Diabetologia (2003) 46:520–523
DOI 10.1007/s00125-003-1055-2

Diabetologia

Short Communication

Norepinephrine release is reduced in cardiac tissue of Type 2 diabetic patients

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Abstract

Aims/hypothesis. The aim of this study was to assess whether cardiac catecholamine release is affected in patients with Type 2 diabetes mellitus.
Methods. A trial tissue was obtained from 19 diabetic (Type 2) and 43 non-diabetic patients undergoing coronary surgery. Endogenous norepinephrine release was examined under baseline conditions as well as during electrical field stimulation (effective voltage 5 V, stimulation frequency 4 Hz, pulse width 2 msec) by high performance liquid chromatography and electrochemical detection. Cardiac function and arterial blood pressure was assessed from coronary angiography.
Results. In atrial tissue from diabetic patients, stimulation-induced norepinephrine release was reduced by 25% compared with non-diabetic patients, while baseline norepinephrine release did not differ between both groups. Preoperative plasma glucose and haemoglobin A_{1C} concentrations were increased in patients with diabetes, however, no relation was found to catecholamine release. Diabetic and non-diabetic patients did not differ regarding left ventricular ejection fraction and arterial blood pressure.
Conclusion/interpretation. Cardiac norepinephrine release is suppressed in patients with Type 2 diabetes which could contribute to sympathetic neuropathy. The difference of norepinephrine release in diabetic and non-diabetic patients was independent of cardiac function and arterial blood pressure. [Diabetologia (2003) 46:520–523]

Keywords Diabetes mellitus, human atrial tissue, norepinephrine release, sympathetic nervous system, ventricular function.

Cardiac sympathetic nerve activity has substantial pathophysiological and prognostic implications in heart disease and certain comorbidities such as diabetes mellitus. In vivo studies using positron-emission tomography have shown regional autonomic imbalance in the myocardium of diabetic patients and impaired vasodilator response of coronary resistance vessels to sympathetic stimulation [1, 2]. Moreover, sympathetic neuropathy has been noted histologically in diabetic cardiac tissue and has been invoked as a cause of impaired cardiac function as well as sudden cardiac death [3]. Reduced plasma norepinephrine concentrations were found in patients with diabetes mellitus, particularly in those with autonomic neuropathy [4]. Conversely, increased plasma norepinephrine concentrations and increased cardiac norepinephrine spillover into plasma indicate sympathetic stimulation in arterial hypertension and human heart failure [5]. Furthermore, in the failing heart, reduction of norepinephrine stores indicates depletion of neurotransmitters in sympathetic nerve endings [5].

So far, the most important aspect of cardiac sympathetic nerve function – namely the release of the neurotransmitter itself – has not been investigated in patients with diabetes mellitus. For that purpose, we examined whether endogenous norepinephrine release

Received: 13 August 2002 / Revised: 11 October 2002
Published online: 22 March 2003

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from sympathetic nerve endings is affected in atrial tissue from Type 2 diabetic patients. Furthermore, we examined the relation of haemodynamic alterations and cardiac catecholamine release in diabetic and non-diabetic patients.

Subjects and methods

The study protocol was approved by the local ethic committee of the Medical University of Lübeck and all patients gave written informed consent.

Atrial tissue was obtained from 19 patients with Type 2 diabetes mellitus (mean duration 5 years; range 1 to 10 years) and 43 non-diabetic patients undergoing heart surgery for coronary bypass grafting. Each diabetic patient received a glucose-reduced diet, 10 patients were treated additionally with oral antidiabetic agents (five patients with glibenclamide, three patients with acarbose, and two patients with metformin). Clinical evidence of diabetic complications (nephropathy, polyneuropathy, retinopathy) were not observed.

Specimens were obtained from the right atrial appendage during venous cannulation for extracorporal circulation and placed in ice-cold Krebs-Henseleit solution immediately after excision. Connective tissue was removed and each specimen was dissected into two to four parts of similar size weighing 50 to 100 mg. Pieces were placed between two electrodes made of platinum wire netting for electrical field stimulation and incubated in 3 ml Krebs-Henseleit solution-filled tubes at 37°C for 45 min without intervention (equilibration period). Incubation medium was saturated with 95% O_2/5% CO_2; the gas flow was adjusted to achieve a pH of 7.4 [6]. Baseline norepinephrine release was measured from the 46th until completion of the 50th min of incubation, thereafter electrical field stimulation (effective voltage 5 V, stimulation frequency 4 Hz, pulse width 2 msec) was carried out for 5 min (from the 51st until completion of the 55th min of incubation). Stimulation-induced norepinephrine release was calculated as cumulative overflow during stimulation and the following washout period of 10 min minus baseline overflow. Norepinephrine release as described has been characterized previously to be exocytotic [6]. Determination was carried out by high performance liquid chromatography with electrochemical detection for quantitative analysis (detection limit 0.1 pmol per gram tissue) [6]. Norepinephrine release of each patient was averaged from the two to four pieces, which were prepared from one specimen, and considered as n=1.

