DIVALENT CATION TRANSPORT IN KIDNEY SLICES
II. MAGNESIUM TRANSPORT IN KIDNEY CORTEX SLICES
AND EFFECTS OF DIURETICS

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Accepted October 24, 1979

Abstract—We examined the properties of Mg\(^{2+}\) transport in rat kidney cortex slices
and the effects of diuretics were also studied. Incubation with 1 mM 2,4-dinitro-
phenol, or under anaerobic conditions, sharply inhibited Mg\(^{2+}\) influx, while markedly
stimulating Mg\(^{2+}\) efflux. Under conditions of hypothermia, partial inhibition of
Mg\(^{2+}\) influx and significant enhancement of Mg\(^{2+}\) efflux were observed. Mg\(^{2+}\)
influx was not affected by ouabain, by altering CaCl\(_2\) concentration in the medium,
or by a change of Ca\(^{2+}\) content in the slices. Incubation with 1 mM ethacrynic acid
or mersalyl depressed Mg\(^{2+}\) influx and stimulated Mg\(^{2+}\) efflux, p-chloromercuribenzoic
acid (5 x 10\(^{-4}\) M) had a similar effect and furosemide had no effect on Mg\(^{2+}\) transport.
These results suggest that Mg\(^{2+}\) influx is mediated by an energy-dependent process
which is dissociated from ouabain-sensitive Na\(^{+}\) transport and Ca\(^{2+}\) flux. Sulfhydryl
groups may be involved in the process of Mg\(^{2+}\) influx.

There is considerable evidence that the bulk of Mg\(^{2+}\) is reabsorbed in renal tubules.
Micropuncture studies indicated that Mg\(^{2+}\) is reabsorbed in the proximal and distal seg-
ments of the nephrons in the dog (1, 2). These investigators also reported that reabsorption
of electrolytes in the proximal nephrons is an isotonic process and that the intratubular
concentration of Mg\(^{2+}\) is low in the distal tubules. In the rat, filtered Mg\(^{2+}\) is reabsorbed
along the tubules (3).

A correlation between renal transport of Mg\(^{2+}\), Ca\(^{2+}\) and Na\(^{+}\) has been reported.
Stop-flow experiments supported the possibility of a similar transport mechanism for Mg\(^{2+}\)
and Ca\(^{2+}\) (4). Infusion of MgCl\(_2\) into the aorta was associated with an increase in tubular
reabsorption of Mg\(^{2+}\) and a definite decrease in Ca\(^{2+}\) and Na\(^{+}\) reabsorption (5).

Diuretics such as ethacrynic acid, furosemide and mersalyl have been shown to increase
Mg\(^{2+}\) excretion (6-8). However, the effects of diuretics on Mg\(^{2+}\) transport of kidney cells
have not been elucidated (9).

As little information is available on the biochemical bases of the Mg\(^{2+}\) transport system
in the kidney, we used kidney cortex slices to elucidate the properties of Mg\(^{2+}\) transport and
to evaluate the effect of diuretics on Mg\(^{2+}\) influx. The possible correlation among Mg\(^{2+}\),
Ca\(^{2+}\) and Na\(^{+}\) transport in the kidney cortex slices is also discussed.

MATERIALS AND METHODS

Preparation of slices: Male Wistar rats, weighing 180 g-250 g were used in all experi-
ments. The kidney cortex was sliced with a razor blade and the slices (0.3–0.4 mm in thickness) were leached at 0°C for 120 min in a solution containing 150 mM NaCl and 5 mM MgSO$_4$ (Mg$^{2+}$-enriched slices) or in a solution containing 150 mM NaCl and 0.1 mM EDTA (Mg$^{2+}$-depleted slices). Mg$^{2+}$-enriched slices were used for Mg$^{2+}$ efflux (decrease in Mg$^{2+}$ content) and Mg$^{2+}$-depleted slices for Mg$^{2+}$ influx (increase in Mg$^{2+}$ content) studies.

