doi: 10.4149/gpb_2013013

GABA-induced vasorelaxation mediated by nitric oxide and $GABA_A$ receptor in non diabetic and streptozotocin-induced diabetic rat vessels

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Abstract. Diabetes has profound, negative effects on the function of arteries and arterioles. Hypertension is considered an independent risk factor for cardiovascular mortality in diabetic patients. The present study was designed to determine whether GABA-induced vasorelaxation in normal and streptozotocin-induced diabetic rat vessels is mediated by nitric oxide and the $GABA_A$ receptor. Diabetes was induced by a single intraperitoneal injection of streptozotocin (STZ) (60 mg/kg). Eight weeks later, superior mesenteric arteries of all groups were isolated and perfused according to the McGregor method. Baseline perfusion pressure of STZ diabetic rats was significantly higher than non-diabetic rats in both intact and denuded endothelium. In the presence of bicuculline, a selective $GABA_A$ receptor blocker, GABA-induced relaxation in intact and denuded endothelium mesenteric beds of non-diabetic and STZ diabetic rats was suppressed. But in the presence of L-NAME, a nitric oxide synthesis inhibitor, although GABA- induced vasorelaxation was not suppressed at a dose of 1 μM of GABA in all groups, this response was suppressed with the other doses of GABA. From the results of this study it may be concluded that the vasorelaxatory effect of GABA is mediated by the $GABA_A$ receptor and nitric oxide in both STZ diabetic and non-diabetic vessels.

Key words: GABA — Diabetes — Mesenteric bed — Vasorelaxation — $GABA_A$ receptor — Nitric oxide

Introduction

Diabetes has profound, negative effects on the function of arteries and arterioles throughout the body. Diabetes-associated dysfunction of resistance vessels is associated with arterial hypertension and vascular occlusive diseases. Diabetes affects arteries and arterioles at the level of both the endothelium and smooth muscle. For example, diabetes causes reduced responsiveness of vascular smooth muscle to dilator agents, predominantly due to impaired potassium channel function (Busija et al. 2004).

Acceptance of the hypothesis that postprandial hyperglycemia has a direct, harmful effect on the cardiovascular system requires, at the very least, a link between acute hyperglycemia and one or more risk factors for cardiovascular disease (Tsai et al. 1994; Jenkins and Klein 1996). Most cardiovascular risk factors are affected directly by an acute increase in glycemia in individuals with diabetes and are modified in the postprandial phase (Tsai et al. 1994; Jenkins and Klein 1996). Hypertension is also considered another independent risk factor for cardiovascular mortality in patients with diabetes (Ozcelikay et al. 2000). Gamma amino butyric acid (GABA) is an inhibitory neurotransmitter which is found in the brain and the pancreas (Reetz et al. 1991; Shi et al. 2000). Some studies showed that, GABA decreases in diabetic patients (Shi et al. 2000; Glad-

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kevich et al. 2006). Our previous study demonstrated that GABA replacement therapy preserves β-cell mass in sever diabetic mice and prevents the development of type I diabetes and in severely diabetic mice, GABA restores β-cell mass and reverses the disease (Soltani et al. 2011).

Some studies have shown that GABA could decrease basal tone and phenylephrine-induced contraction in normal isolated aortic rings (Gimeno et al. 1994), and GABA has a direct effect on vascular smooth muscle (Ozdem and Sadan 1999). Ozdem and co-workers have shown that besides the direct modulatory action that GABA exerts on normal vascular smooth muscle, it also modulates endothelial function (Ozdem 1997). In our previous study, we showed that GABA can induce endothelial vasorelaxation in control and diabetic groups and this effect was mediated by same pathway in both groups. We also showed that GABA had different effects in intact and denuded endothelium and this was related to the endothelium (Farsi et al. 2010). The mechanism of this GABA action is not clear. Some researchers believe that GABA-induced relaxation in normal duodenal smooth muscle is mediated *via* its $GABA_A$ receptor (Zizzo et al. 2007). Some researchers have found that GABA-induced vasorelaxation is not suppressed by $GABA_B$ receptor antagonists in normal vessels (Anwar and Mason 1982).

