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Effects of magnesium, ouabain and bumetanide on ^{86}rubidium uptake in a human atrial cell line

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1 The effects of extracellular magnesium concentrations (0, 0.6, 1.2 mM) on ^{86}Rb (used as an analogue of potassium) uptake were investigated in the Girardi human atrial cell line in the presence and absence of drugs.

2 Increasing extracellular magnesium resulted in significantly higher ^{86}Rb uptake. Compared to uptake in 0.6 mM (the physiological extracellular magnesium concentration), uptake of ^{86}Rb was significantly higher in the 1.2 mM magnesium medium and significantly lower in the magnesium-free medium.

3 Ouabain (10^{-3} M) and bumetanide (10^{-4} M) were added to inhibit, respectively, the Na-K-ATPase and the Na-K-Cl co-transport system in the media containing the three magnesium concentrations. The ouabain-sensitive, bumetanide-sensitive and residual transport were found to be 58%, 29% and 13% of the total uptake in the medium containing 0.6 mM magnesium.

4 The oubain-sensitive ^{86}Rb uptake was inhibited significantly by reducing the magnesium concentrations to zero whereas the bumetanide-sensitive and residual uptake were not significantly affected by different magnesium concentrations.

5 At three different ouabain concentrations (10^{-7} M, 10^{-5} M, 10^{-3} M) studied there was significantly greater uptake of ^{86}Rb in 1.2 mM magnesium compared to uptake in 0 mM magnesium.

6 The present findings indicate that extracellular magnesium is important for ^{86}Rb (potassium) transport in cardiac cells, and suggest that the main effect is on the Na-K-ATPase component of transport.

Introduction

There is a great body of evidence showing the importance of magnesium for potassium homeostasis (Ryan, 1986). Magnesium deficiency has been shown to decrease cell potassium in different cell types. Furthermore, combined magnesium and potassium deficiencies result in greater loss of cell potassium than occurs in simple potassium deficiency, and uncorrected coexisting magnesium deficiency impairs repletion of cell potassium (Ryan, 1986). Also, in studies of the mechanisms behind the antiarrhythmic effects of magnesium in digitalis toxicity, an effect of magnesium on cell potassium has been established (Iseri, 1983). In hypomagnesemic dogs, Seller (1971) found that replacement of magnesium was able to prevent completely egress of myocardial potassium induced by strophanthidin and prevent toxic arrhythmias in about 40% of the animals.

The mechanism(s) involved in the effects of magnesium deficiency on cell potassium in the absence and presence of digitalis have not been fully elucidated. A number of possibilities exist. Direct effects of magnesium deficiency may include increased potassium efflux due to increased membrane fluidity resulting in leaky membranes (Schilling *et al.*, 1980). Magnesium may also influence potassium channels in the

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cell membrane (Shine & Douglas, 1974). Intracellular magnesium has been established as an absolute requirement for the Na-K-ATPase (Skou, 1960). At magnesium concentrations around the physiological range, sodium/potassium exchange in red blood cells increased linearly with increasing intracellular magnesium concentration (Flatman & Lew, 1981). High concentrations of extracellular magnesium have been shown to affect the ouabain-sensitive (Na-K-ATPase component), the bumetanide-sensitive (Na-K-Cl co-transport) and residual (ouabain- and bumetanide-insensitive) routes of transport in human red blood cells (Ellory *et al.*, 1983).

The clinical relevance of the magnesium-potassium interrelationship is most important in the heart. Cardiac cell lines provide models for investigation of the underlying mechanisms. The present studies were carried out using a human atrial cell line (Girardi *et al.*, 1958). The first aim of the experiments was to investigate the effects of 0, 0.6 and 1.2 mM magnesium on the accumulation of ^{86}Rb (a potassium analogue) in Girardi heart cells. The second aim of the study was to investigate if external magnesium may have effects on the ouabain-sensitive (Na-K-ATPase component), the bumetanide-sensitive (Na-K-Cl co-transport) and the residual (ouabain- and bumetanide-insensitive) routes of ^{86}Rb (potassium) uptake in this cultured cardiac cell line. Part of our findings have been presented in a preliminary form (Borchgrevink *et al.*, 1987).