**Incubation conditions:** Leached slices (approximately 400 mg) were incubated at 37°C or 0°C for varying periods in conical flasks with 10 ml Krebs-Ringer bicarbonate buffer (pH 7.4, KRB) containing 118 mM NaCl, 24.9 mM NaHCO$_3$, 1.18 mM KH$_2$PO$_4$, 4.74 mM KCl, 1.18 mM MgSO$_4$, 2.54 mM CaCl$_2$ and 5.56 mM glucose. The incubation medium was bubbled with 95% O$_2$ and 5% CO$_2$, or 95% N$_2$ and 5% CO$_2$ throughout the experiments.

**Estimation of electrolytes:** After incubation, the slices were blotted, weighed and digested overnight with concentrated nitric acid at 50°C. Mg$^{2+}$ and Na$^+$ were estimated with an atomic absorption spectrophotometer (Hitachi 170-10) and a flame photometer (Hitachi 205D), respectively. Fresh kidney cortex used for the experiments contained 6.95±0.27 μmoles Mg/g tissue wet wt. and 63.2±0.71 μmoles Na/g tissue wet wt. (nine determinations).

**Chemicals and presentation of data:** Ethacrynic acid (2,3-dichloro-4-(2-methylene-butyryl)-phenoxyacetic acid) was kindly provided by Japan Merck Banyu Co. and dissolved in KRB, pH 7.4. Ouabain, mersalyl and furosemide were purchased from Merck Chemical Co., Sigma Chemical Co. and Hoechst Japan Co., respectively. Other chemicals used were of reagent grade. Student’s t-test was employed to estimate differences between the means of the control and experimental series. Values were expressed as mean±standard errors (S.E.).

**RESULTS**

**Metabolic dependence of Mg$^{2+}$ transport:** In order to determine whether Mg$^{2+}$ fluxes are energy-dependent processes, we examined the effects of 2,4-dinitrophenol (DNP) and of anaerobic or hypothermic conditions on the Mg$^{2+}$ transport in the slices (Figs. 1, 2). Mg$^{2+}$ content in the slices was 4.42±0.01 μmoles/g tissue wet wt. and 5.84±0.08 μmoles/g after leaching, in the absence or presence of MgSO$_4$, respectively. An increase in Mg$^{2+}$ content in Mg$^{2+}$-depleted and a decrease in Mg$^{2+}$ content in Mg$^{2+}$-enriched slices followed an aerobic incubation of the leached slices in KRB at 37°C. Incubation with 1 mM DNP or under anaerobic conditions resulted in the complete inhibition of Mg$^{2+}$ influx and a substantial increase of Mg$^{2+}$ efflux (Fig. 1), suggesting that Mg$^{2+}$ influx may be mediated by an energy-consuming process. Furthermore, Mg$^{2+}$ influx was partially but significantly inhibited and Mg$^{2+}$ efflux enhanced by incubation under hypothermic (0°C) conditions as compared to incubation at 37°C (Figs. 2A, 2B). These findings support the hypothesis that Mg$^{2+}$ influx is an energy-dependent process.

**Dissociation of Mg$^{2+}$ influx from Na$^+$ transport:** We then investigated whether or not the process of Mg$^{2+}$ influx is coupled with active Na$^+$ efflux. The addition of ouabain
to the incubation medium had no effect on Mg\(^{2+}\) influx, while Na\(^{+}\) efflux was completely inhibited (Table 1), indicating that Mg\(^{2+}\) influx may be dissociated at least from ouabain-sensitive Na\(^{+}\) transport in the kidney cortex slices.

