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The Role of Endothelin-1 and Nitric Oxide in the Pathogenesis of Hypertension in Diabetic Patients

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ABSTRACT

The pathogenesis of renal hypertension has not yet been fully clarified. As the potential role of endothelin-1 (ET-1) and nitric oxide (NO) has been postulated, their concentrations were determined in plasma and urine of diabetic patients. The study included 30 diabetic patients (both IDDM and NIDDM) with initial or advanced diabetic nephropathy (decreased endogenous creatinine clearance, proteinuria) and 20 healthy control subjects. The correlation with blood pressure and other renal function parameters was monitored and compared with the control group. Also, the effect of ACE inhibitors (ACEI) on ET-1 and NO patterns was monitored in correlation with arterial hypertension. In diabetic patients that did not receive ACEI therapy, the increase in plasma ET-1 was associated with both systolic and diastolic blood pressure elevation, whereas in those administered ACEI the increase in plasma ET-1 was associated with a systolic blood pressure decline. In addition, the increase in plasma NO was accompanied by a statistically significant decline of both systolic and diastolic blood pressure in diabetic patients receiving ACEI.

Key words: endothelin-1, nitric oxide, diabetes mellitus, hypertension

Introduction

The pathogenesis of hypertension in diabetes mellitus has not yet been completely elucidated, however, it has been definitely found to be at least in part due to the increase in the intravascular volume of extracellular fluid. Two stages have been identified in the development of diabetic nephropathy, characterized by completely different defects of renal function: hyperfiltration as the main early functional impairment, and progressive reduction of glomerular filtration with the development of chronic renal insufficiency characteristic of the late stage of diabetic nephropathy[1,2]. The major clinical feature of diabetic nephropathy is proteinuria[1–3]. Arterial hypertension develops with the progression of proteinuria and reduction of glomerular filtration. The role of endothelin-1 (ET-1) and nitric oxide (NO) in the pathogenesis of hypertension in diabetes has recently been increasingly investigated. Vascular endothelium plays a crucial role in the vascular tone regulation through the production of vasoactive substances. Vasoactive substances include ET-1 as a vasoconstrictor and NO as a vasodilator.

ET-1 as the most potent vasoconstrictor identified to date enhances sympathetic activity[3–9], increases arterial blood pressure, and decreases plasma flow and glomerular filtration, thus reducing natriuresis and diuresis[10–13]. ET-1 synthesis is stimulated by ischemia, stress, nephrotoxic drugs as well as by various humoral factors such as glucose and insulin. It has been postulated that endothelial activation in diabetes may enhance vascular production of ET-1, which leads to glomerular lesion by increasing vasoconstriction, mesangial cell proliferation and glomerular permeability, and to the development of arterial hypertension[13,14].

NO is a free gaseous radical that is involved in a variety of physiological processes. NO leads to vasodilation, and inhibits vascular smooth muscle proliferation as well as platelet adhesion and aggregation. NO is formed from L-arginine by the action of NO-synthetase in vascular endothelium. It is a potent vasodilator involved in vascular tone regulation[15–17]. In diabetic patients, vascular endothelial dysfunction results in a reduced production or

release of NO[15]. Impaired metabolic control and elevated blood glucose concentrations lead to endothelial dysfunction, which in turn entails a decreased NO production, increased vasoconstriction, and potential development of arterial hypertension in diabetics[16].

The aim of the present study was to determine the concentrations of ET-1 and NO in plasma and urine of diabetic patients with initial or advanced diabetic nephropathy, and to compare them with those in a control group of healthy subjects. The study also addressed the association of ET-1 and NO with arterial hypertension according to the presence or absence of ACE inhibitors (ACEI) in patient therapy.

Patients and Methods

The study included 30 diabetic patients (15 male and 15 female), mean age 65 years, with initial or advanced diabetic nephropathy (decreased endogenous creatinine clearance and proteinuria). The patients were divided into two groups according to type of diabetes mellitus into insulin dependent (IDDM) and non-insulin dependent (NIDDM) diabetes mellitus. All study patients underwent clinical examination, renal ultrasonography (kidney size), and determination of serum hemoglobin, serum creatinine concentrations, creatinine clearance and body mass index (BMI).