Methods

Cell maintenance and growth

Girardi heart cells derived from human atrium (Girardi *et al.*, 1958) were obtained from Flow Laboratories and were grown in Dulbecco's Modified Eagles Medium supplemented with 10% foetal calf serum, glutamine (2 mM), non-essential amino acid (1%) and gentamicin (0.1 mg ml^{-1}). The cells were seeded in 24 multi-well plates (Nunc.) in aliquots of 1 ml each containing 2×10^4 cells.

After 5 days when the cells had reached confluency, the medium was removed and the cells rinsed twice with 0.9 ml of HEPES-Tris buffer of 0 mM magnesium concentration. Medium of the following composition (mM): HEPES 15, NaCl 134, KCl 5.4, $CaCl_2$ 1.0, glucose 11 and Tris buffer to obtain pH of 7.40 was added. Cells were then incubated in medium with one of three different magnesium concentrations (0, 0.6, 1.2 mM) for 120 min at 37°C under an atmosphere of 95% O_2 and 5% CO_2.

^{86}Rb uptake studies

The ^{86}Rb (used as an analogue of K) uptake was investigated. The concentration of ^{86}Rb in each well was 1 μCi ml^{-1}. The uptake was first studied in the three media with different magnesium concentrations in the absence of any drugs. This was defined as total uptake. Uptake was also studied in media containing ouabain (10^{-3} M) and in media containing ouabain (10^{-3} M) and bumetanide (10^{-4} M). After 90 min, ^{86}Rb uptake was stopped by removal of the radioactive medium by suction and rinsing each well three times in ice-cold HEPES-Tris solution. Then 2% sodium dodecyl sulphate solution was added for 40 min. This had the effect of completely lysing the cells and thus releasing radiactivity into the solution in the wells.

The contents of each well were then transferred to counting tubes and their γ-radioactivity measured in an LKB 1282 Compugamma counter for 20 min. In each 24 multi-well plate, 6 wells served as control for cell counting. The cells were trypsinized and counted on an improved Neubauer haemocytometer by use of a phase contrast microscope (Leitz) and the mean number of cells per well was obtained.

Expression of results

The uptake of ^{86}Rb was expressed as counts per min (c.p.m.) per 10^6 cells and a mean of the uptake in the two wells having the same magnesium and drug concentration in each experiment was obtained. All values presented are the mean of 6 or 12 experiments and the s.e.mean is included. The data were subjected to a one-way analysis of variance and subsequently to Student's t test for paired group means. Significant differences between group means were inferred for P values less than 0.05.

Results

The effects of different magnesium concentrations

The results for the effects of extracellular magnesium on the uptake of ^{86}Rb in the absence of any drugs are shown in Table 1. The extracellular magnesium concentration significantly affected ^{86}Rb uptake. Uptake of ^{86}Rb was significantly increased in media containing 0.6 mM magnesium ($P < 0.001$) and 1.2 mM magnesium ($P < 0.001$) compared to uptake in medium containing 0 mM added magnesium. Significant differences in ^{86}Rb uptake were also

Table 1 Effects of extracellular magnesium on total ^{86}Rb uptake

Magnesium concentration (mM)	*^{86}Rb uptake* (c.p.m. 10^{-6} cells)
0.0	758 ± 79
0.6	1104 ± 75*
1.2	1238 ± 37*†

The results shown are mean of 12 experiments ± s.e.mean. * Indicates significant difference ($P < 0.001$) from values in 0.0 mM magnesium medium. † Indicates significant difference ($P < 0.025$) between ^{86}Rb uptake in medium containing 0.6 and 1.2 mM magnesium.

detected between media containing 0.6 mM magnesium and 1.2 mM magnesium ($P < 0.025$).

The effects of magnesium on the ouabain-sensitive, the bumetanide-sensitive, and the residual uptake

Ouabain (10^{-3} M) reduced the ^{86}Rb uptake in the different magnesium media. The addition of bumetanide (10^{-4} M) resulted in further reductions of ^{86}Rb uptake in the three media with different concentrations of magnesium. From these results, the ouabain-sensitive, the bumetanide-sensitive accumulation and the residual ^{86}Rb uptake were calculated and these results are summarized in Figure 1.

