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The effect of zinc and magnesium on calcium uptake into the rat duodenum slices

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Summary. The experiments were carried out on 80 male Wistar rats, divided into four groups as follows: group 1, treated orally with $ZnCl_2$ at a dose of 10 mg Zn^{2+}/kg for 14 days; group 2, control; group 3, $MgCl_2$-treated at a dose of 5 mg Mg^{2+}/kg; group 4, treated with $ZnCl_2$ plus $MgCl_2$ in the same manner as groups 1 and 3. The influx of calcium into the rat duodenum slices was investigated in vitro by the method of Papworth and Patrick. Over a range of calcium concentrations (0–10 mM) the influx of this element was defined as a sum of a saturable term (active transport) and a linear term dependent on concentration (passive transport). In the zinc-treated rats only the saturable term was affected. The study of this term by Lineweaver-Burk plots showed a decrease of the half-saturation constant, K_t, while the maximal value, J_m, remained unchanged. Moreover, magnesium was shown to interact with zinc at gut level because simultaneous oral administration of Mg^{2+} and Zn^{2+} to rats protected them against the inhibition of calcium uptake observed when Zn^{2+} was given alone.

Key words: Metal — Metal interaction — Calcium uptake — Zinc — Magnesium

Introduction

Zinc is an essential element for life but it may also be toxic under conditions of chronic high-level exposure (Shunichi et al. 1987). The mechanisms by which zinc exerts its toxic effects are poorly understood but interference with the normal kinetics and macromolecular binding of the other essential metals, such as Mg, Fe, Ca, Cu etc., may play a significant role. There are numerous examples of interactions between essential inorganic elements (Underwood 1977). A deficiency in one of the antagonized elements can greatly enhance the animals sensitivity to the excess elements and, conversely, high dietary levels of the antagonized element can play a protective role.

More recently, interest has been focused on the influence of essential elements and other nutrients in minimizing the adverse effects of toxic metals. The toxicity of cadmium has been shown to be altered by dietary intakes of Zn, Fe, Mn, Cu, Se, Ca, ascorbic acid, vitamin D and protein (Fox 1974, 1975; Goering and Klaassen 1984; Noda et al. 1978). Cadmium toxicity was enhanced by a deficiency of these nutrients and/or decreased by supplements of them. Moreover, it is well known that low dietary intake of Ca, Zn and Mg increases the susceptibility of animals to lead poisoning (Goyer and Cherian 1977). Magnesium prevents some of toxicological symptoms caused by Cd, Ni, MeHg poisoning (Iturri and Pena 1986; Kasprzak and Poirier 1985; Marier 1986); there are many other references that could be cited.

We found it of interest to study in vitro the kinetics of calcium influx into the duodenum of rats continuously treated with $ZnCl_2$ and, moreover, to study how magnesium administrated together with $ZnCl_2$ altered the process. We found the method of Papworth and Patrick (1970) to be appropriate for our purpose, since it provides a qualitative and quantitative evaluation of the active and passive calcium diffusion and thus allows the nature of the interactions between cations to be specified.

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Materials and methods

Animals used in this study were male Wistar rats initially weighing 50–60 g. The rats were housed in a temperature-controlled room with 12-h cycles of light and dark. The experiments were carried out on four groups of 80 rats on a normal diet as follows. Group 1 (zinc-exposed): the animals were administered with $ZnCl_2$ at a dose of 10 mg Zn^{2+} body mass 6 days a week for 14 days. Group 2 (control). Group 3 (magnesium-treated): 5 mg Mg^{2+}/kg body mass as $MgCl_2$ was administered 6 days a week for 14 days. Group 4 (Mg^{2+} and Zn^{2+}-exposed): the rats were treated with $ZnCl_2$ and $MgCl_2$ in the same way as those of groups 1 and 3.

The kinetics of calcium influx were measured according to the method of Papworth and Patrick (1970) adapted by the authors. Rats were sacrificed 24 h after administration of the last dose. The duodenum was removed, washed briefly in saline at 0° C and dissected into slices (Schachter et al. 1960); twelve duodenal slices were prepared from each rat and allocated between six beakers with 0.9% NaCl. The slices prepared from six rats were mixed and one from each beaker was transferred to the incubation flask filled with 5.0 ml of medium containing 50 mM imidazol, 20 mM glucose, 100 mM D-mannitol, 8 mM sodium phosphate and different concentrations of Ca^{2+} ranging over 0.1–10 mM labelled with ^{45}Ca (specific activity 34.9 mCi/g Ca) and appropriate concentrations of NaCl to produce the same osmolarity: 323 mosmol/l and pH 7.4. In each flask six slices (total fresh mass approximately 200 mg) were incubated for 15 min. After incubation, the slices were washed twice with 0.9% NaCl, blotted and transferred to the counting vials and weighed.

Samples for measuring the simple diffusion into the tissue were prepared in the same manner. The slices were incubated in the medium descibed above with addition of 0.7 mM 2,4-dinitrophenol. In these conditions the saturable component of Ca^{2+} influex was greatly reduced by 2,4-dinitrophenol whereas the linear term remained unchanged.

