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Relaxatory effect of magnesium on mesenteric vascular beds differs from normal and streptozotocin induced diabetic rats

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Abstract

Magnesium deficiency has recently been proposed as a novel factor implicated in the pathogenesis of diabetes complications. Previous studies have shown that magnesium decreases basal tone in normal isolated aortic rings and reduces phenylephrine-induced contraction. The mechanism of this magnesium action is not very well known. The present study was designed to determine the role of endothelium and nitric oxide in magnesium sulfate-induced vasorelaxation in diabetic rat vessels.

Diabetes was induced by a single tail injection of streptozotocin. Eight weeks later, superior mesenteric arteries of control and diabetic animals were isolated and perfused according to the McGregor method. Prepared vascular beds were constricted with phenylephrine to induce 70–75% of maximal constriction. Magnesium sulfate at concentrations of 0.001 M to 0.1 M was added into the medium and perfusion pressure was then recorded. Mesenteric bed baseline perfusion pressure in intact and denuded endothelium of diabetic groups was higher than controls. In all groups, relaxant response to magnesium in mesenteric bed was attenuated after endothelium removal, but a relaxatory effect appears at high concentration. In the presence of N (ω)-nitro-L-arginine methyl ester (L-NAME), magnesium-induced relaxation was significantly suppressed in intact mesenteric bed of control animals but in diabetics, the relaxant response was slightly inhibited. From the results of this study, it can be concluded that magnesium-induced endothelium dependent and endothelium independent vasorelaxation are mediated by nitric oxide in control rats while in diabetic animals other mechanisms may be involved.

Keywords: Diabetes; Mesenteric vascular bed; Nitric oxide; Magnesium; Endothelium dysfunction; (Rat)

1. Introduction

Magnesium deficiency has recently been proposed as a novel factor implicated in the pathogenesis of diabetes complications (Chetan et al., 2002). There is evidence that magnesium deficiency may impair insulin signaling (Reis et al., 2000). Magnesium deficiency has been described in patients with type 1 diabetes (Laurant et al., 1997), but its mechanism is not completely understood (Chetan et al., 2002). Osmotic diuresis may play a role in magnesium loss (Chetan et al., 2002). Hypomagnesemia can also be the cause or a result of diabetes complications (Paolisso and Barbagallo, 1997; Rodriguez-Moran and Guerrero-Romero, 2003). Vascular disease is one of the complicating features of diabetes mellitus in man (Kamata and Kobayashi, 1996). Magnesium deficiency has been shown to produce vascular abnormality in experimental animals (Rude, 1992). Low magnesium causes intracellular calcium elevation followed by vascular contraction and hypertension (Shivakumar and Kumar, 1997). Hypertension is a known cardiovascular risk factor in diabetic patients (Ozcelikay et al., 2000). Magnesium can also change vessel morphology and myogenic tone (Laurant et al., 1999). Previous studies have shown that magnesium could

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decrease basal tone and phenylephrine-induced contraction in normal isolated aortic rings (Gourogoulianis et al., 2001; Yang et al., 2002). The mechanism of this magnesium action is not very well known. Some researchers believe that magnesium-induced vasorelaxation is mediated via its antagonistic action with calcium ions (Ishiguro et al., 1997) and direct vascular smooth muscle effect (Laurant and Touyz, 2000). Altura and Altura (1987) have shown that besides the direct modulatory action that magnesium exerts on normal vascular smooth muscle, it also modulates endothelial function. They emphasised that magnesium-induced vasorelaxation is mediated by nitric oxide (Altura and Altura, 1987; Laurant and Touyz, 2000; Yang et al., 2002). But limited attention has been drawn to the impact of magnesium deficiency on vascular complications of diabetes. Regarding these controversial findings, the present study was designed to determine the endothelium and nitric oxide roles in magnesium sulfate-induced vasorelaxation in diabetic rat vessels.

2. Materials and methods

2.1. Animals

Animals were handled in accordance with the criteria outlined in "Guide for the Care and Use of Laboratory Animals" (NIH US publication 86-23 revised 1985). Male wistar locally produced rats (body weight 180–250 g) were used. All animals were maintained at a constant temperature (22±0.5 °C) with fixed 12:12-h light–dark cycle.

Diabetes was induced by a single tail vein injection of streptozotocin (40 mg/kg). Ten days after streptozotocin injection, fasting blood glucose and magnesium levels were determined using kit (Zistshimi, Tehran, Iran) and spectrophotometer (UV 3100, Shimadzu). Rats with blood glucose levels of ≥14 mmol/L were considered to be diabetic.