The ouabain-sensitive ^{86}Rb accumulation was inhibited significantly by reducing the magnesium

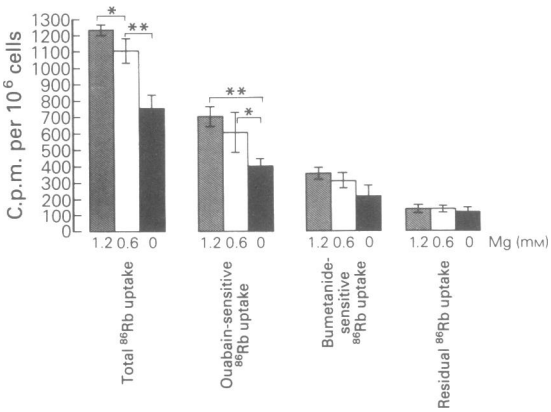

Figure 1 Effects of magnesium (Mg) on the total, ouabain-sensitive, bumetanide-sensitive and residual ^{86}Rb uptake in Girardi heart cells. Values presented are mean of 6 or 12 experiments (total ^{86}Rb uptake) with s.e.mean indicated by vertical lines. *$P < 0.05$, **$P < 0.01$

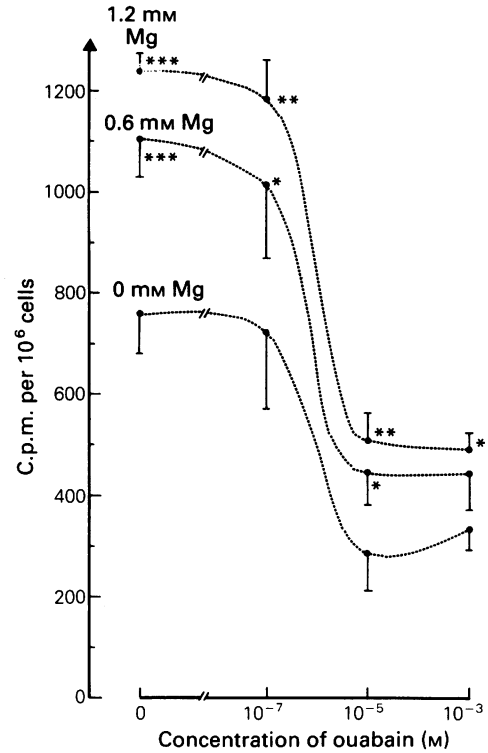

Figure 2 Net uptake of ^{86}Rb of Girardi heart cells at different concentrations of magnesium and ouabain. Values presented are mean of 6 or 12 experiments (0 mM ouabain) with s.e.mean indicated by vertical lines. *$P < 0.05$, **$P < 0.01$ and ***$P < 0.005$ from values obtained in medium containing 0 mM magnesium.

concentration to zero (1.2 mM vs 0 mM: $P < 0.005$, 0.6 mM vs 0 mM: $P < 0.05$). There was a trend towards a decline in the bumetanide-sensitive accumulation with a reduction in magnesium concentration. However, no statistically significant differences were detected in the bumetanide-sensitive ^{86}Rb accumulation as a result of altering the extracellular magnesium concentrations. Likewise, no significant differences were detected in the residual ^{86}Rb in the three media with different magnesium concentrations.

Net uptake of ^{86}Rb at different concentrations of ouabain and magnesium

The effects of different concentrations of ouabain and magnesium on ^{86}Rb accumulation are shown in Figure 2. Ouabain 10^{-7} M did not inhibit the ^{86}Rb net uptake significantly in any of the three different magnesium media. The net uptake of ^{86}Rb in the

media containing 10^{-5} M ouabain was inhibited by 59% ($P < 0.005$), 60% ($P < 0.0005$) and 62% ($P < 0.005$) from appropriate control values at the three concentrations of magnesium, 1.2 mM, 0.6 mM, and 0 mM, respectively. No further inhibition of ^{86}Rb occurred at the higher concentration (10^{-3} M) of ouabain. Thus, it may be concluded that ouabain at 10^{-7} M did not inhibit ^{86}Rb net uptake, whereas ouabain at 10^{-5} M and at 10^{-3} M had maximal inhibitory effects.

At all concentrations of ouabain, there was significantly higher ^{86}Rb accumulation in the medium containing 1.2 mM magnesium compared to ^{86}Rb uptake in the medium containing 0 mM magnesium (10^{-7} M: $P < 0.025$, 10^{-5} M: $P < 0.005$, 10^{-3} M: $P < 0.025$). Also in the 0.6 mM magnesium medium at 10^{-7} M and 10^{-5} M ouabain the ^{86}Rb uptake was significantly higher than in the magnesium-free medium ($P < 0.05$).

