ORIGINAL ARTICLE

The significance of plasma adrenomedullin and calcitonin gene-related peptide concentration in patients with Type 2 diabetes mellitus who are treated for cardiovascular risk factors

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Abstract

OBJECTIVES: To evaluate the significance of plasma adrenomedullin and calcitonin gene-related peptide (CGRP) concentration in patients with Type 2 diabetes mellitus who are treated for hypertension and dyslipidemia.

METHOD: Plasma adrenomedullin and CGRP concentration, transthoracal echocardiography and ABPM were evaluated in 82 patients with Type 2 diabetes mellitus and 41 control subjects with no previous cardiovascular disease. All the subjects had casual blood pressure ≤140/90 mmHg or received antihypertensive medication, were treated by statin if LDL cholesterol was≥3mmol/L, by fibrates if triacylglyceroles≥2 mmol/L.

RESULTS: The mean age was 61±6 in patients with diabetes mellitus and 61±5 years in control subjects (p=0.9). Plasma CGRP was 3.0±1.8 in patients with diabetes mellitus and 2.3±1.0 ng/ml in control subjects (p=0.09). Plasma adrenomedullin was 2.2±0.9 in patients with diabetes mellitus and 2.8±1.1 ng/ml in control subjects (p=0.01). In patients with diabetes mellitus mass index of the left ventricle was significantly higher and the parameters of diastolic function were more deteriorated. Plasma adrenomedullin and CGRP correlated significantly negatively with serum creatinine and positively with mean 24 hours arterial blood pressure in patients with diabetes mellitus but not in control subjects. Plasma adrenomedullin concentration in patients with diabetes mellitus treated for hypertension was significantly reduced.

CONCLUSION: Despite concentration plasma adrenomedullin and CGRP modulation by cardioprotective treatment both neuropeptides remained involved in regulation of hemodynamic and metabolic parameters in patients with Type 2 diabetes mellitus. The low plasma of adrenomedullin in patients with Type 2 diabetic may be marker of the efficient intervention on cardiovascular risk factors.

INTRODUCTION

The elevated plasma adrenomedullin concentration is considered as a marker of unfavourable prognosis in acute as well as chronic disease including diabetes mellitus (Kato *et al.* 2005; Brain & Grant 2004; Ruzicka *et al.* 2001; Malfatto *et al.* 2003). Contrary plasma calcitonin gene-related peptide concentration (CGRP) is decreased according to recent reports in patients with diabetes mellitus (Wang *et al.* 2012; Al-Salam *et al.* 2009). However both plasma adrenomedullin and CGRP concentration may be significantly modified by medication (Krzeminaki K *et al.* 2006; Davidson *et al.* 2012; Del Campo *et al.* 2011). The patients with Type 2 diabetes mellitus are treated often for concomitant hypertension and dyslipidemia.

The aim of this study was to evaluate the impact of correction of cardiovascular risk factors by medicaments (hypertension and dyslipidemia) on plasma adrenomedullin and CGRP concentration in patients with Type 2 diabetes mellitus without any previous cardiovascular accident.

SUBJECTS AND METHODS

The patients with Type 2 diabetes mellitus and control nondiabetic subjects were examined at the Department of Medicine, University Hospital Prague Motol, and Czech Republic. The diagnosis of Type 2 diabetes mellitus was based on clinical findings, fasting blood glucose, C-peptide measurement and a negative finding for anti-beta-cell antibodies at least 1 year prior to enrolment in the study. The patients with Type 2 diabetes mellitus and randomly selected control subjects were included in the study if their casual blood pressure was well controlled regardless of treatment for arterial hypertension – equal or below 140/90. The subjects with a history of cardiovascular or other heart diseases, presence of regional LV kinetic abnormalities or a global LV ejection fraction <55%, a history of severe glomerular filtration rate impairment (<0.5 ml/s/1.73 m^2) and/or microalbuminuria, or a prognosis of a life-limiting disease were excluded.

The study was approved by the Ethics Committee of the University Hospital Motol and undertaken in accordance with the Declaration of Helsinki.