Determination of the plasma glucose and serum creatinine concentration as well as haemoglobin A_{1C} concentration was done in the Clinical Chemistry, University of Lübeck using standard methods. Plasma glucose and serum creatinine of each patient was averaged from two to four preoperative measurements and considered as n=1. Left ventricular ejection fraction and arterial blood pressure was assessed 1 to 7 days prior to cardiac surgery through left ventricular and aortic angiography. Ejection fraction was calculated from planimetric evaluation of end-diastolic and end-systolic volume in the 30° right anterior oblique projection. Heart rate was assessed from a resting electrocardiogram immediately prior to surgery.

Data of each group are expressed as arithmetic means ± SEM and differences were analyzed by unpaired Student's t test. Correlation coefficients were evaluated by the Spearman's test. Categorical variables were compared by means of the two-tailed Fisher's exact test. A p value of less than 0.05 was considered statistically significant.

Table 1. Clinical characteristics of non-diabetic and diabetic patients. *p<0.01, nd = not determined

	Non-diabetic patients (n=43)	Diabetic patients (n=19)
Male : female	33:10	12:7
Age (years)	65±1	69±2
Range (years)	44–83	58–79
Plasma glucose (mg/dl)	117±4	194±7*
Haemoglobin A_{1C} (%)	nd	8.3±0.6
BMI (kg/m^2)	27±1	28±2
Serum creatinine (µmol/l)	103±12	106±10
Left ventricular ejection fraction (%)	63±2	58±5
Mean arterial blood pressure (mmHg)	94±2	95±4
Heart rate (bpm)	73±1	77±4
Medications (% of patients)		
Beta-blocker	60	47
ACE-inhibitor	51	53
Calcium-antagonist	9	11
Digitalis glycoside	9	26

Results

Diabetic patients (n=19) and non-diabetic patients (n=43) did not differ in respect of preoperative age, BMI, serum creatinine concentration, left ventricular ejection fraction, mean arterial blood pressure, and heart rate. Concomitant medications which could have effects on norepinephrine release were equally distributed between both patient groups. Preoperative plasma glucose concentration was approximately 66% higher in patients with diabetes mellitus compared to those without diabetes. The haemoglobin A_{1C} concentration was also increased in diabetic patients (Table 1).

Stimulation-induced norepinephrine release was reduced in atrial tissue from diabetic patients as compared to non-diabetic patients (46±5 vs. 62±4 pmol/g, p<0.05), while baseline norepinephrine release did not differ between both groups (diabetic patients: 14±2 pmol/g; non-diabetic patients: 17±2 pmol/g) (Fig. 1). In both groups, no correlation was found between left ventricular ejection fraction and stimulation-induced norepinephrine release (diabetic patients: r=0.15; non-diabetic patients: r=0.12). However, in diabetic and non-diabetic patients, correlation analysis indicated a positive relation of baseline and stimulation-induced norepinephrine release (diabetic patients: r=0.47, p<0.05; non-diabetic patients: r=0.62, p<0.01). In both groups, no relation was found between plasma glucose concentration and catecholamine release (diabetic patients: baseline norepinephrine r=0.01, stimulation-induced norepinephrine r=–0.07; non-diabetic patients: baseline norepinephrine r=–0.09, stimulation-induced norepinephrine r=0.07). The same was true for