But there has been limited research on the impact of the GABA mechanism on vascular diabetes. Regarding these findings in normal subjects, the present study was designed to determine whether GABA-induced vasorelaxation in normal and streptozotocin-induced diabetic rat (STZ diabetic rat) vessels is mediated by nitric oxide and $GABA_A$ receptors.

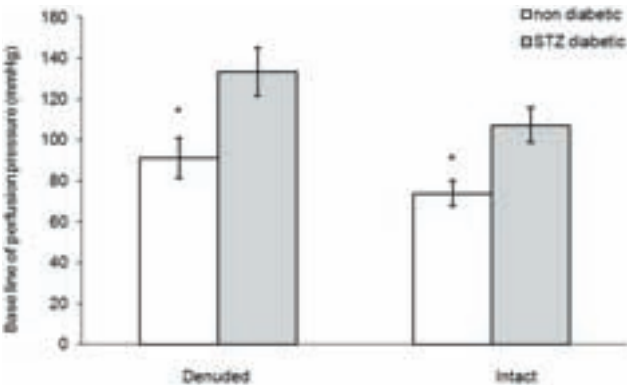

Figure 1. Baseline perfusion pressure (mmHg) of mesenteric vascular beds in non diabetic and STZ diabetic groups with intact and denuded endothelium (6 rats in each group). Data are expressed as mean ± SEM. * Significant difference between STZ diabetic and non diabetic groups with intact and denuded endothelium ($p < 0.001$).

Materials 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 a fixed 12/12-h light/dark cycle. Animals were divided into ten groups; five STZ diabetics and five non-diabetics ($n = 6$ in each group).

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

Diabetes induction

Diabetes was induced with single i.p. injection of STZ, 60 mg/kg. Before intervention and ten days and eight weeks after STZ injection, feeding blood glucose and insulin levels were determined using a glucometer (Ascensia ELITE XL glucometer) and insulin ELISA kit (Crystal chem., Chicago, USA), respectively. Rats with blood glucose levels of 300 mg/dl or above were considered to be diabetic.

Preparation of mesenteric vascular bed

In brief, the abdominal wall was opened, the superior mesenteric artery was exposed and cannulated, and 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 by 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 (MLT0380 ADInstruments). The recording was performed by Power Lab System (16SP, ADInstruments).

After a 30-minwute equilibration, the vascular bed was constricted by Krebs-Henseleit solution containing phenylephrine, an α_1-adrenoceptor agonist (1 mM for intact and denuded diabetic groups and 3 mM for intact and

denuded control groups) to induce 70–75% of maximal vasoconstriction (the doses of phenylephrine were chosen according to the phenylephrine dose response curve, data not shown in the results) then allowed to reach a plateau and stabilize. Increasing concentrations of GABA of 1, 10, 20 and 50 μM were added to the medium every 15 minutes and the perfusion pressure was recorded.

Endothelial denudation

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

Nitric oxide inhibition

To inhibit nitric oxide production, N(ω)-nitro-L-arginine methyl ester (L-NAME), a non-selective nitric oxide synthesis (NOS) inhibitor at a dose of 100 μM was added to the medium 20 min before phenylephrine administration. Then the phenylephrine concentration was adjusted to achieve 70–75% of the maximum contractile response.

$GABA_A$ receptors inhibition

To inhibit $GABA_A$ receptors, bicuculline, a selective $GABA_A$ receptor blocker, at a dose of 25 μM was added to the medium 20 min before phenylephrine administration. Then the phenylephrine concentration was adjusted to achieve 70–75% of the maximum contractile response.

Drugs

The following drugs were used: STZ was obtained from Sigma (USA) and dissolved in 1 ml normal saline immediately before use. GABA, L-NAME, phenylephrine and bicuculline were obtained from Sigma (St. Louis, MO, USA). Ketamine HCl was obtained from Rotexmedica (Trittau, Germany).