Echocardiography

Transthoracal echocardiography, including pulse Doppler and tissue Doppler imaging (TDI) were performed using a Philips Sonos® 7500 cardiac ultrasound unit (Philips Healthcare, Andover, MA, USA). Ejection fraction according to Teicholz formula and index of left ventricular mass were calculated.

Mitral inflow velocity was traced and the following variables of diastolic function were measured and evaluated: peak velocities of early (E) and late (A) transmitral flow, the ratio E/A, and deceleration time (DT). The peak velocities of early (E´) and late (A´) diastolic mitral annular motion (average of septal and lateral values) were determined from TDI recordings, and mitral E/E´ and E´/A´ ratios were calculated. In our laboratory, approximately 4 weeks apart the mean intra-observer difference was 3,6% and inter-observer difference 4.6%.

24-h ambulatory BP monitoring was performed by oscilometry with a portable automated Cardiette bp one (CardiLine, Milan, Italy) with calibration certification; simultaneous 24 hour heart rate monitoring was obtained. The unit was set to take reading every 30 minutes throughout 24 h.

Biochemistry

Biochemical examination of blood samples included glycosylated haemoglobin (HbA_{1c}), lipid analysis and creatinine. HbA_1C was measured using high-performance liquid chromatography and a spectrophotometer detection system (Tosoh G8 analyser; Tosoh Corporation, Tokyo, Japan) with a normal range of 28–45 mmol/L according to the International Federation of Clinical Chemistry and Laboratory Medicine. The measured HbA1c was converted to NGSP network by calculation according to equation NGS*P*= [(0.09148×IFCC)+2.152]

Low-density lipoprotein (LDL) and high-density lipoprotein (HDL) cholesterol were measured using a direct enzymatic method with catalase; triglycerides were measured using the Fossati enzymatic method and serum creatinine (SCr) was measured using an enzymatic method; all of which were performed by an ADVIA® 1800 Clinical Chemistry System (Siemens Medical Solutions, Tarrytown, NY, USA).

Plasma adrenomedullin and CGRP (human) were examined by EIA methods (Phoenix Pharmaceuticals Inc., Burlingame USA).

Statistical evaluation

Continuous variables are given as mean ± SD. Between-group comparisons of continuous parameters were performed by Mann-Whitney test. The χ^2-test was used to compare categorical variables. Relationships between clinical parameters and plasma adrenomedullin and CGRP were assessed using linear regression analysis separately for patients with Type 2 diabetes mellitus and control subjects. A p-value <0.05 was considered to be statistically significant. Data were analysed using the StatGraphics Centurion Data Analysis and Statistical Software, version XV (Statpoint Technologies Inc., Warrenton, VA, USA).

RESULTS

Demographic and clinical data of patients with Type 2 diabetes mellitus and control subjects are summarized in Table 1 and the results of echocardiography examination in Table 2.

Tab. 1. The basic characteristics of patients with Type 2 diabetes mellitus and control subjects

	Diabetes mellitus (n=82)	Control subjects (n=41)	*p*-value
Age (years)	61±6	61±5	0.89
Gender (No of women)	28(34%)	14(28%)	1.0
BMI (kg/m^2)	31±3.7	29±4.6	0.01
FBS (mmol/L)	9.0±2.8	5.6±0.5	<0.01
HbA1c (%NGSP; mmol/L IFCC)	7.6±2.0; 60±16	5.7±0.4; 39±3	<0.01
Serum urea (mmol/L)	6.5±2.2	5.7±1.5	0.19
Serum creatinin (umol/L)	89±19	76±15	<0.01
LDL-cholesterol (mmol/L)	2.8±0.9	3.0±0.8	0.34
HDL-cholesterol (mmol/L)	1.2±0.3	1.5±0.4	<0.01
Triacylglycerols (mmol/L)	2.0±0.9	1.5±0.7	<0.01
Mean 24 hours systolic BP (mm Hg))	133±8	130±9	0.07
Mean 24 hours diastolic BP (mm Hg)	83±6	81±6	0.10

FBS…fasting blood sugar

Tab. 2. Comparison of echocardiography parameters.

