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Mg^{2+}-induced adenosine-receptor mediated relaxations in mesenteric vascular beds of diabetic rats

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Abstract. Our previous studies showed that the magnesium Mg^{2+}-induced relaxations were completely dependent on concentration of nitric oxide (NO) in non-diabetic rat mesenteric vascular beds, in diabetic rats other mechanisms may be involved. The present study was designed to determine the role of adenosine receptor in Mg^{2+}-induced relaxation in streptozotocin (STZ)-induced diabetic rats vessels. Diabetes was induced by the intravenous injection of 60 mg/kg STZ. Eight weeks after diabetes induction, superior mesenteric arteries were isolated and perfused according to the McGregor method. Prepared vascular beds were constricted with phenylephrine to induce 70–75% of maximal constriction (0.001 M). Mg^{2+} at concentrations of 10^{-4} to 10^{-1} M were added into the medium and perfusion pressure was recorded. Theophylline (1 mM), and 3,7- dimethyl-1- propargylxanthine (0.01 μM) were added into medium 20 min before phenylephrine administration. In the presence of theophylline, vasorelaxation induced by high dose of Mg^{2+} (from 0.03 to 0.1 M) was totally suppressed. In presence of N(ω)-nitro-L-arginine methyl ester (L-NAME), the response of Mg^{2+} was completely inhibited at low dose of Mg^{2+}. But, Mg^{2+}-induced relaxation in the presence of adenosine A2a receptor blocker was significantly suppressed in high dose of Mg^{2+}. Mg^{2+}-induced relaxation in the presence of an A2a receptor blocker was not suppressed either by denudation of endothelium or presence of L-NAME. From the results of this study it may be concluded that Mg^{2+}-induced relaxation at high concentrations is mediated by adenosine A2a receptors, but at low concentrations Mg^{2+}-induced relaxation is dependent on NO.

Key words: Diabetes — Magnesium — Adenosine A2a receptor — Nitric oxide — Mesenteric bed

Introduction

Diabetes mellitus is a metabolic disorder resulting from defect in insulin secretion, insulin action or both (Bonner 2000). Despite the fact that it has a high prevalence, morbidity and mortality throughout the world, it is regarded as a non-curable but controllable disease. Vascular disease is one of the complicating features of diabetes mellitus (Kamata and Kondoh 1996; Panus et al. 2003). The prevalence of hypertension in diabetic population is almost twice as compared to non-diabetic general population (Garcia et al. 1974; Jarrett 1989). Hypertension is also considered to be an independent risk factor for cardiovascular mortality in patients with diabetes (Ozcelikay et al. 2000). It has been suggested that alterations in the reactivity of blood vessels to neurotransmitters and circulating hormones are responsible for the cardiovascular complications of diabetes (Christlieb et al. 1976; Ozcelikay et al. 2000).

Our previous studies showed that Mg^{2+} could decrease the basal tone and phenylephrine-induced contraction in

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normal and diabetic isolated aortic rings and mesenteric beds (Soltani et al. 2005a,b). The mechanism of this effect of Mg^{2+} is not very well known. Some researchers believe that Mg^{2+}-induced vasorelaxation is mediated *via* blocking the calcium channel (Altura et al. 1987; Satake et al. 2004; Zhang et al. 2007) and direct smooth muscle effect. Altura and Altura (1987) have shown that besides the direct modulatory action that Mg^{2+} exerts on normal vascular smooth muscle, it also modulates endothelium function. They also reported that Mg^{2+}-induced vasorelaxation is mediated by nitric oxide (NO). But in our previous study (Soltani et al. 2005c), we observed that Mg^{2+}-induced relaxations in the normal vessels were mediated by NO and it also modulated both endothelium and smooth muscle. We also showed that in diabetic rat vessels, Mg^{2+}-induced relaxations were not completely dependent on NO, but smooth muscle and endothelium also play a role. (Soltani et al. 2005c). Adenosine, a vasodilator agent, is formed by the metabolism of ATP and is transported into the extracellular space by various nucleoside transport proteins. Endothelial cell metabolism produces abundant adenosine, which in turn regulates vascular function through its four subtypes of G protein-coupled cell surface receptors (A1R, A2aR, A2bR, and A3R) (Fredholm et al. 2001; Jacobson and Gao 2006). Among these adenosine receptors, A2a receptor has been found to be extensively expressed in vascular endothelial cell and had been widely reported to play a major role in mediating adenosine-induced endothelium-dependent vessel relaxation (Tabrizchi and Bedi 2001; Andersen et al. 2011; Carlström et al. 2011).