Statistical analysis

Data are expressed as mean ± S.E.M. Comparisons between groups were analyzed by student's t-test, and two-way analysis of variance followed by Tukey's test, using SPSS software. $p < 0.05$ was considered significant.

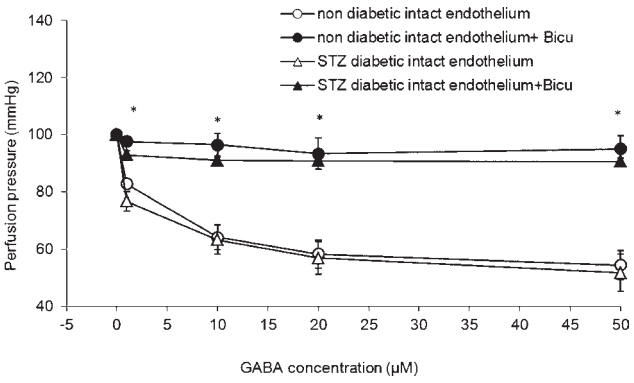

Figure 2. Dose response curves of gamma amino butyric acid (GABA) in mesenteric vascular beds of non diabetic intact endothelium, non diabetic intact endothelium+ Bicuculline (Bicu), STZ diabetic intact endothelium and STZ diabetic intact endothelium + Bicuculline (Bicu) groups. (6 rats in each group, data were expressed as mean ± SEM). * Significant difference between presence and absence of Bicuculline in both STZ diabetic and non diabetic groups ($p < 0.0001$).

Results

No significant differences were found between groups before the intervention. Ten days after STZ injection plasma glucose levels in STZ diabetic group were significantly increased from 107.5 ± 6.9 mg/dl to 402.2 ± 58.9 mg/dl. Eight weeks after diabetes induction, plasma glucose levels remained significantly elevated and plasma insulin level decreased in STZ diabetic rats in compere to non diabetic group (Table 1).

Mesenteric bed response

Baseline perfusion pressure in the STZ diabetic group was significantly ($p < 0.001$) higher than non-diabetic rats in both intact and denuded endothelium (Fig. 1).

GABA at doses of 1–50 μM significantly decreased the perfusion pressure in STZ diabetic and non-diabetic

Table 1. Plasma glucose and insulin concentrations before and after diabetes induction

	Glucose (mg/dl)		Insulin (ng/ml)	
	Before intervention	8 weeks after STZ or saline injection	Before intervention	8 weeks after STZ or saline injection
Non diabetic control	107.44 ± 7.35	110.67 ± 5.1	17.1 ± 0.12	17.2 ± 0.2
Diabetic	107.5 ± 6.9	440.2 ± 7.1*	16.85 ± 0.16	2.3 ± 0.01*

Data are expressed as mean ± S.E.M. ($n = 30$), * $p < 0.0001$.

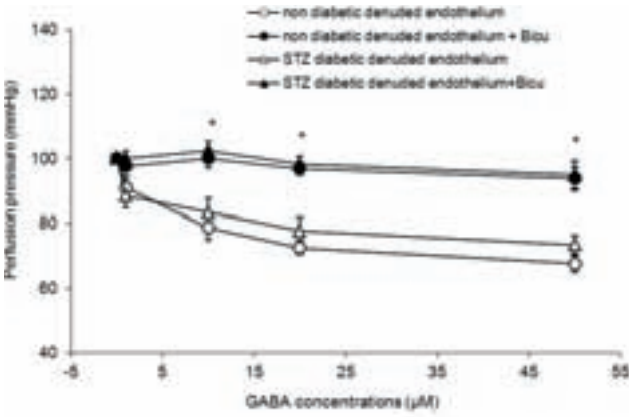

Figure 3. Dose response curves of gamma amino butyric acid (GABA) in mesenteric vascular beds of non diabetic denuded endothelium, non diabetic denuded endothelium+ Bicuculline (Bicu), STZ diabetic denuded endothelium and STZ diabetic denuded endothelium + Bicuculline (Bicu) groups. (6 rats in each group, data were expressed as mean ± SEM). * Significant difference between presence and absence of Bicuculline in both STZ diabetic and non diabetic groups ($p < 0.0001$).

