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Insulin induces opposite changes in plasma and erythrocyte magnesium concentrations in normal man

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Summary. Plasma and erythrocyte magnesium levels were measured by atomic absorption spectrophotometry in 10 healthy volunteers during an oral glucose tolerance test and during an euglycaemic hyperinsulinaemic glucose clamp. At min 180 and 210 of the oral glucose tolerance test, a significant decline in plasma magnesium levels ($p < 0.01$ and $p < 0.05$ respectively) and a significant increase in erythrocyte magnesium levels ($p < 0.01$ and $p < 0.05$ respectively) were observed. Similar changes were seen during the second hour of the glucose clamp, during which euglycaemia (4.1 ± 0.4 mmol/l) was maintained despite hyperinsulinaemia (110-130 mU/l). During in vitro incubations, glucose (5 mmol/l) did not modify erythrocyte magnesium levels. In contrast, erythrocyte magnesium levels were significantly increased ($p < 0.01$) by insulin (100 mU/l), an effect entirely abolished by ouabain ($5 \cdot 10^{-4}$ mol/l). These results suggest that insulin induces a shift of magnesium from the plasma to the erythrocytes both in vivo and in vitro. These data may help to interprete the abnormalities in magnesium circulating levels frequently reported in diabetic patients.

Key words: Glucose clamp, insulin, magnesium, oral glucose tolerance.

Magnesium, the most abundant intracellular divalent cation, is an essential factor in the glucose transport mechanism of cell membranes [1] and a critical cofactor of numerous enzymes in carbohydrate metabolism [2]. While it is usually accepted that plasma magnesium concentration is quite stable in healthy people, hypomagnesaemia is a common finding in diabetic patients [3, 4]. In fact, Mather et al. [4] described the presence of substantial hypomagnesaemia in nearly 25% of a large series of diabetic patients and reported a close relationship between hypomagnesaemia and poor diabetes control. Early studies have indicated that hypomagnesaemia in poorly controlled diabetic patients could be related to increased urinary losses of this cation accompanying glycosuria [5, 6]. Furthermore, studies of De Leeuw et al. [7] have established the existence of a decreased magnesium content in the trabecular bone of insulin-treated diabetic patients as compared to normal controls, a phenomenon also reported in Type 2 (non-insulin-dependent) patients [8]. Surprisingly, very little data is available on the effect of insulin on the regulation of plasma and erythrocyte magnesium levels in healthy subjects [9, 10]. The present study was aimed at investigating a possible rôle of insulin in regulating plasma and erythrocyte magnesium levels in normal man by both classical in vivo (oral glucose tolerance test; glucose clamp) and in vitro experimental techniques.

Subjects and methods

Subjects

Ten healthy young men aged 26 ± 3 years (mean ± SEM; range 19-32 years) volunteered for the study. All were had a normal body mass index (22 ± 1; mean ± SEM) and none had a family history of diabetes or was taking any drug for at least three weeks before starting the experiment. All subjects gave informed consent and the study was approved by the Ethical Committee of our institution. All were consuming a regular weight-maintaining diet.

In vivo experimental protocols

The subjects were studied in the morning starting at 07.00-08.00 h after a 12-h overnight fast. Each subject was tested on two different occasions in random order, separated by at least 1 day. In one experiment, a classical oral glucose tolerance test (OGTT, 75 g glucose) was performed. In the second experiment, an euglycaemic hyperinsulinaemic clamp using the Biostator (Life Science Instruments, Miles Laboratories, Elkhart, Ind., USA) was used. In the euglycaemic hyperinsulinaemic clamp with a fixed insulin infusion rate (100 mU · $kg^{-1} \cdot h^{-1}$) a variable amount of glucose (as a 30% glucose solution sup-

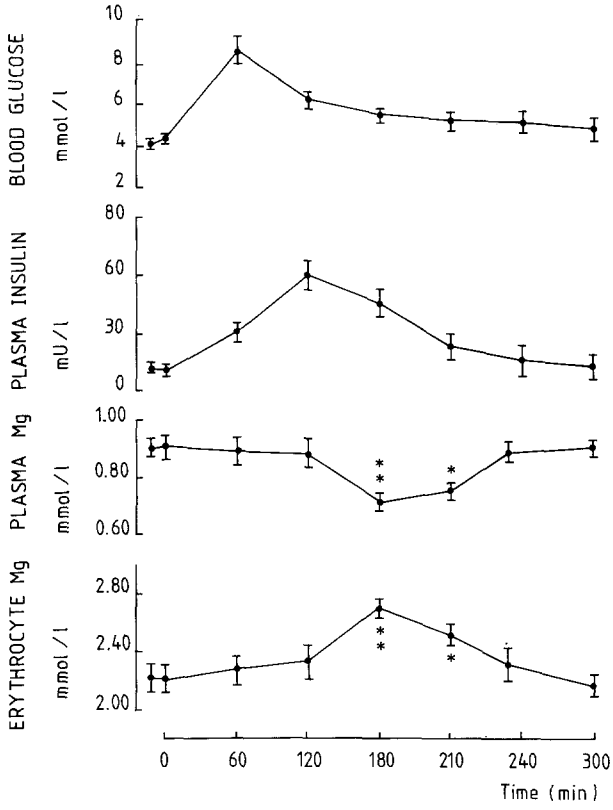

Fig. 1. Changes in blood glucose, plasma insulin, plasma and erythrocyte magnesium levels in response to the ingestion of 75 g glucose. Results are expressed as mean ± SEM ($n = 10$). $^{**}p < 0.01$ and $^{*}p < 0.05$

