

## Relaxatory Effect of Gamma-Aminobutyric Acid (GABA) is Mediated by Same Pathway in Diabetic and Normal Rat Mesenteric Bed vessel

<sup>1</sup>Leila Farsi, \*<sup>2</sup>Mansoor Keshavarz, <sup>3</sup>Nepton Soltani

### Abstract

#### **Objective**(s)

Diabetes related dysfunction of resistance vessels is associated with vascular occlusive diseases. Vasorelaxant agents may have a role in control of diabetic cardiovascular complications. Gamma aminobutyric acid (GABA) has demonstrated to cause vasorelaxation. The present study was designed to determine i) the vasorelaxatory effect of GABA on diabetic vessels and ii) the role of endothelium in GABA-induced vasorelaxation.

#### **Materials and Methods**

After Diabetes induction,. Mesenteric arteries of animals were perfused. Vascular beds were constricted with phenylephrine. GABA (1 to 50  $\mu$ M) was added into the medium and perfusion pressure was then recorded.

#### Results

In all groups of animals, relaxant response to GABA in mesenteric bed appeared. Although diabetes induction did not change mesenteric bed response to GABA, denuded vessels showed a reduced response to GABA both in control and diabetic animals.

#### Conclusion

GABA can induce endothelium dependent vasorelaxation in mesenteric vessels in normal and diabetic rats.

Keywords: Diabetes, Endothelium, GABA, Mesenteric arteries, Rat

<sup>1-</sup>Department of Basic Sciences, Gonabad University of Medical Sciences, Gonabad, Iran

<sup>2-</sup> Department of Physiology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran

<sup>\*</sup>Corresponding authour: Tel/Fax: +98-21-66419484; email: mkeshavarz@sina.tums.ac.ir

<sup>3-</sup> Department of Physiology, School of Medicine, Hormozgan University of Medical Sciences, Bandar Abbas, Iran

## Introduction

Diabetes has profound, negative effects on the function of arteries and arterioles throughout the body. Diabetes related dysfunction of resistance vessels is associated with arterial hypertension and vascular occlusive diseases. Several prospective studies have indicated that hypertension in diabetic population takes place at a rate more than twice compared to the normal population. The hypertension is also considered as an independent risk factor for cardiovascular mortality in patients with diabetes (1). Diabetes affects arteries and arterioles at both the endothelium and smooth muscle levels. It has been suggested that alterations in the reactivity of blood vessels to neurotransmitters and circulating hormones responsible for the cardiovascular are complications of diabetes (1).

Based on the above data, it may be suggested that vasorelaxant agents may have a role in prevention or control of diabetic cardiovascular complications.

GABA is suggested to have a paracrine signal function in neuroendocrine tissues, including the pancreatic islets of Langerhans (2). Some studies showed that GABA could decrease basal tone and phenylephrine-induced contraction in normal isolated aortic rings (3). Holmgaard *et al* study on retina showed the relaxing effect of GABA on retinal arterioles (4). The mechanism of this GABA action is not very well known. Some researchers believe that GABA-induced vasorelaxation is mediated via vascular smooth muscle (5).

However, limited attention has been drawn to the impact of GABA on vascular complication of diabetes. Regarding these controversial findings, the present study was designed to determine i) the vasorelaxatory effect of GABA on diabetic vessels and ii) the role of endothelium in GABA-induced vasorelaxation.

### 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 rats (body weight 180–250 g) were used. All animals were maintained at a constant temperature  $(25\pm1 \ ^{\circ}C)$  with fixed 12:12 hr light-dark cycle. Animals were divided into four groups; two diabetics and two non diabetics (N=7).

## **Diabetes** induction

Diabetes was produced with a single tail injection of streptozotocin (STZ, 40 mg/kg), (6). Ten days after STZ injection, fasting blood glucose levels were determined using a glucometer ACCU-CHEK Active, (ACCU-CHEK, Germany). Rats with blood glucose levels >250 mg/dl were considered to be diabetic. The animals were kept in animal room for eight weeks to develop diabetic vessel complications.

### Preparation of mesenteric vascular bed

All animals were anesthetized by i.p. (intra-peritoneal) injection of ketamine HCl (50 mg/kg). Mesenteric vascular beds were prepared as originally described by McGregor (7). In brief, abdominal wall was opened, superior mesenteric artery exposed, cannulated and gently flushed with pre-warmed (37 °C) modified Krebs Henseleit solution (containing in mM: NaCl; 118, KCl; 4.7, CaCl<sub>2</sub>; 2.5, MgSO<sub>4</sub>; 1.2, glucose; 2, NaHCO<sub>3</sub>; 2.5, NaHPO<sub>4</sub>; 1.2) concomitantly bubbled with a mixture of 95% O<sub>2</sub> and 5% CO<sub>2</sub> (final pH 7.4). The mesentery was isolated from the intestine, and placed in a water-jacked perfusion chamber maintained at 37 °C. The preparation was perfused at 5 ml/min with the modified Kerbs Henseleit solution by a peristaltic pump (Meredos GmbH). The tissue was prevented from drying by superfusion with 0.1 ml/min of the solution. Perfusion pressure was monitored via a T tube inserted between the pump and the inflow cannula was connected to a pressure (ADInstruments, MLT0380). transducer Recording was done with Power Lab System (4SP, ADInstruments, ML760).