**Effect of Ca\(^{2+}\) on Mg\(^{2+}\) influx:** The effect of altering the concentration of CaCl\(_2\) on Mg\(^{2+}\) content in the slices is shown in Fig. 3. Mg\(^{2+}\) content increased from 4.20±0.19 \(\mu\)moles/g slice to 5.59±0.21 \(\mu\)moles/g slice in the absence of CaCl\(_2\) in the medium during 30 min incubation. The addition of CaCl\(_2\) (concentrations of up to 5 mM) to the medium had no effect on Mg\(^{2+}\) influx, while Ca\(^{2+}\) content in the slices correlated with Ca\(^{2+}\) concentration in the medium. We examined Mg\(^{2+}\) influx in Ca\(^{2+}\)-depleted and Ca\(^{2+}\)-enriched slices containing 1.00±0.14 \(\mu\)moles Ca/g slice and 7.57±0.34 \(\mu\)moles Ca/g slice, respectively, before incubation (at zero time, data not shown). Irrespective of Ca\(^{2+}\) content in the slices, there was no significant difference between Mg\(^{2+}\) influx in Ca\(^{2+}\)-depleted and Ca\(^{2+}\)-enriched slices.

**Effects of diuretics and p-chloromercuribenzoic acid (PCMB):** We examined the effects of diuretics and PCMB on Mg\(^{2+}\) transport in kidney cortex slices (Tables 2, 3). The Mg\(^{2+}\) content of control slices was increased from 3.55±0.08 \(\mu\)moles/g slice to 4.73±0.12 \(\mu\)moles/g
slice during 30 min incubation (Table 2). Ethacrynic acid (final concentration, 1 × 10^{-3} M) and mersalyl (1 × 10^{-3} M) significantly depressed, PCMB (5 × 10^{-4} M) completely inhibited Mg^{2+} influx. However, furosemide (1 × 10^{-3} M) showed no effect on Mg^{2+} influx. On the other hand, Mg^{2+} efflux in Mg^{2+}-enriched slices was stimulated by the addition of ethacrynic acid, mersalyl or PCMB to the medium, while furosemide (1 × 10^{-3} M) had no effect on Mg^{2+} efflux (Table 3). These data suggest that the sulfhydryl groups may be involved in the process of Mg^{2+} influx.

**DISCUSSION**

Aerobic incubation in KRB at 37°C produced a Mg^{2+} influx in Mg^{2+}-depleted and Mg^{2+} efflux in Mg^{2+}-enriched kidney cortex slices. However, incubation with 1 mM DNP, under anaerobic or hypothermic conditions inhibited Mg^{2+} influx. Preliminary results in our laboratory indicate that inulin space of kidney cortex slices varies according to experimental conditions during incubation. Even though Mg^{2+} content after incubation was expressed...
as μmoles/g of intracellular fluid, calculated on the basis of inulin space of the experimental conditions, Mg²⁺ influx was inhibited by DNP, under anaerobic or hypothermic conditions (data not shown). These results suggest that Mg²⁺ influx is dependent on metabolic energy. DNP and an anaerobic condition inhibit the production of cellular ATP and produce a decrease of ATP content in the slices. Reduction of cellular ATP content may lower the activity of ATP hydrolysis responsible for Mg²⁺ transport, if this system is indeed present in the kidney cortex. Incubation of slices under hypothermic conditions resulted in only a partial inhibition of Mg²⁺ influx, while the addition of DNP to the incubation medium or incubation under anaerobic conditions completely inhibited the Mg²⁺ influx. We have no explanation for this phenomenon. The process of Mg²⁺ influx may to some extent be resistant to hypothermic conditions. We hypothesize that the stimulation of Mg²⁺ efflux by DNP, anaerobic or hypothermic conditions may be related to the apparent inhibition of Mg²⁺ influx by these treatments, since Mg²⁺ transport from medium to cells or vice versa takes place in both Mg²⁺-enriched and Mg²⁺-depleted slices.

Alternatively, if Mg²⁺ transport is coupled with other ion transports, then Mg²⁺ is secondarily transported from the medium to the cells. Ouabain, a specific inhibitor of (Na⁺ + K⁺)-ATPase, did not affect Mg²⁺ transport, while this compound completely inhibited active Na⁺ transport, suggesting that the Mg²⁺ transport and ouabain-sensitive Na⁺ systems