plemented with 0.26 mEq KCl) was infused to maintain normoglycaemia during the experiment. Blood samples for glucose, insulin, plasma and erythrocyte magnesium determinations were collected, after basal values (−10 and 0 min), every 60 min for 5 h in the OGTT and every 20 min for 2 h in the euglycaemic clamp.

In vitro experimental protocol

In each fasted subject, a blood sample was collected into heparinized tubes. To separate the erythrocytes from plasma, the blood samples were centrifuged (5000 rpm × 15 min) and the precipitate washed at least three times using isotonic saline solution. Subsequently, the erythrocytes were incubated for 90 min in a Krebs-Ringer buffer which had the following ionic composition (mmol/l): NaCl (122), KCl (4.8), $CaCl_2$ (2.5), $MgCl_2$ (1.2), and $NaHCO_3$ (20). The solutions were continuously gassed with a mixture of 95% O_2 and 5% CO_2 to maintain a pH of 7.4 and the temperature kept at 37 °C. To this solution were added, when required, insulin (0.1 U/l), glucose (5 mmol/l) and ouabain (0.5 mmol/l). After the incubation, the erythrocytes were treated as described below to determine their magnesium concentration.

Analytical procedures

Plasma glucose was determined immediately after completion of the experiment using a Beckman Auto-Analyzer (Beckman, Geneva, Switzerland). Plasma insulin was measured by radioimmunoassay (Bio-Data, Milan, Italy). Erythrocyte magnesium concentrations were determined after isolation of the erythrocytes from whole blood by centrifugation (5000 rpm for 15 min) and addition of 3 ml of deionized water to 2 ml of precipitate. To 0.2 ml of this mixture 5 ml of 0.75 mmol/l EDTA was added; after standing 30 min, the solution was centrifuged and the supernatant was used for the assay. Plasma and erythrocyte magnesium concentrations were determined in triplicate by atomic absorption spectrophotometry using a Perkin-Elmer apparatus (Perkin-Elmer Co, Norwalk, Conn, USA).

Materials

Ouabain, sodium chloride, kalium chloride, calcium chloride, magnesium chloride and sodium bicarbonate were obtained from Sigma (St. Louis, Mo, USA). Porcine insulin (Sigma) was prepared as a stable solution in 3 mmol/l HCl (1 mg/ml) and added to the incubation medium just before use.

Statistical evaluation

After analysis of variance, statistical comparisons were performed using the paired Student's t-test, choosing $p < 0.05$ as the level of significance.

Results

Oral glucose tolerance

As shown in Figure 1, ingestion of glucose induced normal increments in blood glucose and plasma insulin. A significant decline in plasma magnesium was recorded at 180 ($p < 0.01$) and 210 min ($p < 0.05$) while, in contrast, significant and similar increases in erythrocyte magnesium levels were recorded at the same time points.

Glucose clamp

Despite hyperinsulinaemia averaging 110-130 mU/l, blood glucose was clamped close to its initial value and averaged 4.1 ± 0.4 mmol/l during the second hour of the experiment (Fig. 2). Plasma magnesium, which was stable at 0.85-0.90 mmol/l during the first hour of the test, progressively declined during the second hour, reaching 0.70 ± 0.04 ($p < 0.05$) and 0.65 ± 0.03 mmol/l ($p < 0.01$) at minutes 100 and 120 respectively. Again, clearcut opposite changes in erythrocyte magnesium levels were observed at the same time points.

In vitro studies

Results are depicted in Table 1. Insulin (100 mU/l) significantly increased erythrocyte magnesium levels ($p <$ 0.01) while glucose alone had no effect. Moreover, glucose did not significantly modify the effect exerted by insulin while, in contrast, the latter was completely abolished by ouabain (0.5 mmol · l).

Discussion

Several recent studies have shown that magnesium plasma levels may be low in diabetic patients, and have suggested that hypomagnesaemia is associated with diabet-