In the pilot study a cumulative concentrationresponse curve for phenylephrine, (from 0.000001 to 0.1 M) was carried out in mesenteric bed 30 min post equilibration to obtain proper phenylephrine concentration. Each phenylephrine dose was added into the medium and perfusion pressure was recorded for 15 min.

In the main experiments, vascular effects of GABA were investigated. After equilibration, to

induce 70-75% of maximal vasoconstriction, the vascular bed was constricted by phenylephrine Krebs Henseleit solution and then allowed to reach a plateau. GABA (1 to  $50 \,\mu$ M) was added into the medium every 15 min and perfusion pressure was recorded.

To clarify the role of endothelium, denuded vessels were made with 5 min perfusion of distilled water and were used in a series of experiments (8).

### Drugs

Streptozotocin (STZ) obtained from Pharmacia & Upjohn (Kalamazoo, Michigan, USA) was dissolved in 1 ml cold normal saline immediately before use. GABA was purchased from MERK (Germany). Phenylephrine was taken from Sigma (St. Louis, MO, USA), ketamine HCl was also taken from Rotexmedica (Trittau, Germany).

### Statistical analysis

Data were expressed as Mean±SEM. Comparisons between groups were made by student's t-test, and two-way analysis of variance followed by Tukey's HSD (Honestly Significant Difference) *post hoc* test. P < 0.05was considered significant. SPSS software was used to analyze data.

### **Results**

No significant difference was found between groups before the intervention. Ten days after STZ injection blood glucose levels were significantly increased from  $111\pm7.6$  to  $276.4\pm10.7$  mg/dl (P< 0.05). Eight weeks after diabetes induction, plasma glucose levels remained significantly elevated in diabetic rats.



Figure 1. Baseline perfusion pressure in all groups. Mesenteric bed perfusion pressure of all four groups (intact control and diabetic and denuded control and diabetic) were compared in basal conditions. Data are expressed as mean $\pm$ SEM (n= 28).

\* Significant difference with all other groups (P < 0.05).



Figure 2. Concentration-response curves of GABA in controls and diabetics (intact and denuded endothelium) groups. The tissues were precontracted with phenylephrine. Data are expressed as mean $\pm$ SEM (n= 28).

\* Significant difference with denuded control (P < 0.05). # Significant difference with denuded diabetic (P < 0.05).

#### Mesenteric bed responses

Significant difference was found between baseline perfusion pressure in control and diabetic groups (Figure 1). Diabetes induction caused an increase in perfusion pressure. Perfusion pressure was increased in denuded mesenteric bed vasculature both in control and diabetics (Figure 1).

Pretreatment with phenylephrine (0.003-0.006 mol/l), increased the perfusion pressure to 114.8±7.66 mmHg (70-75% of basic contraction). GABA caused a concentration-dependent relaxation in all mesenteric bed vessels (Figure 2).

Although diabetes induction doesn't change mesenteric bed response to GABA, denuded vessels showed a severe reduced response to GABA both in control and diabetic animals (Figure 2).

### Discussion

In this study we used diabetes model induced by STZ. In this model we induced diabetes by a single i.v. injection of STZ (50 mg/kg), that is similar to type I diabetes mellitus (9) and insulin production is reduced due to B cell destruction. To be sure that irreversible diabetes was induced, the rats with blood glucose more than 250 mg/dl were considered to be diabetic.

Previous studies confirmed that vascular complications of diabetes in rat occurred 2 months after diabetes induction (6). Due to increased vascular tone and decreased lumen size the peripheral vascular resistance is increased which is the basal hemodynamic abnormality in hypertension. As expected, in our study baseline perfusion pressure in diabetic mesenteric beds were higher than controls. Although the etiology of vascular disorder in diabetes is not fully understood, it has been suggested that alteration in the sensitivity or reactivity of vascular smooth muscle to neurotransmitters and circulating hormones may cause or contribute to diabetic hypertension (10). Several studies have suggested that endothelial dysfunction may have a role in this difference (6, 11).

Endothelium denudation caused baseline perfusion pressure to increase both in control and diabetic mesenteric beds (Figure 1). Presumably if endothelial dysfunction had been the sole factor of diabetic hypertension here, then both denuded endothelium groups should have had equal perfusion pressures. But our findings showed that perfusion pressure was higher in diabetic denuded endothelium compared to its control. So it seems other factors such as atherosclerosis and vessel elasticity reduction in combination with endothelium dysfunction may be involved (12,13).

Our study showed that GABA has relaxatory

effect on mesenteric bed. Some studies have shown the vasodilatory effect of GABA on ovarian blood vessels in normal rat when it was locally administered (14) and a tonic dilatory influence by endogenous GABA on cerebral microvasculature in the normal rats was also reported (15).

GABA could also decrease mesenteric perfusion pressure both in control and diabetic animals in the same degree. So we can come to this conclusion that GABA may act via a common pathway which is unaffected by diabetes.

Denuded endothelium reduced responsiveness of vessels to GABA in controls and diabetics. It has been postulated that endothelium may play role in relaxatory effect of GABA on mesenteric bed.

## Conclusion

From the results of this study we may conclude that GABA can induce endothelium dependent vasorelaxation in normal and diabetics rats. Further studies should be carried out to elucidate precise mechanisms.

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#### Leila Farsi et al

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