ET-1 and NO were determined in serum and morning urine sample. ET-1 was determined by ELISA using an ET test kit with enzyme immunologic analysis designed for direct determination of ET in biological fluids[5].

Determination of NO includes measurement of its stable product (NO_2) based on Griess reaction and colorimetric determination of stained product concentration. Urine nitrites were also determined by use of Griess reagent. Nitrite concentration was assessed by reading the reaction solution absorption at 540 nm[18]. Protein was quantitatively determined in 24-h urine.

Based on funduscopy findings, used to assess the presence of microangiopathic lesions, patients were divided into two groups with proliferative and nonproliferative diabetic retinopathy. Glycated hemoglobin (HbA_{1c}) was used for diabetic control assessment. Blood pressure was monitored throughout the study period. Elevated

blood pressure was recorded in 16 and normal blood pressure in 14 patients. Data on the duration of diabetes mellitus, patient habits (cigarette smoking) and ACEI therapy were obtained from patient histories. Control group included 20 healthy subjects (5 male and 15 female), mean age 63 years. Body mass index in this group was between 25–30 (mean 27).

Statistical analysis was performed by use of Kolmogorov-Smirnov goodness of fit test, to determine whether the distribution of values of a particular variable differed significantly from the normal gaussian distribution. On determination of significance of interval variable differences, Mann-Whitney nonparametric test was used for differences between two independent groups, and Wilcoxon nonparametric test for differences between dependent samples.

Results

A higher plasma concentration of ET-1 was measured in diabetic patients as compared with control subjects (10.63 pg/mL *vs.* 8.10 pg/mL); however, the difference did not reach statistical significance (p=0.23). Urine concentration of ET-1 was also higher in diabetic patients than in control subjects (12.05 pg/mL *vs.* 9.66 pg/mL); this difference was not statistically significant either. Plasma concentration of NO was statistically significantly higher in diabetics than in healthy control subjects (7.49 μmol/L *vs.* 5.88 μmol/L; p=0.0001). Urine concentration of NO was 13.61 μmol/L in diabetic patients and 11.02 μmol/L in control subjects; the difference was not statistically significant (p=0.312). The main clinical laboratory parameters of diabetic patients and control subjects are shown in Tables 1 and 2, respectively.

There was no statistically significant difference in plasma and urine concentrations of ET-1 and NO between IDDM and NIDDM patients. These two groups showed no statistically significant differences in the level of blood pressure, diabetes duration, proteinuria and HbA_{1C}. These variables yielded no statistically significant differences between the subgroups of diabetic patients with proliferative and nonproliferative retinopathy.

In the group of diabetic patients there were 87% of nonsmokers and 13% of cigarette smokers. Kidney size

TABLE 1
CLINICAL LABORATORY FINDINGS IN DIABETIC PATIENTS

	Plasma endothelin (pg/mL)	Plasma NO (μmol/L)	Urine endothelin (pg/L)	Urine NO (μmol/L)	Age (yrs)	Kidney size (mm)	Creatinine (μmol/L)	Creatinine Clearance (μmol/L)
N	30	30	30	30	30	30	30	30
Arithmetic mean	10.6367	7.4950	12.0583	13.6190	64.33	107.1667	190.80	29.5
Range	30.19	4.17	20.50	61.44	36	63	550	80
Minimum	3.91	5.48	4.33	5.36	45	85.00	50	8
Maximum	34.1	9.65	24.83	66.80	81	148.00	600	90