### TABLE 2. Effect of diuretics and PCMB on Mg²⁺ influx

| Treatments      | Mg²⁺ content* (μmoles/g slice wet wt.) |
|-----------------|----------------------------------------|
| Control         | 4.73±0.12 (15)                         |
| Mersaly (1×10⁻³ M) | 3.54±0.05** (4)                      |
| Ethacrynic acid (1×10⁻³ M) | 4.02±0.07*** (4)                |
| Furosemide (1×10⁻³ M) | 4.83±0.09 (4)                      |
| PCMB (5×10⁻⁴ M) | 3.12±0.08** (4)                      |

Initial Mg²⁺ content was 3.55±0.08 μmoles/g slice (16). Mean values±S.E. are given for the numbers of experiments shown in parentheses. *Mg²⁺ content was determined after 30 min incubation in KRB at 37°C. **Significantly different from control, p<0.005. ***Significantly different from control, p<0.01.

### TABLE 3. Effect of diuretics and PCMB on Mg¹⁺ efflux

| Treatments      | Mg¹⁺ content* (μmoles/g slice wet wt.) |
|-----------------|----------------------------------------|
| Control         | 5.59±0.08 (16)                         |
| Mersaly (1×10⁻³ M) | 4.18±0.10** (4)                      |
| Ethacrynic acid (1×10⁻³ M) | 4.70±0.11** (4)                |
| Furosemide (1×10⁻³ M) | 5.46±0.16 (4)                      |
| PCMB (5×10⁻⁴ M) | 2.71±0.06** (4)                      |

Initial Mg¹⁺ content was 6.67±0.08 μmoles/g slice (16). Mean values±S.E. are given for the numbers of experiments shown in parentheses. *Mg¹⁺ content was determined after 30 min incubation in KRB at 37°C. **Significantly different from control, p<0.005.
are different.

We previously reported that Ca\textsuperscript{2+} efflux from kidney cortex slices is dependent on metabolic energy (10). Our present results show that Mg\textsuperscript{2+} influx was unaffected by the addition of CaCl\textsubscript{2} to the medium and was not associated with the change of Ca\textsuperscript{2+} content at the start of the incubation. These observations support the suggestion that Mg\textsuperscript{2+} influx is not related to Ca\textsuperscript{2+} flux.

Diuretics such as mersalyl, ethacrynic acid and furosemide inhibit tubular reabsorption of Mg\textsuperscript{2+} (6–8). In addition, ethacrynic acid and furosemide have been shown to inhibit glycolysis (11) and oxidative phosphorylation of dog kidney mitochondria (12). Ethacrynic acid reduced cellular ATP levels (13). Eknoyan et al. (12) have indicated that the 50\% inhibitory molar concentration for ethacrynic acid on oxygen consumption by isolated mitochondria was 6.2 \times 10^{-4} and for furosemide 1.5 \times 10^{-3}. We did not determine the effect of furosemide on Mg\textsuperscript{2+} transport, but a higher concentration of this compound may produce such an effect.

We reported recently that ethacrynic acid and mersalyl at the same concentrations as used in the present investigation, reduced cellular ATP content (10). Therefore, we assume that the inhibition of Mg\textsuperscript{2+} influx by mersalyl or ethacrynic acid observed in the present study, is due to the diminution of cellular ATP. There is, however, an alternative hypothesis which suggests that both diuretics and PCMB directly affect the Mg\textsuperscript{2+} membrane transport process in the kidney cortex. Gemba and Nishimura (14) reported that Mg\textsuperscript{2+}-activated ATPase of rat kidney cortical microsome is inhibited by mersalyl, but not furosemide. Therefore, in kidney cortex slices, Mg\textsuperscript{2+}-activated ATPase may be responsible for Mg\textsuperscript{2+} transport.

Further investigations are underway to elucidate whether Mg\textsuperscript{2+} influx is implicated in Mg\textsuperscript{2+}-activated ATPase. In addition, it is of importance to clarify which flux of Mg\textsuperscript{2+} at the slice level corresponds to Mg\textsuperscript{2+} reabsorption in vivo.

Acknowledgements: We thank Prof. K. Yamamoto of Osaka City University for pertinent discussions and reading of the manuscript. Thanks are also due to Miss S. Nakano, a student of our college, for assistance with the experiments.

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