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ScienceDirect
The International Journal of Biochemistry & Cell Biology 40 (2008) 747–754
IJBCB
www.elsevier.com/locate/biocel

Diabetes induces changes of catecholamines in primary mesangial cells

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Received 9 April 2007; received in revised form 8 October 2007; accepted 10 October 2007
Available online 22 October 2007

Abstract

Diabetes mellitus is a frequent cause of kidney function damage with diabetic nephropathy being predominantly related to glomerular dysfunction. Diabetes is capable of interfering with distinct hormonal systems, as well as catecholamine metabolism. Since mesangial cells, the major constituent of renal glomerulus, constitute a potential site for catecholamine production, the present study was carried out to investigate alterations in catecholamine metabolism in cultured mesangial cells from the nonobese diabetic mouse, a well-established model for type I diabetes. We evaluated mesangial cells from normoglycemic and hyperglycemic nonobese diabetic mice, as well as cells from normoglycemic Swiss mice as control. Mesangial cells from normoglycemic mice presented similar profiles concerning all determinations. However, cells isolated from hyperglycemic animals presented increased dopamine and norepinephrine production/secretion. Among the studied mechanisms, we observed an upregulation of tyrosine hydroxylase expression accompanied by increased tetrahydrobiopterin consumption, the tyrosine hydroxylase enzymatic cofactor. However, this increase in synthetic pathways was followed by decreased monoamine oxidase activity, which corresponds to the major metabolic pathway of catecholamines in mesangial cells. In addition, whole kidney homogenates from diabetic animals also presented increased dopamine and norepinephrine levels when compared to normoglycemic animals. Thus, our results suggest that diabetes alters catecholamine production by interfering with both synthesizing and degrading enzymes, suggesting a possible role of catecholamine in the pathogenesis of acute and chronic renal complications of diabetes mellitus.

Keywords: Catecholamine; Mesangial cell; Diabetes; Nonobese diabetic mice

Abbreviations: BH_4, tetrahydrobiopterin; DA, dopamine; DDC, dopa decarboxylase; DβH, dopamine β-hydroxylase; EPI, epinephrine; Hh, hyperglycemic NOD mice; MAO, monoamine oxidase; MC, mesangial cells; Nn, normoglycemic NOD mice; NE, norepinephrine; NOD, nonobese diabetic mice; Ophe, *O*-Phenantroline; S, Swiss mice; TH, tyrosine hydroxylase; HPLC, high-performance liquid chromatography.

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doi:10.1016/j.biocel.2007.10.016

1. Introduction

Diabetes mellitus is a frequent cause of kidney function damage with diabetic nephropathy being currently viewed as a predominantly glomerular process followed by secondary tubular loss (Barthelmebs, Mayer, Thomas, Grima, & Imbs, 1995; Jeansson, Granqvist, Nyström, & Haraldsson, 2006). Mesangial cells are the major constituents of the renal glomerulus. They play a pivotal role in the regulation of glomerular filtration rate and participate in the development of functional and morphological glomerular abnormalities. Indeed, mesangial cells constitute a potential site for catecholamine production, expressing all synthesizing and degrading enzymes (Di Marco et al., 2003; Pizzinat et al., 2003).

Catecholamines are well-known classical hormones that play an important role in the regulation of a variety of renal physiological functions, such as sodium and water metabolism and arterial pressure control, and a consequent causative role in several common diseases such as hypertension, dyslipidemia (metabolic syndrome), and diabetes (Leese & Vora, 1996; Umrani & Goyal, 2002; Zeng et al., 2007). Although the precise pathophysiological roles and mechanisms remain vague, they could involve regulation of catecholamine synthesis and secretion.

Tissue or cellular concentration of catecholamines depends, in part, on the activity of the amine synthesizing or degrading enzymes. Among the synthesizing enzymes, tyrosine hydroxylase (TH) is the rate-limiting step in catecholamine synthesis cascade. Other enzymes, such as dopa decarboxylase (DDC), dopamine β-hydroxylase (DβH), and phenylethanolamine *N*-methyltransferase (PNMT) are also involved. On the other hand, the mitochondrial enzyme monoamine oxidase (MAO) represents one of the major metabolic pathways for biogenic amine degradation, being the predominant catecholamine-degrading enzyme expressed in mesangial cells (Lackovic, Salkovic, Kuci, & Relja, 1990; Pizzinat et al., 2003).

In several experimental models, diabetes mellitus is accompanied by an increase in the concentrations of catecholamine in the brain, heart and pancreas (Adeghate, Ponery, & Sheen, 2001; Ganguly, Beamish, Dhalla, Innes, & Dhalla, 1987; Lackovic et al., 1990). However, there is no information available on the activation status of catechomine system in mesangial cells in diabetes.

Hence, the current study was carried out to investigate catecholamine metabolism in cultured mesangial cells from nonobese diabetic (NOD) mouse, a well-established model of spontaneous insulin-dependent diabetes mellitus (type I) (Jeansson et al., 2006). Herein we showed that diabetes alters catecholamine production by interfering with expression and/or activity of both synthesizing- and degrading-enzymes. Additionally, we observed that the kidney from diabetic animals also presented increased DA and NE levels.

2. Materials and methods

Experiments were performed with female NOD mice, a strain that spontaneously develops insulin-dependent type I diabetes mellitus. The mice were kept on standard food and water was available ad libitum. All animals procedures and experiments were approved by the Institutional Ethics Committee and all efforts were made to minimize animal suffering.

2.1. Experimental groups

Groups studied: (1) Swiss female mice (S): used as control, blood glucose <110 mg/dl (<6 mmol/l); (2) female normoglycemic NOD mice (Nn): blood glucose <110 mg/dl (<6 mmol/l); and (3) female hyperglycemic NOD mice (Hn): blood glucose >300 mg/dl (>17 mmol/l). Conversion SI unit: mg/dl of glucose × 0.0555 = mmol/l of glucose.

Animals ages (S, Nn and Hn) ranged from 24 to 28 weeks. The animals used in this protocol were purchased from Jackson Laboratories (Bar Harbor, ME, USA).

2.2. Measurement of blood glucose levels

Blood glucose levels were measured using *ACCU-CHEK® Sensor* for each animal.

2.3. Mesangial cell culture

The culture was performed as previously described (Di Marco et al., 2003). Macrodissected cortex was submitted to a serial sieving and mesangial cells were obtained from collagenase-treated isolated glomeruli in order to remove the epithelial cell component. The cells were plated on RPMI 1640 supplemented with 20% fetal bovine serum (FBS), 50 U/ml penicillin, 2.6 mM acid HEPES and 2 mM glutamine. The cultures were allowed to develop in a CO_2 incubator (5% CO_2, 95% air) at 37 °C. Before the experiments, the cells were incubated without fetal bovine serum for 24 h. The medium and scraped cells were stored at −80 °C. Cells were used from the 4th up to the 8th passage and were characterized according to the following criteria: (1) morphological aspect of stellate cells; (2) positive immunofluorescence stain of cellular fibronectin (clone