N – number of diabetic patients, NO – nitric oxide

TABLE 2
DESCRIPTIVE STATISTICS DATA IN CONTROL GROUP

Group		Plasma endothelin (pg/mg)	Plasma NO (μmol/L)	Kidney size (mm)	Creatinine (μmol/L)	Creatinine Clearance (μmol/L)	Systolic blood pressure (mm Hg)	Diastolic blood pressure (mm Hg)
Control	N	20	20	20	20	20	20	20
	Arithmetic mean	8.103	5.8875	105.70	88.25	106	122.25	81.25
	Median	7.180	5.6800	105.00	85.50	103	120.00	80.00

N – number of control subjects, NO – nitric oxide

TABLE 3
CLINICAL LABORATORY FINDINGS IN DIABETIC PATIENTS

	Systolic blood pressure (mm Hg)	Diastolic blood pressure (mm Hg)	Diabetes duration (yrs)	Proteinuria (g/24 h)	HbA$_{1c}$ (%)
N	30	30	30	30	30
Arithmetic mean	160.17	92.00	10.17	2.3263	7.60
Range	90	60	22	8.15	10.4
Minimum	130	60	2	0.25	3.4
Maximum	220	120	24	8.40	13.8

N – number of diabetic patients, HbA$_{1c}$ – glycated hemoglobin

determined by echosonography was 107.16 mm in the diabetic group and 105.70 mm in the control group, the difference being not statistically significant (p=0.856). Serum Hb concentration was statistically significantly lower in diabetics than in control subjects (118.10 g/L *vs.* 133.65 g/L; p=0.002), whereas serum creatinine concentration statistically significantly higher in the former (190.88 μmol/L *vs.* 88.25 μmol/L; p=0.004). Creatinine clearance in diabetic patients was 29,5 μmol/. In the control group the creatinine clearance was 106 μmol/, which was statistically significant difference (p=0.004). (Table 1).

There is no statistically sisgnificant difference in body mass index between diabetic patients (BMI=27) and healthy control (25). The mean blood pressure level was 160/92 mm Hg in diabetics and 120/80 mm Hg in control subjects, yielding a statistically significant difference (p= 0.0001). There was a positive correlation between diabetes mellitus and both systolic (rs 0.369; p=0.0001) and diastolic (rs 0.269; p=0.001) blood pressure (Table 3).

Sixteen (53.3%) diabetics received ACEI therapy, whereas 14 (46.7%) did not receive this medication. An increase in systolic blood pressure with the rise in plasma ET-1 was observed in the latter, as indicated by the high value of the regression coefficient square for square regression R^2=0.4239, yielding a curve that followed the value distribution of the study variables (Figure 1). The
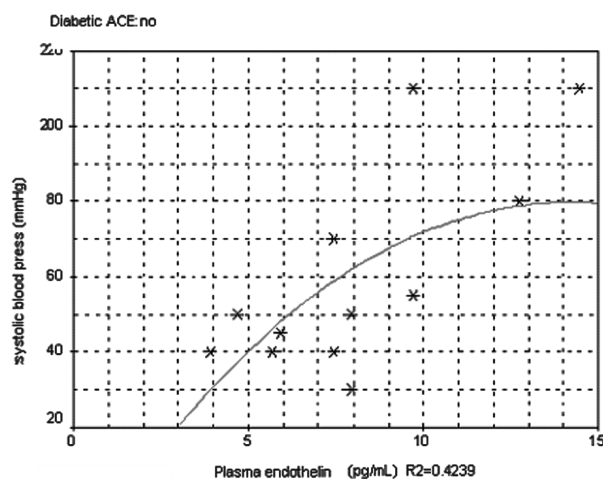

Fig. 1. Systolic blood pressure levels according to plasma endothelin-1 (ET-1) in diabetic patients not receiving ACEI.
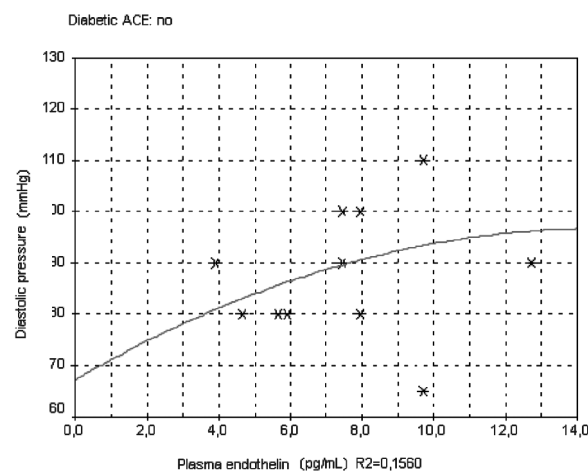