Regarding those above findings, the present study was designed to determine the role of adenosine receptor in Mg^{2+}-induced vasorelaxation in streptozotocin (STZ)-induced diabetic rats.

Material and Methods

Animals

The animals were handled in accordance with the criteria outlined in the Guide for Care and Use of Laboratory Animals (NIH US publication 86-23 revised 1985).

Locally produced male Wistar 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. Animals were divided into six groups ($n = 6$ in each group). All animals received 60 mg/kg of STZ (i.p.) to induce diabetes. Ten days after STZ injection, feeding blood glucose levels were determined using a glucometer (Ascensia ELITE XL). Rats with blood glucose levels above 300 mg/dl were considered to be diabetic. They were kept in animal room for eight weeks.

Preparation of mesenteric vascular bed

All animals were anesthetized by ketamine hydrochloride injection (50 mg/kg, i.p.) and the mesenteric vascular bed was prepared as originally described by McGregor (McGregor 1965). In brief, abdominal wall was opened, superior mesenteric artery was exposed and cannulated, gently flushed with 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) concomitantly bubbled with a mixture of 95% O_2 and 5% CO_2 (final pH 7.4), and warmed to 37°C. The mesentery was isolated from the intestine, and placed in a water-jacked perfusion chamber maintained at 37°C. The preparation was perfused at 1 ml/min with modified Krebs-Henseleit solution by a peristaltic pump (Meredos GmbH). The tissue was prevented from drying using superfusion with 0.1 ml/min modified Krebs-Henseleit solution. Perfusion pressure was monitored *via* a T-tube inserted between the pump and the inflow cannula. This was connected to a pressure transducer (MLT0380AD Instruments). The recording was performed by Power Lab System (16SP, AD Instruments). After 30-minute equilibration, the vascular bed was constricted by Krebs-Henseleit solution containing phenylephrine, an α_1-adrenoceptor agonist (0.001 M, the doses of phenylephrine were chosen according to the phenylephrine dose response curve, data not shown in the results) to induce 70–75% of maximal vasoconstriction, then allowed to reach a plateau and stabilize. Mg^{2+} at concentrations from 10^{-4} to 10^{-1} M was added into the medium and perfusion pressure was recorded. Drug concentrations increased every 15 minutes.

Endothelial denudation

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

Nitric oxide inhibition

To inhibit NO production, L-NAME (10^{-4} M), a non-selective nitric oxide synthesis (NOS) inhibitor was added into medium 20 min before phenylephrine administration. Then phenylephrine concentration was adjusted to achieve 70–75% of maximum contractile response.

Adenosine receptors inhibition

To inhibit adenosine receptors, a non-specific adenosine receptor blocker theophylline (1 mM), and a selective adenosine A2a receptor blocker 3,7-dimethyl-1-propargylxanthine (0.01 μM) were added into medium 20 min

before phenylephrine administration. Then phenylephrine concentration was adjusted to achieve 70–75% of maximum contractile response.

Drugs

STZ was obtained from Pharmacia & Upjohn (USA) and dissolved in 1 ml normal saline immediately before use. Magnesium sulfate, L-NAME, phenylephrine, theophylline and 3,7-dimethyl-1-propargylxanthine were obtained from Sigma (St. Louis, MO, USA). Ketamine HCl was obtained from Rotexmedica (Trittau, Germany).

Statistical analysis

Data expressed as mean ± S.E.M. The comparisons between groups were analysed by one-way and two-way analysis of variance followed by Tukey's test, using SPSS software. $p < 0.05$ was considered significant.

Results

There was no significant difference between groups in blood glucose eight weeks after diabetes induction.

Fig. 1 shows mesenteric bed perfusion pressure responses of diabetic animals to magnesium sulfate in presence and absence of non-specific adenosine receptor blocker (theophylline). When magnesium sulfate was added cumulatively (10^{-4}–10^{-1} M), perfusion pressure was reduced in intact endothelium mesentric bed, reached to a relative steady state and decreased again until its maximum response at concentration 10^{-1} M. In the presence of theophylline (1 mM), Mg^{2+}-induced relaxations in mesenteric beds by high dose of Mg^{2+} were totally suppressed and perfusion pressure was not changed, but in low concentration of Mg^{2+} the perfusion pressure decreased (Fig. 1). In the other hand, in the presence of L-NAME (10^{-4}M), Mg^{2+} -induced vasorelaxation in diabetic intact endothelium could not be completely inhibited and perfusion pressure was reduced especially at high Mg^{2+} concentration (Fig. 1).