groups with intact endothelium in a dose-dependent manner (Fig. 2). After endothelial denudation GABA decreased perfusion pressure in both STZ diabetic and non-diabetic groups (Fig. 3). There was no significant difference between the slopes of percentage response curves in STZ diabetic and non-diabetic groups with intact and denuded endothelium (Fig. 2 and 3). The relaxatory effect of GABA in both STZ diabetic and non-diabetic groups started with a dose of 1 μM of GABA and it reached steady state at a dose of 20 μM (Fig. 2 and 3).

In the presence of bicuculline (2.5 mM), GABA-induced relaxation in intact endothelium mesenteric beds of non-diabetic and STZ diabetic rats was suppressed and perfusion pressure in both groups did not change (Fig. 2). Significant differences were observed at GABA concentrations of 1 to 50 μM in the presence and absence of bicuculline in non-diabetic group with intact endothelium (Fig. 2). We also obtained significant differences in STZ diabetic group with intact endothelium in presence and absence of bicuculline (Fig. 2)

In the present study we showed that in the presence of bicuculline (2.5 mM), GABA-induced relaxation denuded endothelium mesenteric beds of non-diabetic and STZ diabetic rats was suppressed and perfusion pressure in both groups did not change (Fig. 3). Significant differences were seen at GABA concentrations of 1 to 50 μM in the presence and absence of bicuculline in STZ diabetic group with denuded endothelium (Fig. 3). We also achieved significant differences in non-diabetic group with denuded endothelium in presence and absence of bicuculline (Fig. 3).

Our results in Fig. 4 showed that there is no significant difference between dose response curve of GABA in mesenteric vascular bed with intact and denuded endothelium in non diabetic and STZ diabetic rats in presence of bicuculline.

In the presence of L-NAME (100 μM), GABA-induced relaxation in intact mesenteric beds of non-diabetic and STZ diabetic animals was suppressed at a dose 10 to 50 μM and perfusion pressure in both groups did not change (Fig. 5). A significant difference was not observed at a GABA concentration of 1 μM in the presence and absence of L-NAME in both STZ diabetic and non-diabetic groups with intact endothelium (Fig. 5).

Discussion

In our previous study, we have shown that GABA can induce endothelium vasorelaxation in control and STZ diabetic groups and this effect was mediated by same pathway and we also showed that GABA had different effect in intact and denuded endothelium and this act was related to the endothelium (Farsi et al. 2010). The mechanism of this GABA action is not very well known. The present study was designed to determine whether GABA-induced vasorelaxation in non-diabetic and STZ-induced diabetic rat vessels is mediated by nitric oxide and $GABA_A$ receptors.

The results of this study indicate that the baseline perfusion pressure of STZ diabetic group was significantly ($p < 0.001$) higher than non-diabetic rats in both intact and denuded endothelium. This finding is agreement with our previous study (Soltani et al. 2005a,b; Farsi et al. 2010). In the present study, we showed that in the presence of bicuculline, GABA-induced relaxation in intact and endothelium-