Discussion

The results of the present experiments indicate that extracellular magnesium is a factor in regulating cellular potassium (^{86}Rb) uptake. Since a concentration higher than the physiological magnesium level increased the ^{86}Rb uptake and a magnesium-free medium reduced the accumulation, it may be concluded that high extracellular magnesium stimulates and low concentrations inhibit potassium uptake. Shine & Douglas (1974) found that extracellular magnesium had effects on potassium balance; 16 mM magnesium abruptly decreased ^{42}K efflux from rat isolated blood-perfused interventricular septa. The present study establishes that concentrations of extracellular magnesium around the physiological range are important for the potassium homeostasis.

In investigating the mechanisms whereby external magnesium may influence ^{86}Rb uptake a dose of ouabain sufficient to inhibit Na-K-ATPase completely, was used. This dose of the cardiac glycoside reduced the total ^{86}Rb accumulation by 58% in the 0.6 mM magnesium medium, indicating that more than half of the ^{86}Rb uptake was dependent on Na-K-ATPase. The addition of bumetanide (10^{-4} M), which has been shown to block the sodium, potassium and chloride co-transport system sensitive to diuretics acting on the loop (Murphy *et al.*, 1983), produced a further reduction of the ^{86}Rb uptake, indicating that 29% of the uptake was dependent on this transport system. When comparing the ouabain-sensitive and the bumetanide-sensitive accumulation, and the residual uptake in media with three different magnesium concentrations (Figure 1), it was found that the ouabain-sensitive uptake was inhibited significantly in the magnesium-free medium.

The finding that altering the external magnesium concentration has an effect upon Na-K-ATPase is in apparent contrast to previous studies. Skou (1960) and Flatman & Lew (1981) concluded that magnesium affects the enzyme at the inner surface of the cell membrane. Three studies (Shine & Douglas, 1975; Specter *et al.*, 1975; Flatman & Lew, 1981) did not find any evidence for a change in the activity of Na-K-ATPase on alteration of extracellular magnesium. Specter *et al.* (1975) found that magnesium did not change digitalis-inhibited Na-K-ATPase when the enzyme complex was isolated from dog hearts. Shine & Douglas (1975) showed that magnesium administration could decrease the net ^{42}K loss induced by acetylstrophanthidin in rabbit septa without altering the depressed influx of ^{42}K produced by the glycoside. They suggested that the effects of magnesium on potassium losses during digitalis administration arises from a direct action of magnesium on potassium channels in the sarcolemmal membrane. However, these investigators used very high concentrations of magnesium (> 10 mM) in their experiments with rabbit septa.

In experimental magnesium deficiency in laboratory animals, including dogs, rats, and hamsters, an increase in sodium in cardiac tissue has been demonstrated without any decrease in myocardial magnesium (Chang *et al.*, 1985; Borchgrevink & Jynge, 1987). Thus, it has been hypothesized that the reduced extracellular magnesium level results in reduced activity of Na-K-ATPase, with a resulting increase in intracellular sodium of the cardiac myocytes (Chang & Bloom, 1985).

While our present studies with the Girardi heart cell line suggest that manipulation of extracellular magnesium alters potassium transport by reducing the Na-K-ATPase component, the possibility of a contribution by an effect on intracellular magnesium must be kept in mind. The heart cells were exposed to the different magnesium concentrations for a total of 3.5 h. This may have been sufficient to alter an intracellular magnesium compartment responsible for modulation of the Na-K-ATPase activity.

There is also evidence that magnesium may affect digitalis-binding to cell membranes. In a study on the effects of magnesium deficiency on myocardial tritiated digoxin uptake and retention in mice, Goldman *et al.* (1971) found that hearts of magnesium-deficient mice retained more digoxin than controls. Ryan & Ryan (1983) discovered that [^{3}H]digoxin binding to human erythrocyte membranes was increased in the absence of magnesium. However, Specter *et al.* (1975) concluded that magnesium deficiency did not increase myocardial uptake of digitalis.

In the present studies, altering extracellular magnesium levels did not significantly affect the

bumetanide-sensitive Na-K-Cl co-transport system, though there was a trend towards reduced bumetanide-sensitive transport in the media with lower magnesium concentrations. No effects of extracellular magnesium were detected on the residual component of ^{86}Rb uptake.

