+}\)]\(_i\) \[66\], indicating the absence of Na\(^+\)/Mg\(^{2+}\) antiport under these conditions.

In later experiments, [Mg\(^{2+}\)]\(_i\) and [Na\(^+\)]\(_i\) were measured with microelectrodes in frog skeletal muscle fibers using the more specific ETH 5214 for [Mg\(^{2+}\)]\(_i\) measurement \[67\]. Increasing [Mg\(^{2+}\)]\(_o\) to 10 or 20 mM in Ringer solution increased [Mg\(^{2+}\)]\(_i\) and decreased [Na\(^+\)]\(_i\), dependent on [Mg\(^{2+}\)]\(_o\), indicating reversed Na\(^+\)/Mg\(^{2+}\) antiport. Partial depolarization by increasing [K\(^+\)]\(_o\) up to 12.5 mM had no effect on the alterations of [Na\(^+\)]\(_i\) and [Mg\(^{2+}\)]\(_i\). These results may indicate that an electroneutral Na\(^+\)/Mg\(^{2+}\) antiport is operating in skeletal muscle when Mg\(^{2+}\) homeostasis is changed \[67\].

Final remarks

Cell membranes are poorly permeable to Mg\(^{2+}\). When control (non-Mg\(^{2+}\)-loaded) cells were incubated in Mg\(^{2+}\)-free NaCl medium, Mg\(^{2+}\) efflux was usually not detectable. Under these conditions, of the reviewed cell types, only lymphocytes and Ehrlich ascites tumor cells expressed a low rate of Mg\(^{2+}\) efflux. Therefore, to study Mg\(^{2+}\) efflux in other cell types, the physiological Mg\(^{2+}\) homeostasis must be changed by Mg\(^{2+}\) loading or by hormones or effectors.

Only in a few studies was the Na\(^+\)/Mg\(^{2+}\) antiport measured directly. In most studies the Na\(^+\)/Mg\(^{2+}\) antiport was deduced from the changes in [Mg\(^{2+}\)]\(_i\), or [Mg\(^{2+}\)]\(_o\), plus [Na\(^+\)]\(_i\). In addition, the inhibition of changes in [Mg\(^{2+}\)]\(_i\), and [Na\(^+\)]\(_i\), by amiloride, imipramine and quindine, or their prevention in Na\(^+\)-free medium, were taken as evidence for a Na\(^+\)/Mg\(^{2+}\) antiport.

In the indirect studies, the stoichiometric ratio of the Na\(^+\)/Mg\(^{2+}\) antiport could not be measured due to buffering of Mg\(^{2+}\), due to compartmentation of intracellular Mg\(^{2+}\) and Na\(^+\) and due to interaction of other Na\(^+\) transporters. Moreover, there is an interference of intracellular Ca\(^{2+}\) and H\(^+\) with intracellular Mg\(^{2+}\).

As far as studied, the Na\(^+\)/Mg\(^{2+}\) antiport was reversible when the driving Na\(^+\) and Mg\(^{2+}\) gradients were reversed.

The kinetic properties and regulation of the Na\(^+\)/Mg\(^{2+}\) antiport were studied only in a few cell types.

For the reviewed cell types, the kinetics and stoichiometric ratio of the Na\(^+\)/Mg\(^{2+}\) antiport were only investigated directly and in more detail in Mg\(^{2+}\)-loaded thymocytes. Thus the existence of two Na\(^+\)/Mg\(^{2+}\) antiporters was assumed: a high Na\(^+\) affinity 1 Na\(^+\)/1 Mg\(^{2+}\) antiporter and a low Na\(^+\) affinity 2 Na\(^+\)/1 Mg\(^{2+}\) antiporter. In smooth muscle cells, a 3 Na\(^+\)/1 Mg\(^{2+}\) antiporter was discussed.

As far as studied, Na\(^+\)/Mg\(^{2+}\) antiport in non-Mg\(^{2+}\)-loaded cells can be activated by intracellular Mg\(^{2+}\) and by phosphorylation in most nucleated cell types by PKA, in a renal cell line by PKC as in erythrocytes \[1\]. It is probable that there exist isoforms of the Na\(^+\)/Mg\(^{2+}\) antiporter in various cell types as well as in the same cell type, as was discussed for erythrocytes \[1\]. The existence of isoforms will be proven if the chemical structure of the various Na\(^+\)/Mg\(^{2+}\) antiporters is identified.

The Na\(^+\)/Mg\(^{2+}\) antiport seems to be present in all cell types, although some investigators did not detect it due to their experimental conditions.

The function of Na\(^+\)/Mg\(^{2+}\) antiport is to perform efflux of Mg\(^{2+}\), to establish a low and constant [Mg\(^{2+}\)]\(_i\). In special cell types, the Na\(^+\)/Mg\(^{2+}\) antiport is involved in transcellular Mg\(^{2+}\) transport e.g. in gastrointestinal tract, kidney and placenta.

References

2. Günther T, Vormann J. Na\(^+\)-dependent Mg\(^{2+}\) efflux from Mg\(^{2+}\)-loaded rat thymocytes and HL 60 cells. Magnes Trace Elem 1990; 9: 279-82.


39. Watanabe M, Konishi M, Ohkido I, Matsuful S. Enhanced sodium-dependent extrusion of magnesium...


58. Tashiro M, Konishi M. Intracellular free magnesium in guinea-pig heart, studied with Mg2+-selective microelectrodes and fluorochromes. Experiment Physiol 1999; 84: 105-10.