Fig. 2. Diastolic blood pressure levels according to plasma endothelin-1 (ET-1) in diabetic patients not receiving ACEI.

subgroup of diabetic patients not receiving ACEI showed a rise in diastolic pressure with ET-1 increase (Figure 2); regression coefficient for square model $R^2=0.1560$. The subgroup of diabetic patients treated with ACEI showed a marked systolic pressure decline with the rise in plasma ET-1 (Figure 3), as indicated by the regression coefficient square for square regression $R^2=0.1474$, and a nonsignificant decline of diastolic blood pressure ($R^2=0.0725$).

The group of diabetic patients as a whole, irrespective of ACEI therapy, showed a mild decrease of systolic blood pressure with the increase in plasma NO, however, without statistical significance ($R^2=0.0096$) (Figure 4). In the subgroup of diabetics receiving ACEI, there was a statistically significant decrease in systolic blood pressure with the increase in plasma NO; square of regression coefficient for linear model $R^2=0.1402$ (Figure 5). This subgroup showed an obvious tendency of diastolic blood

pressure decline with the increase in plasma NO; model $R^2=0.1065$ (Figure 6). The subgroup of diabetic patients not receiving ACEI showed a mild increase in systolic blood pressure and an increase in diastolic blood pressure with the rise in plasma NO, yet without statistical significance ($R^2=0.1115$ and $R^2=0.2806$, respectively) (Figures 7 and 8).

Discussion

Diabetic nephropathy is the most common cause of chronic renal insufficiency. Initially it manifests with microalbuminuria. The mechanisms involved in the occurrence of microalbuminuria should be properly understood to be able to prevent renal complications in diabetic patients (14). Vascular endothelium plays a crucial role in the regulation of vascular tone by producing vasoactive substances, i.e. vasodilators such as NO and
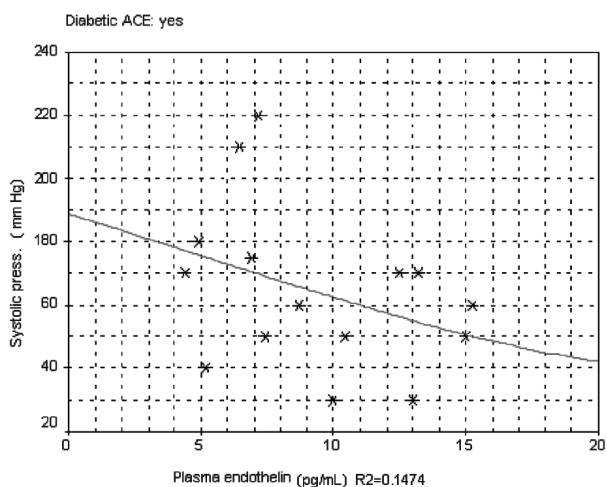

Fig. 3. Systolic blood pressure levels according to plasma endothelin-1 (ET-1) in diabetic patients receiving ACEI.
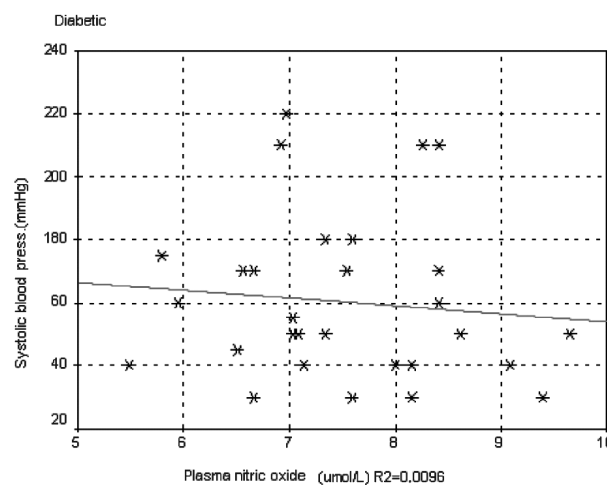