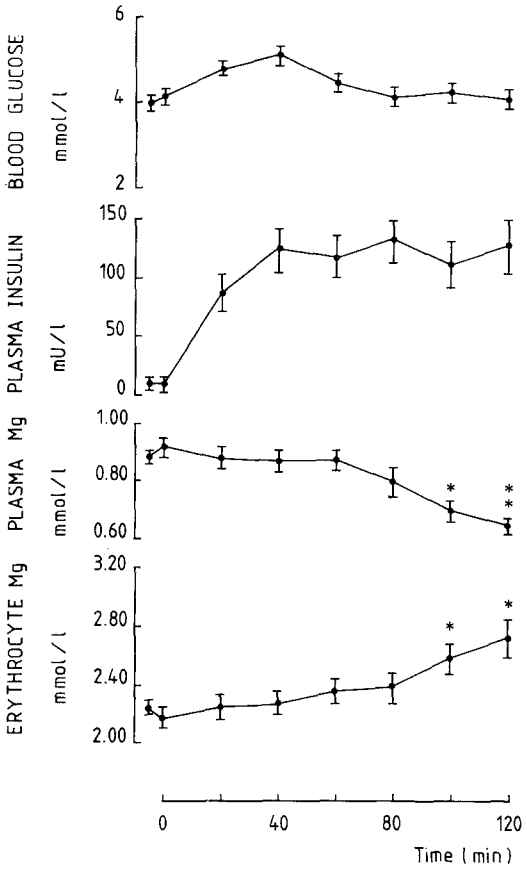

Fig. 2. Changes in blood glucose, plasma insulin, plasma and erythrocyte magnesium levels during a 2-h hyperinsulinaemic euglycaemic glucose clamp. Results are expressed as mean ± SEM ($n = 10$). $^{**}p < 0.01$ and $^{*}p < 0.05$

Table 1. Erythrocyte magnesium levels (mmol/l)

1. Buffer alone	2.60 ± 0.08
2. Glucose (5 mmol/l)	2.63 ± 0.12
3. Insulin (100 mU/l)	2.82 ± 0.08[a]
4. Glucose (5 mmol/l) + Insulin (100 mU/l)	2.77 ± 0.05[b]
5. Glucose (5 mmol/l) + Insulin (100 mU/l) + Ouabain (0.5 mmol/l)	2.61 ± 0.09

Results are expressed as mean ± SEM. Number of incubations was 10 in each series. [a] $p < 0.01$; [b] $p < 0.05$

ic micro- or macroangiopathy. In fact, low plasma magnesium levels have been reported during or after ketoacidosis in Type 1 (insulin-dependent) diabetic patients [5, 6] or in poorly controlled diabetic patients [4], and are often attributed to increased urinary losses [5, 6]. Decreased bone magnesium reserves have been reported in both Type 1 [7] and Type 2 [8] diabetic patients. A possible role of hypomagnesaemia in the development of diabetic microangiopathy was suggested by the study of McNair et al. [11]. These authors have reported definite hypomagnesaemia in a group of 71 insulin-treated diabetic outpatients, and have shown that hypomagnesaemia was most pronounced in a subgroup of patients having the most severe degree of retinopathy. Since the two subgroups (minor retinopathy versus severe retinopathy) were comparable regarding known risk factors implicated in diabetic retinopathy, the authors concluded that hypomagnesaemia appeared to be an additional risk factor in the development and progress of this complication. On the grounds of these observations, cautious administration of magnesium salts has been suggested as a possible means to slow down the development of diabetic retinopathy [12], although this suggestion was considered premature by Mather and Levin [13]. Moreover, hypomagnesaemia has been suggested to be involved in the development of atherosclerosis and ischaemic heart disease [14], conditions frequently associated with diabetes mellitus [15].

Data on erythrocyte magnesium levels in diabetic patients are scarce. Levin et al. [16] reported that erythrocyte magnesium content was similar in diabetic and in healthy subjects; Fuji et al. [17] observed slightly decreased erythrocyte magnesium levels in patients with proliferative retinopathy while, in contrast, Vanroelen et al. [18] reported that a particular group of 13 male diabetic patients with marked macroangiopathy had higher erythrocyte magnesium than a control group of 45 diabetic men without macroangiopathy. Recently Ratzmann [10] reported a 10–20% decline in plasma magnesium levels after the intravenous injection of 0.1 U of insulin in healthy subjects, and suggested that this effect may result from the transport of extracellular magnesium into the intracellular space of the insulin-dependent tissues. The present study confirms the fall in plasma magnesium occurring after oral glucose loading already reported by Rosenbloom [9], and describes the occurrence of a mirror rise in erythrocyte magnesium levels. In order to document a possible shift of magnesium from the plasma to the erythrocytes, further studies were performed. The hyperinsulinaemic normoglycaemic glucose-clamp technique [19] permits a massive insulin-mediated glucose utilization despite maintenance of blood glucose at its basal level. Under these conditions plasma magnesium levels significantly declined while erythrocyte magnesium contemporaneously increased, clearly suggesting that hyperinsulinaemia, and not hyperglycaemia, is responsible for the shift of magnesium from the plasma to the erythrocytes. Such a view was supported by our in vitro studies, which showed that glucose per se had no effect on erythrocyte magnesium. In contrast, insulin significantly increased the erythrocyte magnesium content. Furthermore, ouabain, a classical ATPase inhibitor, completely inhibited the effect of insulin on magnesium erythrocyte accumulation. This suggests that, as for other systems, the ATPase-dependent pump system is involved in the mechanism by which magnesium enters the cell under the effect of insulin [20, 21]. Our data suggest that the strong inverse relationship between plasma magnesium and blood glucose previously reported in insulin-treated diabetic patients and in patients receiving oral hypoglycaemic therapy [4] may in fact depend on changes in insulin circulating levels.

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