In conclusion, the present results with the Girardi heart cell line support other findings that magnesium plays an important role in modulating cellular potassium (^{86}Rb) transport. The present study indicates that concentrations of extracellular magnesium around the physiological range are important for the potassium homeostasis. The main mechanism appears to be via an effect on the ouabain-sensitive or Na-K-ATPase component of transport.

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References

BORCHGREVINK, P.C. & JYNGE, P. (1987). Acquired magnesium deficiency and myocardial tolerance to ischemia. *J. Am. Coll. Nutr.*, **6**, 355–363.

BORCHGREVINK, P.C., CASEY, P., COUNIHAN, T.B. & RYAN, M.P. (1987). The effects of magnesium on ^{86}Rb uptake in cultured heart cells. *Ir. J. Med. Sci.*, **156**, 302–303.

CHANG, C. & BLOOM, S. (1985). Interrelationship of dietary Mg intake and electrolyte homeostasis in hamsters: 1. Severe Mg deficiency, electrolyte homeostasis and myocardial necrosis. *J. Am. Coll. Nutr.*, **4**, 173–185.

CHANG, C., VARGHESE, P.J., DONERY, J. & BLOOM, S. (1985). Magnesium deficiency and myocardial infarct size in the dog. *J. Am. Coll. Cardiol.*, **5**, 280–289.

ELLORY, J.C., FLATMAN, P.W. & STEWART, G.W. (1983). Inhibition of human red cell sodium and potassium transport by divalent cations. *J. Physiol.*, **340**, 1–17.

FLATMAN, P.W. & LEW, V.L. (1981). The magnesium dependence of sodium-pump-mediated sodium-potassium and sodium-sodium exchange in intact human red cells. *J. Physiol.*, **315**, 421–446.

GIRARDI, A.J., WARREN, J., GOLDMAN, C. & JEFFERIES, B. (1958). Growth and CF antigenicity of measles virus in cells derived from human hearts. *Proc. Soc. Exp. Biol. Med.*, **98**, 18–23.

GOLDMAN, R.H., KLEIGER, R.E., SCHWEIZER, R.E. & HARRISON, D.C. (1971). The effect on myocardial ^{3}H-digoxin of magnesium deficiency. *Proc. Soc. Exp. Biol. Med.*, **136**, 747–749.

ISERI, L.T. (1983). Potassium, magnesium and digitalis toxicity. In *Potassium: Its Biologic Significance.* ed. Whang, R. pp. 125–136. Boca Raton: C.R.C. Press.

MURPHY, J.J., RYAN, M.P. & TIERNEY, C. (1983). Investigation of diuretic-sensitive ion transport system in rat thymocytes. *Br. J. Pharmacol.*, **80**, 697P.

RYAN, M.P. (1986). The role of magnesium in potassium deficiency. In *Potassium in Cardiovascular and Renal Medicine—Arrhythmias, Myocardial Infarction and Hypertension.* ed. Whelton, P.K., Whelton, A. & Walker, W.G. pp. 23–42. New York, Basel: Marcel Dekker, Inc.

RYAN, M.F. & RYAN, M.P. (1983). The effects of magnesium on the binding of ^{3}H-digoxin to the membranes of intact red blood cells. *Ir. J. Med. Sci.*, **152**, 219–220.

SELLER, R.H. (1971). The role of magnesium in digitalis toxicity. *Am. Heart J.*, **82**, 551–556.

SCHILLING, K., BORNIG, H., COMME, G. & HOPPE, H. (1980). Effect of magnesium dependent cell membrane alterations on the transport of K^+ in Ehrlich ascites tumour cells. *Acta Biol. Med.*, **39**, 177–184.

SHINE, K.I. & DOUGLAS, A.M. (1974). Magnesium effects on ionic exchange and mechanical function in rat ventricle. *Am. J. Physiol.*, **227**, 317–324.

SHINE, K.I. & DOUGLAS, A.M. (1975). Magnesium effect on rabbit ventricle. *Am. J. Physiol.*, **228**, 1545–1554.

SKOU, J.C. (1960). Further investigations on a Mg^{++} and Na^+-activated adenosintriphosphatase, possibly related to the active, linked transport of Na^+ and K^+ across the nerve membrane. *Biochim. Biophys. Acta*, **42**, 6–23.

SPECTER, M.J., SCHWEIZER, E. & GOLDMAN, R.H. (1975). Studies on magnesium's mechanism of action in digitalis-induced arrhythmias. *Circulation*, **52**, 1001–1005.

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