Effect of Insulin on NO Production by Monocytes from Patients with Metabolic Cardiovascular Syndrome

T. E. Suslova, A. V. Sitozhevskii, O. N. Ogurkova, O. V. Gruzdeva, T. S. Fedorova*, and R. S. Karpov

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Basal and insulin-induced production of NO by monocytes significantly increased in patients with metabolic cardiovascular syndrome. Plasma insulin concentration in these patients was below the control. No intergroup differences were found in C-peptide concentration. A negative correlation was revealed between insulin-induced NO production by monocytes and C-peptide/insulin ratio in patients. The role of monocytes on the regulation of vascular tone via NO production in patients with metabolic cardiovascular syndrome is discussed.

Key Words: *NO; insulin; monocytes; metabolic cardiovascular syndrome*

Insulin resistance is a general pathogenetic mechanism of various components of metabolic cardiovascular syndrome (MCS) including non-insulin-dependent diabetes mellitus, dyslipidemia, and arterial hypertension [2,10]. Changes in tissue sensitivity to insulin can impair NO synthesis and secretion. According to modern notions, this compound maintains homeostasis in the vascular wall and regulates vascular tone and cell adhesion [3,7]. The mechanisms of hormonal regulation of paracrine NO production are poorly understood.

Here we studied the effect of insulin on NO production by peripheral blood monocytes and evaluated the relationship between this process and plasma insulin concentration in patients with MCS.

MATERIALS AND METHODS

We examined 18 men (45-50 years) with non-insulin-dependent diabetes mellitus, coronary heart disease, and arterial hypertension. The duration of the disease was 5-8 years. The patients received hypotensive, hypoglycemic, and antianginal drugs. The control group included 15 healthy donors (men, 40-47 years).

Monocytes were isolated from the suspension of mononuclear leukocytes by the method of selective adhesion in flat-bottom flasks. Peripheral blood mononuclear leukocytes were isolated on a Histopaque density gradient (ρ=1.077 g/cm^3) [4]. Monocytes were cultured in complete RPMI medium. Basal and insulin-induced production of NO was assayed by the concentration of stable NO metabolites (nitrite anions) in supernatants of a 1-day-old monocyte culture using Griess reagent. Insulin concentration in the medium was 0.03, 0.30, 0.10, 1.00, and 10.00 nM. The reference group included monocytes cultured in complete nutrient medium with NO synthase inhibitor N$^{\omega}$-monomethyl-L-arginine (1 μmol/ml) and insulin in the specified concentrations.

The concentrations of insulin and C-peptide in blood plasma were measured by enzyme immunoassay with Dako diagnostic kits. The significance of differences was evaluated by Mann—Whitney test. Correlation analysis included Spearman correlation test.

RESULTS

Basal NO production by cultured monocytes from patients with MCS was much higher than in healthy do-

Institute of Cardiology, Tomsk Research Center, Siberian Division of the Russian Academy of Medical Sciences; *Siberian State Medical University, Tomsk. ***Address for correspondence:*** liv@cardio.tsu.ru. T. E. Suslova

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nors (Table 1). The intensity of basal NO production reflects functional activity of monocytes and metabolic prehistory of cells without hormonal stimulation *in vitro*. Insulin added to the medium in low physiological concentration (0.03 nM) increased NO production in subjects of both groups. Further increase in insulin concentration was accompanied by stimulation of NO production, which reached a maximum at a hormone concentration of 0.3 nM. Insulin in a concentration of 10 nM suppressed NO production by cultured monocytes from healthy donors and patients with MCS.

Basal NO production by cultured monocytes was high in patients with MCS. These data illustrate higher degree of cell priming with pathological metabolic factors during cardiovascular syndrome. The observed differences can also result from greater lability of monocytes from patients under changing *in vitro* conditions. The intensity of NO production increased in the presence of insulin, which was probably associated with NO synthase activation in monocytes. Our assumption is confirmed by published data that insulin similarly modulates NO synthase activity in endotheliocytes, smooth muscle cells, and platelets [7-9]. The biphasic effect of insulin was observed in various types of cells [10,11]. This effect *in vivo* underlies the mechanism of regulation of NO synthesis by monocytes under conditions of significant variations in blood hormone concentration. Biological activity of insulin is impaired in patients with MCS. These changes affect NO synthesis by endotheliocytes and smooth muscle cells, which can produce dysfunction of endotheliocytes [6,8].

Insulin concentration in patients with MCS was lower than in healthy donors (Table 2), which the C-peptide/insulin ratio increased in patients with MCS. It should be emphasized that C-peptide concentration did not differ in patients and healthy donors. Blood insulin concentration depends on a variety of factors. The blood content of insulin is determined by the ratio between production and utilization of this hormone. C-peptide concentration serves as a criterion of insulin formation from proinsulin. The amount of circulating C-protein is similar to insulin concentration. However, C-protein half-life surpasses that of the hormone [1,3]. The decrease in insulin concentration and increase in the C-peptide/insulin ratio in patients with MCS probably reflect impairment of insulin reception in peripheral tissues and intensive degradation of this hormone in the liver.

A negative correlation was revealed between insulin-induced production of NO by monocytes and C-peptide/insulin ratio in patients with MCS (R=-0.75, $p \le 0.05$). Insulin concentration in culture medium of cells from these patients was 0.3 nM. In patients with MCS and non-insulin-dependent diabetes mellitus, the cells operate under conditions of relative insulin deficiency [5,6,11]. The increase in basal and insulin-induced NO production in these patients probably underlies the compensatory mechanism maintaining homeostasis in the vascular wall. These changes are observed under conditions of decreased insulin-dependent NO production by endotheliocytes and smooth muscle cells. Monocytes are probably the major factor determining NO concentration during insulin resistance. NO maintains equilibrium between vasoconstriction and vasodilatation. Our results suggest that monocytes are involved in the regulation of vascular tone in patients with MCS. This effect is realized via complex mechanisms of paracrine and autocrine NO production in the vascular wall. Insulin *in vitro* produces a modulatory effect, which probably underlies physiological mechanisms of regulation of NO synthesis by monocytes or changes in this mechanism under conditions of insulin resistance *in vivo*.

TABLE 1. Basal and Insulin-Induced NO Production in the Supernatant of Cultured Peripheral Blood Monocytes from Patients with MCS and Healthy Donors ($M \pm m$)

Insulin concentration in culture medium, nM	NO concentration, nmol NO_2^-/ml per 10^6 cells	
	healthy donors (n=15)	patients (n=18)
Sample without insulin	20.1±7.1	32.5±5.7*
0.03	22.7±1.2	35.5±5.2*
0.1	28.2±6.0	48.5±7.3*
0.3	23.7±3.1	47.5±5.6*
1	21.6±3.1	39.7±7.5
10	19.0±4.3	38.6±6.7*

Note. Here and in Table 2: *$p \le 0.05$ compared to healthy donors.

TABLE 2. Blood Concentrations of Insulin and C-Protein and C-Peptide/Insulin Ratio in Patients with MCS and Healthy Donors ($M \pm m$)

Parameter	Healthy donors (n=15)	Patients (n=18)
Insulin, pmol/liter	92.8±17.5	47.0±5.0*
C-peptide, pmol/liter	624.1±85.6	663.4±70.4
C-peptide/insulin	7.2±0.6	15.0±1.6*

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