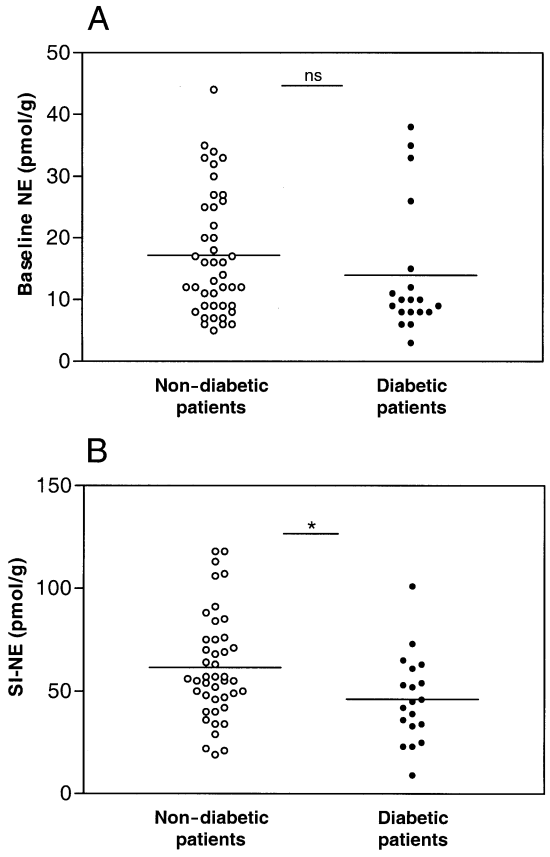

Fig. 1A, B. Baseline norepinephrine (NE) release (**A**) and stimulation-induced NE (SI-NE) release (**B**) in non-diabetic (n=43) and diabetic (n=19) patients. Means of groups are represented by solid lines and differences were analyzed by unpaired Student's t test. * $p<0.05$, ns=not significant

haemoglobin A_{1C} concentration and norepinephrine release in diabetic patients (baseline norepinephrine r=–0.34, stimulation-induced norepinephrine r=0.06).

Discussion

The principal finding of our study is that there is a decrease in the quantity of norepinephrine made available during stimulation of sympathetic nerves in the myocardium of patients with Type 2 diabetes mellitus. Exocytotic norepinephrine release from sympathetic nerve endings was reduced by 25% in atrial tissue from diabetic as compared to non-diabetic patients. Our finding is consistent with observations in rat atrial tissue and rabbit vascular tissue indicating that stimulation-induced norepinephrine release is decreased in diabetic animals [7, 8]. It was concluded that the cardiac sympathetic neurotransmission is functionally impaired, since baseline norepinephrine release and total tissue-content of norepinephrine did not differ among diabetic animals and control animals [7]. To support this contention, ultrastructural studies of human cardiac atrial nerve endings have shown more axons with intra-axonal signs of degeneration, such as altered mitochondria and short membrane fragments in diabetic patients compared to non-diabetic patients [3]. Therefore, it is conceivable that the ATP-dependent intra-vesicular storage of norepinephrine is affected resulting in a reduced availability of norepinephrine for exocytotic release. Another line of evidence indicating that exocytotic norepinephrine release is selectively impaired in diabetes mellitus derived from functional investigations, which documented a decreased inotropic response of diabetic atrial tissue to electrical field stimulation [7]. Notably, the tyramine-induced inotropic response did not differ among diabetic and non-diabetic tissue, documenting that nonexocytotic release of norepinephrine from axoplasma was not affected.

This study is in keeping with the hypothesis of a functionally-impaired sympathetic neurotransmission in diabetic human atrial tissue, since only electrically-evoked norepinephrine release was suppressed while baseline release was not affected. Reduced cardiac norepinephrine release could represent a pathophysiological link to attenuated chronotropic and inotropic responses to sympathetic nerve stimulation, which have been described in diabetic animals previously [7, 9]. Accordingly, in diabetic patients, left ventricular ejection fraction in response to exercise is decreased while resting ejection fraction is not affected [10]. In contrast, it is conceivable that suppression of sympathetic activity has beneficial effects as the heart is less prone to arrhythmogenic effects of catecholamines.

It was a limitation of our study that regional distribution of sympathetic function was not studied in the human heart. Since the regional transmitter release might differ in humans, these findings cannot be directly extrapolated on cardiac tissue other than atrial myocardium.

In conclusion, in atrial tissue of patients with Type 2 diabetes mellitus exocytotic norepinephrine release from sympathetic nerve endings is reduced and this could contribute to sympathetic neuropathy. The difference of norepinephrine release in diabetic and non-diabetic patients was independent of age, plasma glucose, renal function, cardiac function, arterial blood pressure, and heart rate.

Acknowledgements. We are grateful to A. Constantz, D. Kemken, C. Krause, and I. Stölting for their technical assistance.

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