	Diabetes mellitus (n=82)	Control subjects (n=41)	*p*-value
LVEF (%)	60±2	61±3	0.56
E/A	1.0±0.3	1.1±0.3	0.12
E´ (cm/s)	7.5±1.7	8.5±1.6	<0.01
A´ (cm/s)	10.9±1.7	10.6±1.8	0.39
E´/A´	0.7±0.1	0.8±0.2	<0.01
E/E´	10.2±2.2	8.7±2.1	<0.01
LV mass index (g/m2)	114±18	103±16	<0.01

LVEF – left ventricular ejectin fraction
LV – left ventricle

The comparison of hypertension and dyslipidemia treatment between patients with Type 2 diabetes mellitus and control subjects is presented in Table 3.

Plasma CGRP was 3.0±1.8 in patients with Type 2 diabetes mellitus and 2.3±1.0 ng/ml in nondiabetic patients (p=0.09). Plasma adrenomedullin was 2.2±0.9 in patients with Type 2 diabetes mellitus and 2.8±1.1 ng/ml in control subjects (p=0.01).

Fifty-nine patients with Type 2 diabetes mellitus received hypolipodemic drugs (72%). Their plasma adrenomedullin concentration was 2.3±1.0 comparing to 1.9±0.7 ng/L in 23 untreated subjects (p=0.2) and plasma CGRP was 3.2±1.5 comparing to 2.5±0.8 ng/L respectively (p=0.1).

Tab. 3. Comparison of the application of antihypertensive and hypolipidemic drugs.

	Diabetes mellitus (n=82)	Control subjects (n=41)	*p*-value
Treatment for hypertension (No/%)	67 (87%)	15 (36%)	<0.01
Treatment for dyslipidemia (No/%)	59 (72%)	13 (32%)	<0.01
Statins (No/%)	52 (63%)	20 (24%)	<0.01
Fibrates	11 (13.5%)	3 (7.3%)	0.32
ACE inhibitors or sartans(No/%)	56 (68%)	9 (22%)	<0.01
Beta blockers (No/%)	28 (34%)	6 (15%)	<0.01
Calcium channel blockers (No/%)	22 (27%)	6 (15%)	0.38
Diuretics (No/%)	30 (37%)	2 (5%)	0.04
Another hypotensive drugs (No/%)	9 (11%)	0 (0%)	0.08

Sixty-six patients with Type 2 diabetes mellitus were treated for hypertension (80%). Their plasma adrenomedullin concentration was 2.0±0.8 comparing with 3.1±1,2 ng/ml in 16 normotensive patients (p<0.001). CGRP in patients with Type 2 diabetes mellitus who were treated for hypertension was 2.9±1.3 comparing to 3.6±1.3ng/L in normotensive patients (p=0.18).

In patients with Type 2 diabetes mellitus plasma adrenomedullin correlated negatively with HbA1c (r=–0.230; p=0.03) serum creatinine (r=–0.473; p<0.01) and positively with 24 hours mean diastolic blood pressure (r=0.313; p=0.004) while CGRP negatively correlated with serum creatinine (r=–0.366; p<0.01) and positively with 24 hours mean systolic (r=0.265; p=0.015) and 24 hours mean diastolic pressure (r=0.265; p=0.015).

In control subject no correlation of plasma adrenomedullin with clinical and echocardiography parameters was detected. Plasma CGRP concentration correlated with E´ (r=0.487; p<0.01) and E´/A´ (r=0.360; p=0.03).

DISCUSSION

The concentration of plasma adrenomedullin is elevated in acute as well as chronic diseases due to endothelial dysfunction (Hosomi *et al.* 2004). It is considered to be a marker of prognosis in some clinical conditions (Nicholls et al 2003; Eto *et al.* 2003; Li & Du 2003).

In the previous studies the elevation of plasma adrenomedullin concentration was documented in patients suffering from Type 1 as well as Type 2 diabetes mellitus in comparison to subjects without diabetes mellitus (Ruzicka *et al.* 2004; Lim *et al.* 2007). The higher con-

centration of adrenomedullin was also found in subjects who developed later hypertension, condition that is frequently present in patients suffering from diabetes mellitus (Kato *et al.* 2006).