Fig. 4. Systolic blood pressure levels according to plasma nitric oxide (NO) in diabetic patients not receiving ACEI.
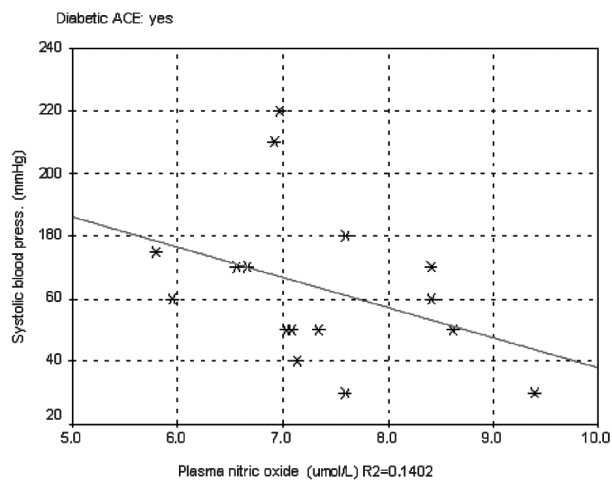

Fig. 5. Systolic blood pressure levels according to plasma nitric oxide (NO) in diabetic patients receiving ACEI.
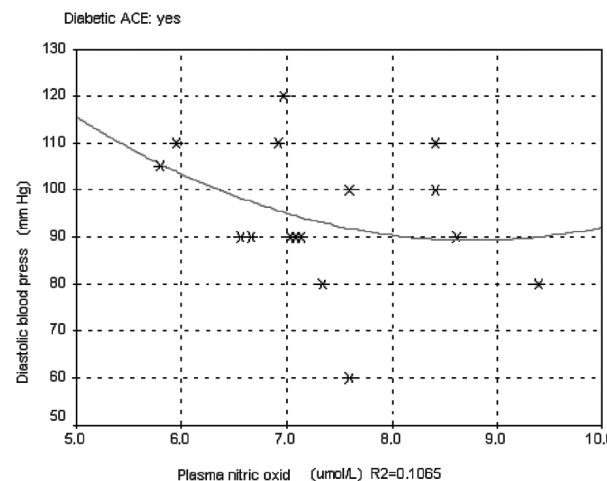

Fig. 6. Diastolic blood pressure levels according to plasma nitric oxide (NO) in diabetic patients receiving ACEI.
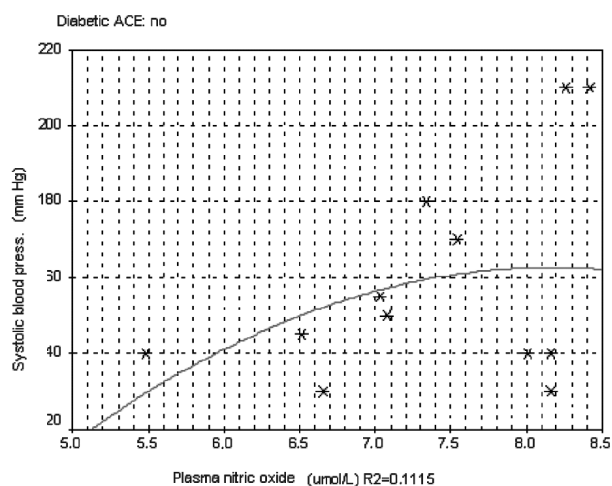
Fig. 7. Systolic blood pressure levels according to plasma nitric oxide (NO) in diabetic patients not receiving ACEI.
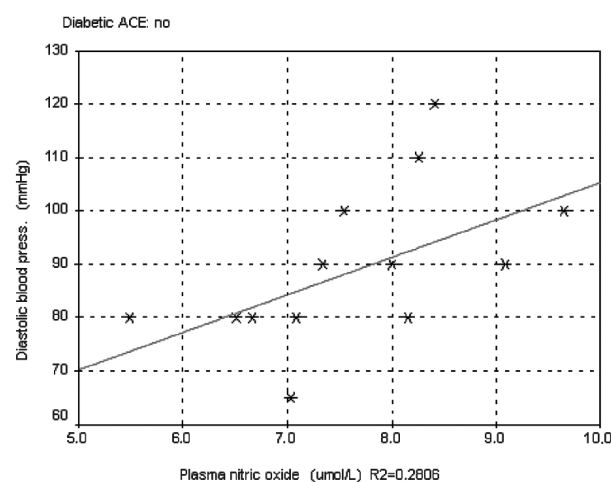