After endothelium denudation, the perfusion pressure in mesenteric bed was not changed up to 0.06 M of Mg^{2+}concentration, but in concentration 0.1 M, a significant relaxation was seen (Fig. 2). As Fig. 2 shows, with the presence of A2a receptor blocker, Mg^{2+}-induced relaxation in mesenteric bed was not suppressed up to 0.06 M of Mg^{2+}, but in 0.1 M level, a significant vasodilatation between groups was observed.

The result in Fig. 3 show that in the presence of both A2a receptor blocker and L-NAME, Mg^{2+}-induced vasorelaxation was not suppressed. We observed a significant difference between diabetic intact endothelium with the presence of L-NAME and diabetic intact endothelium with the presence of both L-NAME and A2a receptor blocker groups. The results in Fig. 3 also indicate that, the Mg^{2+}-induced relaxation in mesenteric beds in the presence of A2a receptor blocker was not suppressed at low dose of Mg^{2+}, but it is significantly suppressed at high Mg^{2+} concentration.

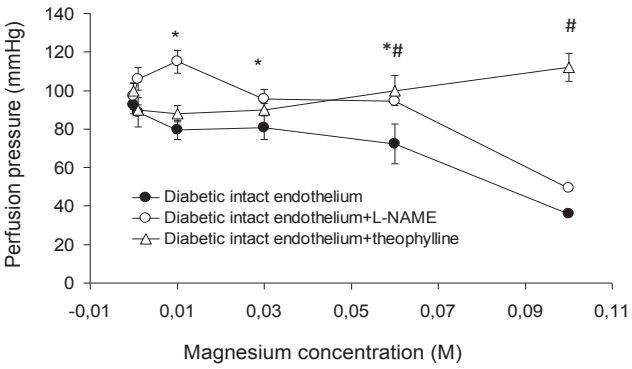

Figure 1. Dose response curves of magnesium sulfate in mesenteric vascular bed of diabetic intact endothelium, diabetic intact endothelium in presence of L-NAME (non-specific NO synthesis) and diabetic intact endothelium in presence of theophylline (non-specific adenosine receptor blocker) groups. Data were expressed as mean ± SEM ($n = 6$). * Significant difference between diabetic intact endothelium in presence and absence of L-NAME ($p < 0.05$); # significant difference between diabetic intact endothelium in presence of theophylline and other groups ($p < 0.001$).

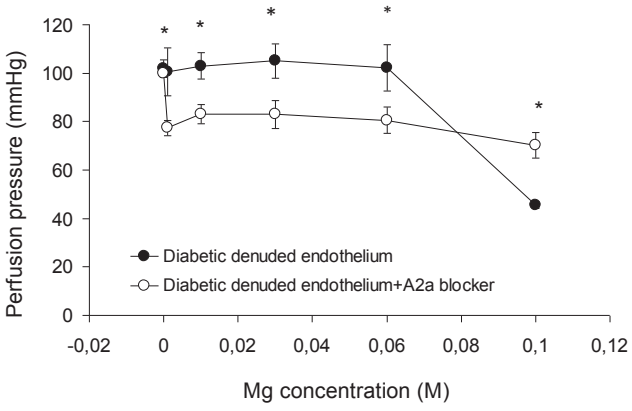

Figure 2. Dose response curves of magnesium sulfate in mesenteric vascular bed of diabetic denuded endothelium in presence and absence of A2a adenosine receptor blocker. Data were expressed as mean ± SEM ($n = 6$). * Significant difference between diabetic denuded endothelium in presence and absence of A2a adenosine receptor blocker ($p < 0.001$).

Discussion

Mg^{2+} administration can decrease vascular tone and produce vasorelaxation in normal and diabetic vessel (Soltani et al. 2005b,c; Zhang et al. 2007). Our previous studies (Soltani et al. 2005a), showed that plasma magnesium level in diabetic rats was significantly lower than non diabetic ones and administration of Mg^{2+} could prevent diabetes vascular complications. According to our previous studies (Soltani et al. 2005a,b,c), Mg^{2+} supplementation produced vasorelaxation in both diabetic and non-diabetic mesenteric vascular beds. We also concluded that Mg^{2+} induced relaxations in non-diabetic mesenteric beds mediated by NO, but in diabetic rats other mechanisms may be involved. We designed this study to investigate the possible mechanism of Mg^{2+}-induced relaxation on diabetic mesenteric vascular beds. Two new findings of this study are developed: a) Mg^{2+}-induced relaxation at high concentrations is mediated by adenosine A2a receptors, but at low concentrations Mg^{2+}-induced relaxation is dependent on NO. b) When both NOS synthesis and A2a adenosine receptors are blocked, Mg^{2+} can induce vasorelaxation *via* direct effect on smooth muscle.