Eight weeks later, all animals were anesthetized by i.p. injection of ketamine HCl (50 mg/kg) and mesenteric vascular beds were prepared as originally described by McGregor (1965).

2.2. Preparation of mesenteric vascular bed

In brief, abdominal wall was opened, superior mesenteric artery was exposed, cannulated and gently flushed with warmed (37 °C) modified Krebs Henseleit solution (containing in mM: NaCl; 118, KCl: 4.7, $CaCl_2$; 2.5, $MgSO_4$; 1.2, glucose; 2, $NaHCO_3$; 2.5, $NaHPO_4$; 1.2) bubbled with a mixture of 95% O_2 and 5% CO_2 (final pH 7.4). The mesenteric bed was isolated from the intestine and placed in a water-jacket perfusion chamber maintained at 37 °C. Preparations were perfused at 5 mL/min of modified Krebs Henseleit solution by a peristaltic pump (Meredos). Tissues were prevented from drying by superfusion with 0.5 mL/min modified Krebs Henseleit solution. Perfusion pressure was monitored via a T tube inserted between the pump and the inflow cannula connected to a pressure transducer (MLT0380, ADInstruments). Pressure recording was done by Power Lab System (4SP, ADInstruments).

After 30-min equilibration, the vascular bed was constricted by Krebs–Henseleit solution containing phenylephrine, an α_1-adrenoceptor agonist (0.003 M for intact and 0.006 M for denuded group), to induce 70–75% of maximal vasoconstriction then allowed to reach a plateau and stabilize. Phenylephrine concentration was adjusted to make the same initial perfusion pressure in intact and denuded endothelium of control and diabetic beds. Magnesium sulfate at concentrations from 0.001 to 0.1 M was added into the medium and perfusion pressure was recorded. Drug concentrations were increased every 15 min.

2.3. Denudation of endothelium

To achieve endothelial denudation, the preparation was perfused with distilled water for 5 min (Wagner et al., 1999).

2.4. Nitric oxide inhibition

To inhibit nitric oxide production, N (ω)-nitro-L-arginine methyl ester (L-NAME), a non selective nitric oxide synthase (NOS) inhibitor was added into medium 20 min before Phenylephrine administration. Then Phenylephrine concentration was adjusted to achieve 70–75% of maximum contractile response.

2.5. Drugs

The following drugs were used: Streptozotocin (Pharmacia & Upjohn Kalamazoo, USA), Phenylephrine, Magnesium sulfate, L-NAME (Sigma, St. Louis, MO, USA) and Ketamine HCl (Rotexmedica, Trittau, Germany).

Streptozotocin dissolved in 1 mL normal saline immediately before use. Phenylephrine, Magnesium sulfate and L-NAME were dissolved in perfusate medium.

2.6. Statistical analysis

Each mesenteric bed was used for one experiment. Data were expressed as mean±S.E.M. Comparisons between groups were analyzed by student's *t*-test, and two-way analysis of variance (ANOVA) followed by Tukey post-hoc test. $P<0.05$ was considered significant.

3. Results

No significant differences existed among groups before the intervention. Glucose plasma level was significantly elevated in streptozotocin-treated animals; though magne-

Table 1
Glucose and magnesium concentration in diabetic and control rats, 8 weeks after diabetes induction (data expressed as mean±S.E.M.)

	Glucose (mmol/L)	Magnesium (mmol/L)
Control (n=10)	5.73±0.68	1.36±0.20
Diabetic (n=10)	17.30±2.36*	0.83±0.05*

* P<0.05.

sium was significantly decreased compared to nondiabetic rats (Table 1).

3.1. Endothelium role in Mg-induced relaxation of mesenteric bed

Baseline mesenteric bed perfusion pressure of diabetic group was significantly higher than control in both intact and denuded endothelium (Fig. 1).

In control animals as shown in Fig. 2A, when magnesium sulfate was added cumulatively (0.001–0.1 M), perfusion pressure was reduced in intact endothelium mesenteric beds, reached to a relative steady state and decreased again until its maximum response at 0.1 M. But in denuded endothelium mesenteric beds, perfusion pressure was increased at first, reached to a relative steady state and then decreased until its maximum response at 0.1 M. Significant differences were observed between intact and denuded endothelium at 0.01, 0.03, 0.06 and 0.1 M magnesium sulfate concentrations.

Fig. 2B shows intact and denuded mesenteric bed perfusion pressure responses of diabetic animals to magnesium sulfate. Intact endothelium mesenteric bed of diabetic rats shows similar pattern as control. In denuded ones, no change was observed in perfusion pressure up to 0.06 M of magnesium sulfate but in 0.1 M magnesium concentration a significant relaxation was seen.

