GABA-induced vasorelaxation mediated by nitric oxide and GABA<sub>A</sub> 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<sub>A</sub> 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<sub>A</sub> 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<sub>A</sub> receptor and nitric oxide in both STZ diabetic and non-diabetic vessels.

**Key words:** GABA — Diabetes — Mesenteric bed — Vasorelaxation — GABA<sub>A</sub> 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-
kevich et al. 2006). Our previous study demonstrated that GABA replacement therapy preserves β-cell mass in severely 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.

### 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).

![Figure 1](attachment:figure1.png)

**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).
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.

**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

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<thead>
<tr>
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<th>Glucose (mg/dl)</th>
<th>Insulin (ng/ml)</th>
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<tbody>
<tr>
<td></td>
<td>Before intervention</td>
<td>8 weeks after STZ or saline injection</td>
</tr>
<tr>
<td>Non diabetic control</td>
<td>107.44 ± 7.35</td>
<td>110.67 ± 5.1</td>
</tr>
<tr>
<td>Diabetic</td>
<td>107.5 ± 6.9</td>
<td>440.2 ± 7.1*</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± S.E.M. \( (n = 30) \), \( * p < 0.0001 \).
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 at a dose 10 to 50 µM and perfusion pressure in both groups did not change (Fig. 3). 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 GABAA receptors.

The results of this study indicate that the baseline perfusion pressure of STZ diabetic group was significantly (p < 0.0001) 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 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).

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 bicuculline.

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).
shown in Fig. 4, it is possible that the GABAA receptor is Cl channel; an increase in intracellular Cl causes relaxation.

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


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