Fig. 8. Diastolic blood pressure levels according to plasma nitric oxide (NO) in diabetic patients not receiving ACEI.

prostacycline, and vasoconstrictors such as thromboxane A2 and ET-1. The synthesis of ET-1 is stimulated by ischemia, stress, nephrotoxic drugs as well as by a variety of humoral substances such as glucose and insulin. It has been postulated that endothelium activation in diabetes can increase the vascular production of ET-1, which then leads to glomerular lesion by increasing the vasoconstriction, mesangial cell proliferation and glomerular permeability[1,13,14]. Both *in vivo* and *in vitro* studies suggest that NO-mediated vasodilation can be reduced in diabetes mellitus. NO is a potent vasodilator that is involved in the regulation of vascular tone in humans. Normal vascular endothelium prevents development of atherosclerosis by the formation and release of NO. Besides its vasodilating action, NO inhibits proliferation of smooth muscle vasculature as well as platelet adhesion and aggregation[15].

Hyperglycemia leads to dysfunction of the vascular adrenergic system, impairment of NO synthesis, and impaired response of NO system cells through a reversible mechanism that includes superoxide anions. In uncontrolled diabetes mellitus, endothelial dysfunction results in a reduced NO production and potential development of hypertension[16]. In our study, the group of diabetic patients had higher plasma and urine concentration of ET-1 as compared with control subjects, although the difference was not statistically significant. The study included patients with hypertension, uncontrolled diabetes mellitus, obesity, and variable serum creatinine levels. Hypertension, poor metabolic control, obesity and decreased glomerular filtration are known to influence the activity of ET-1[14].

Vermes et al. found an increased plasma ET-1 in NIDDM patients with proteinuria and normal plasma ET-1 in NIDDM patients with microalbuminuria[19]. Recent studies have been focused on the role of circulating ET-1 in early diabetic nephropathy. Elevated plasma concentrations of ET-1 have been reported in NIDDM pa-

tients with or without microalbuminuria[20], suggesting that the vascular production of ET-1 might be enhanced by endothelial activation in diabetes mellitus, thus increasing the vasoconstriction, mesangial cell proliferation and glomerular permeability, which ultimately lead to glomerular lesion[19,20]. In experiments on rats with impaired renal function, plasma ET-1 concentration was normal, whereas urinary excretion of ET-1 was increased[20]. De Mattia *et al*. measured circulating and urine ET-1 concentrations in NIDDM patients with or without microalbuminuria, and compared ET-1 concentration between hypertensive and normotensive patients. They found higher levels of circulating ET-1 in NIDDM patients irrespective of the presence or absence of micro- albuminuria[14]. These data support the hypothesis according to which NIDDM influences vascular production of ET-1 *via* early endothelial activation and before the occurrence of microalbuminuria. The 24-h urinary excretion of ET-1 was considerably reduced in NIDDM patients with or without microalbuminuria. Urine concentration of ET-1 showed no correlation with plasma ET-1, urinary albumin excretion, or creatinine clearance. In this study, the values of creatinine and blood glucose were normal, in contrast to our study, where elevated levels of creatinine, blood glucose and HbA_{1c} were recorded.