Intact and denuded endothelium mesenteric beds of diabetic rats also show significant differences in response

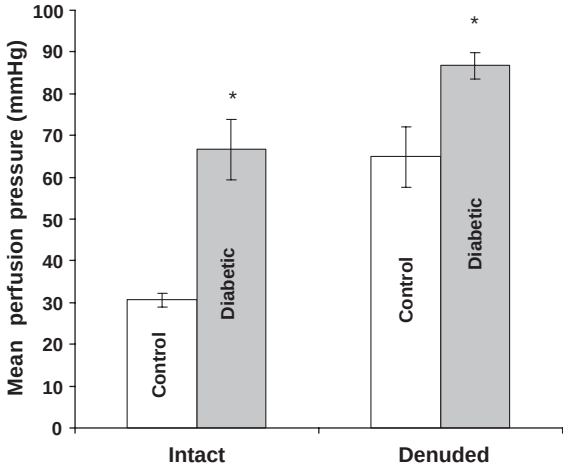

Fig. 1. Baseline perfusion pressure (mm Hg) of mesenteric vascular bed in control and diabetic rats with intact and denuded endothelium (6 rats in each group and data expressed as mean±S.E.M.). *P<0.05.

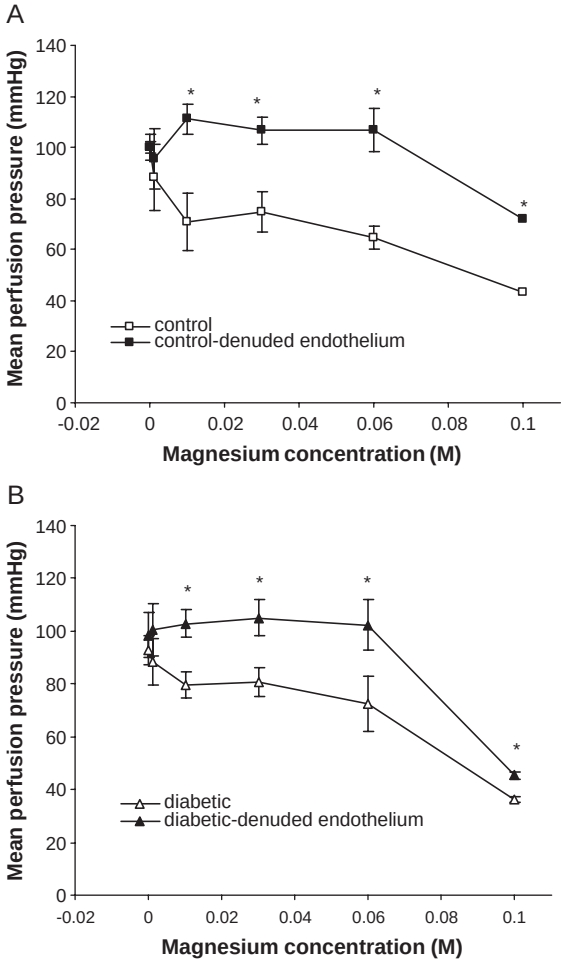

Fig. 2. Dose–response curves of magnesium sulfate in mesenteric vascular bed of (A) control and (B) diabetic rats with intact and denuded endothelium. After precontraction with phenylephrine, magnesium sulfate-induced endothelium–dependent relaxation in mesenteric bed regarding to magnesium sulfate response, there was significant difference between denuded and intact endothelium in each group. (6 rats in each group and data expressed as mean±S.E.M.). *P<0.05.

to magnesium sulfate concentrations of 0.01, 0.03, 0.06 and 0.1 M.

3.2. Nitric oxide role in Mg-induced relaxation of mesenteric bed

In the presence of L-NAME (0.0001 M), Mg-induced relaxation in intact mesenteric beds of control animals was totally suppressed and perfusion pressure remained unchanged. Significant differences were observed at magnesium sulfate concentrations of 0.01, 0.03, 0.06 and 0.1 M in the presence and absence of L-NAME (Fig. 3). In the diabetic beds L-NAME (0.0001 M) could not completely inhibit the response to magnesium. However, significant differences were existed in comparison to L-NAME absence (Fig. 3). Although there was no significant difference in magnesium sulfate relaxatory response in intact endothelium of control and diabetic