The uptake of ^{45}Ca was measured by the liquid scintillation method. The rate of influx was expressed as μmol $Ca^{2+} \times g^{-1} \times h^{-1}$. According to Papworth and Patrick (1970), Ca influx over a range of calcium concentrations was analyzed into saturable (J_1) and linear (J_2) components as follows:

$$J = \frac{J_m[Ca^{2+}]}{K_t + [Ca^{2+}]} + P[Ca^{2+}] \text{ or } J = J_1 + J_2$$

where: $J = Ca^{2+}$ influx, $[Ca^{2+}] = Ca^{2+}$ concentration, J_m = maximum value of $[Ca^{2+}]$ influx, K_t = half-saturation constant for saturable component (carrier) and P = constant for linear component (simple diffusion).

Statistical analysis. Regressions were measured over a set of five points, each of which represented the average of four experimental values. Student's t test was used to examine the relationship of variables, and the linearity of the regressions was tested by variance analysis. Regression line equations were calculated using the method of least squares.

Results and discussion

The influx of calcium was determined over a range of concentrations in the incubation medium. The rate of influx was defined by a two-component expression comprising a saturable (Michaelis-Menten) term (J_1) and a second term linear with concentration (J_2). The linear component may be attributed to simple diffusion into the tissue, where P is the permeability constant. There is no evidence to suggest that this second component can be saturated at high concentrations (Papworth and Patrick 1970).

The results obtained for Ca influx into the duodenal slices in the control and experimental groups are shown in Fig. 1. From the resulting curves it may be concluded that both transfer mechanisms are involved in the transport of calcium. There was no difference in the total calcium influx, J, between the control and the Mg^{2+}-treated animals, and the control and the $Mg^{2+} + Zn^{2+}$-treated rats, whereas a 2-week treatment with Zn^{2+} (10 mg/kg) caused a decrease in this parameter as compared to control.

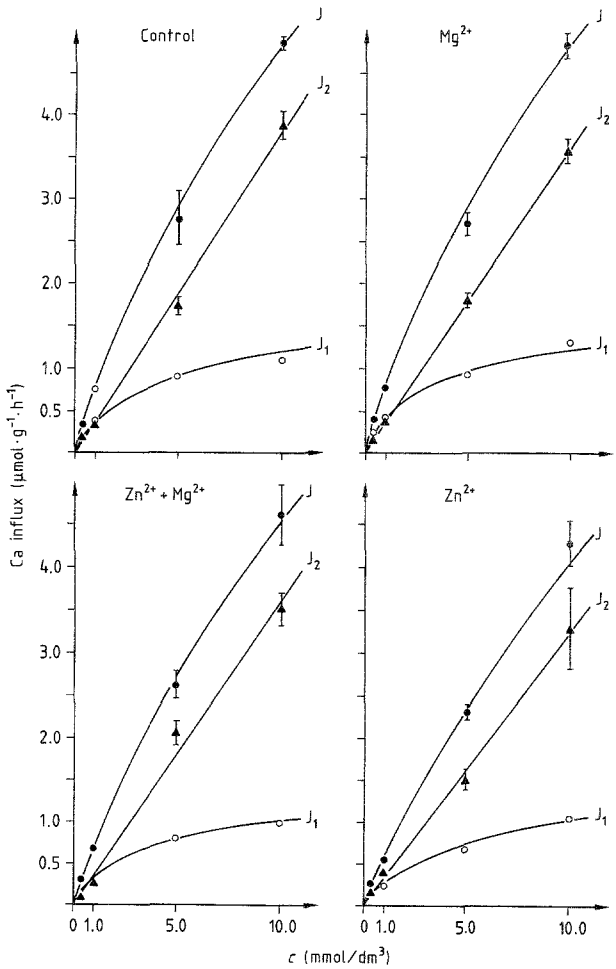

Fig. 1. Calcium influx into duodenal slices in control and treated animals. J, total calcium influx; J_1, saturable component; J_2, linear component (passive transport). Means ±SE of four experiments

Table 1. Active transport (J_1): linear regression equations, maximal calcium influx value (J_m) and half-saturation constant (K_t) according to the method of Lineweaver and Burk. Passive transport (J_2): constant P