In the present study, we found a positive correlation between plasma ET-1 and blood pressure in the group of diabetic patients. Diabetic patients were divided into subgroups according to the presence or absence of ACEI therapy. The subgroup of diabetic patients not receiving ACEI showed a positive correlation between plasma ET-1, and both systolic and diastolic blood pressure. In the subgroup of diabetic patients receiving ACEI, however, the correlation between plasma ET-1 and systolic blood pressure was negative.

In our study, diabetic patients showed higher plasma NO concentration than control subjects. Rodrigez-Manas *et al*. assessed the impact of glycemic control on the NO

system function in type 1 diabetes mellitus and found NO response dysfunction to be only present in the early stage of the disease (duration of less than 3 years), in patients without clinical evidence for vascular disease[16]. In diabetics, intensive oxidative stress was observed in the very early stage of the disease, including children and adolescents. Oxidative stress is associated with the metabolic state in diabetes[21]. Oxidative stress and NO inactivation by the vascular superoxide anion O_2 play the key role in the pathogenesis of vascular diseases, including hypertension, atherosclerosis, hypercholesterolemia, diabetes mellitus, cardiac insufficiency, etc. In most cases, a rise in O_2 decreases endothelial vascular relaxation mediated by NO, thus inactivating endogenous NO[21].

In the present study, a mild yet statistically nonsignificant decrease in systolic blood pressure with the increase in plasma NO was observed in the group of diabetic patients as a whole. However, a statistically significant decrease of both systolic and diastolic blood pressure with the increase in plasma NO was recorded in the subgroup of diabetic patients receiving ACEI.

In conclusion, the present study demonstrated the correlation of plasma ET-1 and NO concentrations with systolic and diastolic blood pressure to depend on the presence or absence of ACEI therapy. Plasma ET-1 and NO were determined during the treatment with antihypertensive agents (mostly patients with severe and uncontrolled hypertension). Study results suggested the relationship of blood pressure with ET-1 and NO to be modified by ACEI therapy. Additional studies are needed with ET-1 and NO determined before the introduction of ACEI treatment, in order to more precisely identify the correlation of these parameters with blood pressure.

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ULOGA ENDOTELINA-1 I NITRIČKOG OKSIDA U PATOGENEZI HIPERTENIZIJE DIJABETIČARA

SAŽETAK

Patogeneza bubrežne hipertenzije nije u potpunosti jasna, a određenu ulogu mogu imati endotelin-1 (ET-1) i nitrički oksid (NO)(1). Zbog toga smo u ovom istraživanju određivali koncentraciju ET-1 i NO u plazmi i u urinu u dijabetičara. U istraživanje je uključeno 30 ispitanika sa šećernom bolešću (IDDM i NIDDM) s početnom ili razvijenom dijabetičkom nefropatijom (smanjen klirens endogenog kreatinina, proteinurija) i 20 zdravih kontrolnih ispitanika. Pratila se povezanost s krvnim tlakom i drugim parametrima bubrežne funkcije te uspoređivala s kontrolom. Također, smo pratili učinak ACE – inhibitora (ACEI) na odnos ET-1 i NO u korelaciji s arterijskom hipertenzijom. U bolesnika sa šećernom bolešću koji nisu koristili ACEI u terapiji uočen je porast plazmatske koncentracije ET-1 s porastom sistoličkog i dijastoličkog krvnog tlaka, dok u dijabetičara koji su liječeni ACEI s porastom plazmatske koncentracije ET-1 postoji tendencija pada sistoličkog krvnog tlaka. U dijabetičara liječenih ACEI, utvrđen je statistički značajan pad sistoličkog i dijastoličkog krvnog tlaka s porastom plazmatske koncentracije NO.