Transient receptor potential vanniloid 1 and its neuropeptide CGRP were found to be impaired in diabetes mellitus (Zhang *et al.* 2012; Li & Peng 2002; Du *et al.* 2004; Bowles *et al.* 2003). In the recent study CGRP plasma concentration was significantly lower in patients with diabetes mellitus mainly if they had coronary artery disease in comparison with subject without diabetes mellitus (Wang *et al.* 2012). According to this report CGRP may have a role in the pathogenesis of coronary artery disease with diabetes.

However the patients with Type 2 diabetes mellitus have a high prevalence of hypertension and dyslipidemia. These cardiovascular risk factors are currently corrected with the very potent drugs like ACE inhibitors, sartans, beta-blockers, statins, fibrates etc. In many previous studies these medicaments were shown to modulate plasma level of adrenomedullin as well as CGRP (Mishima *et al.* 2003; Kawasaki *et al.* 1999; Zhang *et al.* 2009; Nguyen *et al.* 2012).

It was reason why in our study the significance of plasma concentration of adrenomedullin and CGRP was evaluated in the patients with Type 2 diabetes mellitus who had no previous cardiovascular accident with well-compensated blood pressure and dyslipidemia by the above-mentioned medication.

The patients with Type 2 diabetes mellitus in our study had comparable values of arterial blood pressure and LDL cholesterol with control subjects due to antihypertensive and hypolipidemic treatment. However their index of left ventricular mass was significantly higher and echocardiography parameters of the left ventricular diastolic function were more abnormal. Laboratory results were also more deteriorated mainly serum creatinine level. All these changes are associated with the more serious cardiovascular prognosis.

However plasma adrenomedullin in patients with diabetes mellitus was significantly lower comparing to control subjects and was associated with treatment of hypertension. Nevertheless in the small group of patients with Type 2 diabetes mellitus who were not treated for hypertension plasma adrenomedullin concentration was higher than in control subjects. No impact of dyslipidemia treatment on plasma adrenomedullin was detected. In patients with Type 2 diabetes mellitus the significant correlations of plasma adrenomedullin with serum creatinine level and mean 24 hours diastolic pressure were present while no such correlations were seen in control subjects. In spite of the significant decrease of plasma adrenomedullin concentration in patients with Type 2 diabetes mellitus due to treatment of hypertension these correlations support the physiological significance of adrenomedullin.

Plasma concentration CGRP was unexpectedly higher in patients with Type 2 diabetes mellitus comparing to control subjects but the difference was not significant. In patients with Type 2 diabetes mellitus CGRP concentration significantly correlated with serum creatinine and mean 24 systolic and diastolic blood pressures while no such associations were found in control subjects.

The explanation why plasma CGRP concentration was not lower in our patients with Type 2 diabetes mellitus in comparison with control subjects is not clear. We may anticipate the influence of medication because all patients with Type 2 diabetes mellitus took at least one drug either for treatment of hypertension or dyslipidemia but no significant evidence for impact of treatment was revealed.

The interesting positive correlation was discovered between movement of mitral valve in early diastole E´ and plasma CGRP concentration in control subjects. It means the release of CGRP may lead to the improvement of diastolic relaxation of the left ventricle in patients without diabetes mellitus.

CONCLUSION

Despite concentration plasma adrenomedullin and CGRP modulation due to cardioprotective treatment both neuropeptides remained involved in regulation of hemodynamic and metabolic parameters in patients with Type 2 diabetes mellitus without any previous cardiovascular accident that are treated for hypertension and (or) dyslipidemia. The low plasma of adrenomedulin and relatively high CGRP concentration in our patients with Type 2 diabetic may be markers of the efficient intervention on cardiovascular risk factors.

LIMITATIONS

Apart from the small size of subjects included into study the effect of the individual drugs on plasma adrenomedullin and CGRP concentration could not be evaluated because majority of patients with Type 2 diabetes mellitus have used combination of medications both for hypertension and dyslipidemia. Nevertheless the impact of the application of antihypertensive treatment on plasma adrenomedullin concentration in patients with Type 2 diabetes mellitus was documented at least.

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