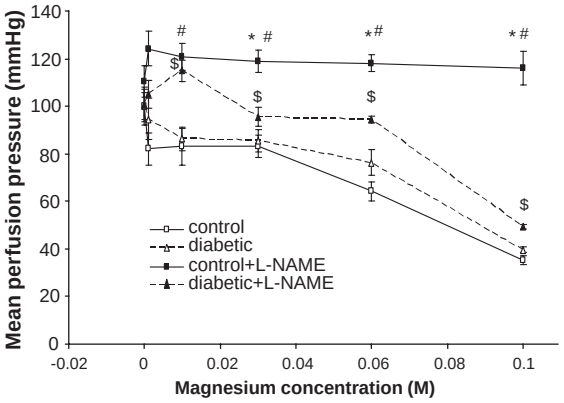

Fig. 3. Dose–response curves of magnesium sulfate in control, diabetic, control+L-NAME and diabetic+L-NAME mesenteric vascular beds, pre-constricted with phenylephrine. L-NAME was added to the medium 20 min before the administration of phenylephrine (6 rats in each group and data expressed as mean±S.E.M.). *, Difference between control+L-NAME and diabetic+L-NAME groups ($P<0.05$). #, Difference between control and control+L-NAME groups ($P<0.05$). $, Difference between diabetic and diabetic+L-NAME groups ($P<0.05$).

groups, Mg-induced relaxation in the presence of L-NAME in control group was significantly higher than diabetic beds (Fig. 3).

4. Discussion

Magnesium deficiency has recently been proposed as a novel factor implicated in the pathogenesis of diabetes complications (Chetan et al., 2002). Shivakumar and Kumar (1997) showed that magnesium deficiency produced vascular lesion. Yang et al. (2002) also showed that administration of magnesium can decrease vascular tone and produce vasorelaxation in normal aortic rings. We hypothesized that magnesium might have a pivotal role in vascular tone of diabetics. So we designed this study to investigate the possible mechanisms of this action.

In the present study, we have observed that magnesium plasma level was significantly decreased in diabetic rats. Some recently conducted studies have shown that plasma concentration of ionized magnesium has markedly been reduced in patients with atherosclerosis, hypertension, and diabetes mellitus (Morrill et al., 1997). But the underlying mechanism is not completely understood. Osmotic diuresis clearly accounts for a portion of magnesium loss. It is believed that glucosuria in diabetes impairs magnesium reabsorption in renal tubules (Chetan et al., 2002, Valk, 1999).

As expected, in our study baseline perfusion pressure in denuded endothelium mesenteric bed was higher than those with intact endothelium. This difference seems to be related to endothelial cells (Malmasjo et al., 1998). Baseline perfusion pressure in intact and denuded endothelium of diabetic groups was higher than controls. This finding is in accordance with endothelium dysfunction (Laight et al., 2000) and atherosclerosis in diabetes (Diederich et al., 1994; Kiff et al., 1991).

Acute magnesium administration induces hypotension through vasorelaxation, which is observed in various vascular beds. Increased extracellular magnesium concentration decreases vascular resistance and improves peripheral, renal, coronary and cerebral blood flow (Laurant and Touyz, 2000). Some investigators have suggested that magnesium vasorelaxatory effect is a consequence of direct action on vascular smooth muscle cells (Laurant and Touyz, 2000; Touyz, 2003), though others have shown that the relaxant effect of magnesium on normal rat aortic rings was mediated via endothelium (Altura and Altura, 1987; Longo et al., 2001; Yang et al., 2002). Our results show that magnesium not only has an endothelium dependent modulatory function but also a direct smooth muscle effect in high concentrations. Magnesium relaxatory effect in mesenteric beds of both normal and diabetic rats was attenuated after endothelium removal but a relaxatory effect appears at high concentration. This may suggest that such relaxation is mediated by endothelium, however a non-endothelium mediated component is also evident at very high concentrations in mesenteric beds from both normal and diabetic rats (Fig. 2).

In the present study, we also showed that inhibition of nitric oxide production markedly suppressed magnesium–induced relaxation. It is likely that magnesium-induced relaxation in intact mesenteric bed of control animals is mediated by nitric oxide system. This is in accordance with endothelium–dependent magnesium function. Yang et al. (2002) have shown that endothelium-dependent magnesium-induced relaxation in intact rat aortic rings was mediated by endogenous nitric oxide in a concentration-dependent manner. L-NAME cannot completely suppress magnesium-induced relaxation of diabetic beds (Fig. 3). Therefore, it seems that magnesium-induced relaxation in intact diabetic mesenteric bed is not mediated only by nitric oxide system (Fig. 3).

From the results of this study it may be concluded that magnesium can induce endothelium dependent and endothelium independent vasorelaxation. The relaxatory effect of magnesium sulfate in normal mesenteric beds mediated by nitric oxide, but in diabetic rats other mechanisms may be involved.

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