The results of this study have shown that acute Mg^{2+} administration induces vasorelaxation in diabetic mesenteric beds. This finding has been reflected in our pervious studies (Soltani et al. 2005b,c) as well. However, inhibition of NO production by L-NAME couldn't completely suppress

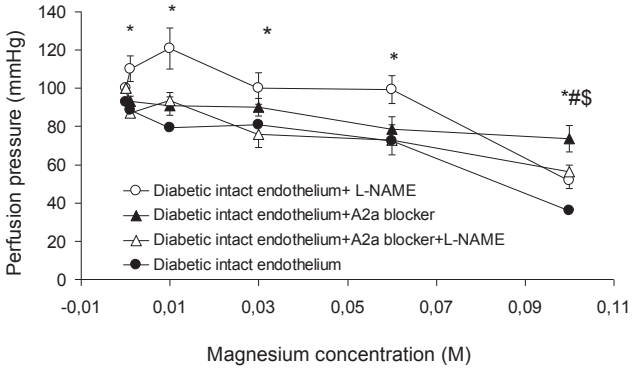

Figure 3. Dose response curves of magnesium sulfate in mesenteric vascular bed of diabetic intact endothelium, diabetic intact endothelium in presence of L-NAME (non-specific NO synthesis) and A2a adenosine receptor blocker and diabetic intact endothelium in presence of L-NAME + A2a adenosine receptor blocker. Data were expressed as mean ± SEM, $n = 6$). * Significant difference between diabetic intact endothelium in presence of L-NAME and other groups ($p < 0.001$); # significant difference between diabetic intact endothelium in presence of A2a adenosine receptor blocker and diabetic intact endothelium in presence of L-NAME + A2a adenosine receptor blocker group ($p < 0.001$); $ significant difference between diabetic intact endothelium in presence and absence of A2a adenosine receptor blocker ($p < 0.001$).

Mg^{2+}-induced relaxation in diabetic mesenteric beds. As we can see in Fig. 1, with the presence of L-NAME, Mg^{2+}-induced vasorelaxation completely suppressed with low concentration of Mg^{2+}, but with high concentration of Mg^{2+} a significant vasodilatation was observed comparing to diabetic intact endothelium group. We also observed that Mg^{2+}-induced relaxation at high concentrations was suppressed by theophylline – a non-specific adenosine receptor blocker. So it seems that Mg^{2+}-induced relaxations at low concentrations is mediated by NO, but adenosine receptors are involved at high concentrations of Mg^{2+} vasodilatation. Some researchers suggested that the vasorelaxatory effect of adenosine is mediated *via* activation of A2a adenosine receptors (Andersen et al. 2011; Carlstrom et al. 2011).

In the present study, we observed that high concentration of Mg^{2+}-induced vasodilatation was suppressed with the presence of adenosine A2a blocker. From the results shown in Figures 1 and 3 we can determine some possible mechanisms involved in the vasodilatory effect of high concentrations of Mg^{2+} mediated by the adenosine A2a receptor.

In this study, we obtained that the Mg^{2+}-induced relaxation at low concentrations was endothelium-dependent and it was inhibited by endothelium removal. Although we expected that in the presence of both adenosine A2a receptor blocker and endothelium removal the Mg^{2+}-induced relaxation would be totally suppressed, but we interestingly observed a significant vasorelaxation by acute Mg^{2+} administration. Our results in Fig. 3 support this finding because with the presence of L-NAME and adenosine A2a receptor blocker, acute Mg^{2+} administration could decrease perfusion pressure. Although the responsible mechanisms have not been understood (Soltani et al. 2005c), some possible mechanisms that could have been involved are direct effect of Mg^{2+} on smooth muscle and decrease the myofilament sensitivity to calcium. Some researchers (Satake et al. 2004; Zhang et al. 2007) showed that Mg^{2+}-induced relaxation in the non-diabetic vessel was mediated *via* blocking the calcium channel; Mg^{2+} probably influences intracellular free calcium ion concentration which is fundamental in smooth muscle contraction. In vascular smooth muscle cells, Mg^{2+} antagonizes calcium ion by inhibiting transmembrane calcium transport and calcium entry (Satake et al. 2004; Zhang et al. 2007). From the results of this study it may be concluded that Mg^{2+}-induced relaxation at high concentrations is mediated by adenosine A2a receptors in the diabetic vessels but Mg^{2+}-induced relaxations at low concentration were dependent on NO.

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