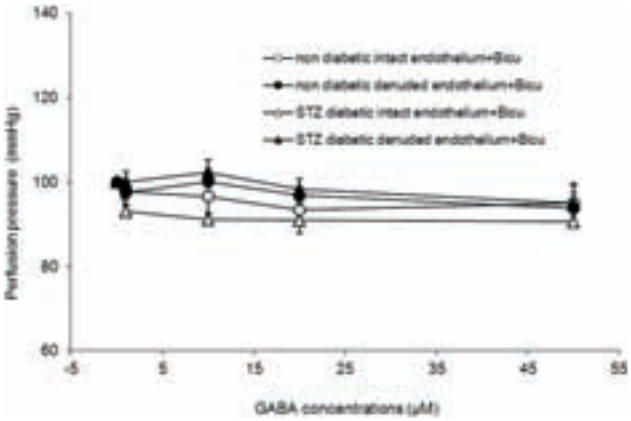

Figure 4. Dose response curve of gamma amino butyric acid (GABA) in mesenteric vascular bed with intact and denuded endothelium in non diabetic and STZ diabetic rats in presence of Biciculline (6 rats in each group, data expressed as mean ± SEM)

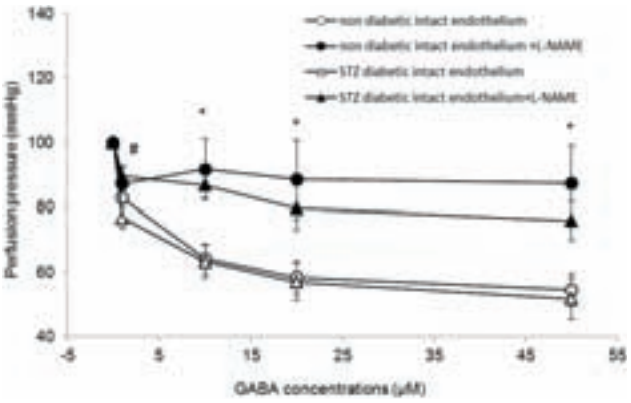

Figure 5. Dose response curve of gamma amino butyric acid (GABA) in mesenteric vascular beds with intact endothelium in non diabetic and STZ diabetic rats in presence and absence of L-NAME (6 rats in each group, data expressed as mean ± SEM). # Significant difference between response to Phenylephrine and dose 1 μM of GABA in presence and absence of L-NAME in each group ($p < 0.01$). * Significant difference between presence and absence of L-NAME in both STZ diabetic and non diabetic groups in dose 10, 20 and 50 μM of GABA ($p < 0.0001$).

denuded mesenteric beds of non-diabetic and STZ diabetic animals was suppressed and perfusion pressure in both groups did not change. So it seems that GABA-induced vasorelaxations in non-diabetic and STZ diabetic animals are mediated by the $GABA_A$ receptor, which is presented in this study for the first time. $GABA_A$ receptor is an activated Cl channel; an increase in intracellular Cl causes relaxation in the vessel (Wang et al. 2000; Bhatt et al. 2005). As we have shown in Fig. 4, it is possible that the $GABA_A$ receptor is located on the smooth muscle beside the endothelial cell, because there are no significant differences in perfusion pressure with presence of bicuculline in STZ diabetic and non-diabetic groups after endothelial denudation.

Our results have shown that in the presence of L-NAME, GABA-induced relaxation in intact mesenteric beds of non-diabetic and STZ diabetic rats was suppressed at doses of 10 to 50 μM (Fig. 5). We did not observe significant differences at a GABA concentration of 1 μM in the presence of L-NAME in both STZ diabetic and non-diabetic groups with intact endothelium (Fig. 5); on the other hand, in the absence of L-NAME the most marked relaxatory effect of GABA was observed at the 1 μM concentration. So it may be inferred that the low-dose GABA relaxatory effect is not mediated by the nitric oxide system.

In conclusion, our results support the hypothesis that the GABA relaxatory effect is mediated by the $GABA_A$ receptor and the nitric oxide system in both STZ diabetic and non-diabetic vessels.

Acknowledgements. This study was supported by the vice-Chancellor for research, Hormozgan University of Medical Science, Bandar Abbas, Iran. We thank P. Waltl (author AID in the Eastern Mediterranean) for improving the English language in the manuscript. We also thank Mr Mostafa Kazemi for technical assistance.

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Received: July 18, 2012
Final version accepted: October 18, 2012