Group of animals	Active transport (J_1)			Passive transport (J_2)
	Regression equations $\frac{1}{J_1}=\frac{1}{J_m}+\frac{K_t}{J_m}\frac{1}{[Ca^{2+}]}$	J_m $\mu mol \times g^{-1} \times h^{-1}$	K_t $mmol \times l^{-1}$	P $ml \times g^{-1} \times h^{-1}$
Control	$\frac{1}{J_1}=0.98+1.47\frac{1}{[Ca^{2+}]}$	1.02 (0.72–1.23)	1.50 (1.05–1.81)	0.38 ± 0.08
Mg^{2+}-treated	$\frac{1}{J_1}=0.98+1.30\frac{1}{[Ca^{2+}]}$	1.02 (0.80–1.49)	1.33 (1.02–1.89)	0.36 ± 0.02
Zn^{2+}-treated	$\frac{1}{J_1}=0.97+2.94\frac{1}{[Ca^{2+}]}$	1.03 (0.84–1.45)	3.04 (2.46–4.26)	0.32 ± 0.06
Zn^{2+} and Mg^{2+} treated	$\frac{1}{J_1}=0.96+1.84\frac{1}{[Ca^{2+}]}$	1.04 (0.85–1.25)	1.92 (1.56–2.30)	0.36 ± 0.13

Values in parentheses are 95% confidence intervals. The relationship and linear regression between variables, tested by Student's t-test and variance analysis, are accepted with a probability of error, α, of ± 0.05

An analysis of the two components J_1 and J_2 reveals that the passive calcium diffusion does not appear to be changed (Fig. 1, Table 1) thus a decrease in the saturable component is responsible for reduction in the total calcium influx in rats. In fact, an analysis of the active influx of calcium into duodenal slices using the Lineweaver-Burk diagrammatic representation (Fig. 2, Table 1) shows that the main variation in J_1 is a consequence of variation in K_t but not J_m. Comparisons of the saturation constant K_t (Table 2) indicate that significant differences were observed between the control and Zn^{2+}-exposed groups, the Zn^{2+}-exposed and the $Zn^{2+}+Mg^{2+}$-exposed and finally the Mg^{2+}-treated and the Zn^{2+}-treated animals. Therefore the change in the half-saturation constant, K_t, in the Zn^{2+}-exposed animals might be related to a decrease in the affinity of the carrier system for the calcium. Furthermore, administration of Mg^{2+} to the Zn^{2+}-exposed animals restores K_t to a value comparable to that in the control and the Mg^{2+}-treated animals. Thus magnesium prevents the influence of zinc on the active transport of calcium.

It is well known that calcium and zinc are antagonistic. It was shown that a single high oral administration of zinc caused a hypocalcemic effect which is based mainly on an increase in the gastric calcium secretion mediated by acetylcholine (Yamaguchi et al. 1981). Yamaguchi et al. (1983)

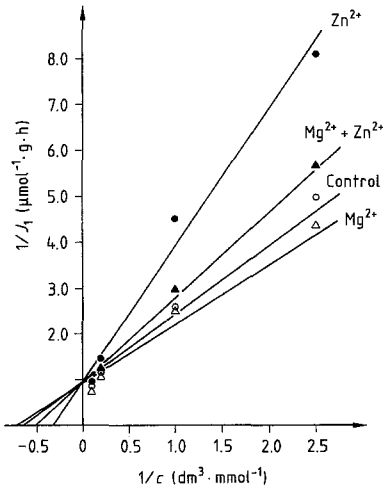

Fig. 2. Lineweaver-Burk representation of active calcium influx in control and treated animals

Table 2. Results of comparison of regression

Comparison	P	J_m	K_t
Control vs Zn^{2+}-treated	n.s.	n.s.	s. $K_t(Zn^{2+}) > K_t$ (control)
Control vs Mg^{2+}-treated	n.s.	n.s.	n.s.
Control vs $Zn^{2+}+Mg^{2+}$	n.s.	n.s.	n.s.
Zn^{2+}-treated vs $Zn^{2+}+Mg^{2+}$	n.s.	n.s.	s. $K_t(Zn^{2+}) > K_t(Zn+Mg)$
Mg^{2+}-treated vs Zn^{2+}-treated	n.s.	n.s.	s. $K_t(Zn^{2+}) > K_t(Mg^{2+})$
Mg^{2+}-treated vs $Zn^{2+}+Mg^{2+}$	n.s.	n.s.	n.s.

n.s., non-significant difference at the level 5%; s., significant difference at the 5% level

reported that zinc-induced hypocalcemia might cause bone resorption, which is primarily mediated by the action of the parathyroid hormone and related to calcium homeostasis in the rat. A report (Brewer et al. 1979) that high zinc levels can inhibit calmodulin function may also explain why increasing dietary zinc resulted in bone calcium loss. Moreover, zinc deficiency also affects calcium metabolism resulting in bone abnormalities in the embryos of chicks and rats (Soares et al. 1987).

As has been shown in this study, zinc affects calcium transport in the rat intestine. The biochemical mechanism(s) of this metal action is unknown and can not be discerned from the present results. They can only suggest a hypothesis that a direct effect of zinc on the calcium-binding protein is as likely as an indirect action of this element on the 1α,25-dihydroxycholecalciferol-dependent carrier protein. Therefore, the present study clearly indicates that magnesium can decrease the influence of zinc on calcium transport. The competition between the physiological divalent cations is quite complex, so further investigations on the metabolic interactions of zinc with calcium and magnesium are required to elucidate the mechanisms underlying the antagonistic effects of these metals.

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