Acta Diabetol (2000) 37:55-60

ORIGINAL

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Plasma epinephrine and norepinephrine response to stimuli in autonomic neuropathy of type 2 diabetes mellitus

Received: 28 May 1999 / Accepted in revised form: 28 August 2000

Abstract The objective of this study was to examine epinephrine and norepinephrine plasma levels in patients with clinical type 2 diabetes mellitus, at different stages of autonomic neuropathy. Eighteen patients were classified in groups without (n = 6) and with early (n = 6), definite (n = 3) and severe (n = 3) neuropathy. Blood catecholamine levels were measured after the Valsalva maneuver, cold exposure and orthostatic tests. The norepinephrine basal levels were lower in patients with severe neuropathy (0.4 ± 0.2 nmol/l), compared with the group with no neuropathy (1.3 ± 0.5 nm/l, p = 0.034), or with early neuropathy (1.3 ± 0.7 nm/l, p = 0.035). After the Valsalva maneuver, no increase was found in the group with severe alteration. In patients without neuropathy, cold exposure induced a peak of norepinephrine at 5 min (Δ = 1.9 ± 1.6 nmol/l). The increase was lower in groups with definite and severe damage. In patients with definite or moderate neuropathy, the orthostatic test induced minimal or no response. The epinephrine response to the maneuvers was not significant, and no differences were found among the groups. Norepinephrine basal levels and cold responses are diminished in patients with definite and severe autonomic neuropathy. This provides further evidence on their impaired response to stress. The comparable epinephrine levels in patients with or without autonomic neuropathy indicates that adrenal medullar function is not significantly altered.

Key words Autonomic neuropathy • Type 2 diabetes • Catecholamine

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Introduction

The sympathetic nervous system includes pathways involved in the physiological response to diverse physical, environmental, and behavioral stressors [1]. The impairment of this system as a complication of diabetes mellitus induces diverse clinical manifestations such as postural hypotension, impotence, and alterations in diverse visceral functions. Furthermore, autonomic neuropathy is associated with increased mortality both in type 1 and type 2 diabetes mellitus [2]. Plasma catecholamine levels are valuable indices of sympathetic nervous activity. Some reports indicate that patients with advanced diabetic autonomic neuropathy have diminished plasma norepinephrine [3] as well as enhanced responses to epinephrine and norepinephrine infusion [4]. However, the catecholamine response to stimuli in patients with diverse extents of autonomic damage has not been sufficiently explored [5].

The sympathetic nervous system response to diverse stressors has been assessed by means of a variety of challenges such as sustained handgrip, cold exposure [6], the Valsalva maneuver, and postural changes [7]. In this work we studied epinephrine and norepinephrine responses to different stimuli in patients with type 2 diabetes mellitus at different clinical stages of autonomic neuropathy.

Material and methods

Subjects

We studied 18 patients with type 2 diabetes mellitus that accepted inclusion in the study after detailed information of the purpose, procedures and risks involved. The project was reviewed and accepted by the institutional ethics committee. All patients gave written informed consent. Patients were recruited from the Medical Research Institute. The criteria for inclusion were: less than 60 years of age, with the diagnosis of diabetes after 30 years of age, and without pregnancy. All patients had a normal electrocardiogram (ECG), and no clinical evidence of thyroid disease or previous myocardial infarction. No patient was under recent treatment with α or β adrenergic blockers, chronic analgesics, diuretics, antidepressive medication, phenothiazines or nitrates. None reported smoking or chronic alcoholism. No patient received insulin treatment.

Clinical studies

At baseline, a clinical history was taken for all patients, including age, years since diagnosis, and actual and previous medication. Diet and use of hypoglycemic agents were also registered. Height, body weight and body mass index were collected. Sitting blood pressure was taken after 5 min of rest. A conventional 12-lead resting ECG was recorded.

Autonomic function tests for classification of neuropathy. The classification of neuropathy was carried out as described by Ewing and Clark [8], based on the response to five tests: supine position test, expiration-to-inspiration test (E/I), Valsalva maneuver, handgrip test and orthostatic test. These tests were carried out at 0800-01000 h, in a warm and quiet room. The R-R intervals were calculated from lead II of the electrocardiogram as follows:

1. The *supine position test* evaluated changes in the pulse rate after one minute lying (normal, 1.4 or more; borderline, 1.01-1.03; abnormal, 1.00 or less).
2. The *expiration-to-inspiration test* (E/I), in which the patient breathed with maximum vital capacity at a respiratory cycle of 10 s, for 60 s in supine position. For this test pulse changes were analyzed after six breathing cycles. During each cycle, the ratio of the longest to the shortest R-R interval was calculated and the mean of six ratios was taken as the E/I ratio (normal, 15 beats/min or more; borderline, 11-14 beats/min; abnormal, 10 beats or less).
3. In the *Valsalva maneuver* the patients made a 15-s forced expiration against a sphygmomanometer at 70% of their maximum capacity. The Valsalva index was obtained from the longest R-R postmaneuver interval divided by the shortest R-R interval during the maneuver (normal, 1.21 or more; abnormal, 1.20 or less).
4. The *handgrip test* was done measuring the strength of the handgrip on the cuff of the sphygmomanometer for a 30-s period (normal, 16 mmHg or more; borderline, 11-15 mmHg; abnormal, 10 mmHg or less).
5. For the *orthostatic test*, the subjects actively stood up after a 5-min resting period in the supine position (normal, 10 mmHg or less; borderline, 11-29 mmHg; abnormal, 30 mmHg or more). Systolic (SBP) and diastolic blood pressures (DBP) were measured with a calibrated sphygmomanometer, at the end of the resting period and at 1 and 10 min in standing position. The presence of symptoms was inquired and changes in SBP were registered.

According to the results, the autonomic impairment of the patients was classified as: *stage 1*, without clinical neuropathy, (control group); *stage 2*, early neuropathy; *stage 3*, definite neuropathy and *stage 4*, severe neuropathy.

Autonomic function tests of blood catecholamine changes. After classification, in a different session, a heparinized catheter was placed in the antecubital vein, at 0800-01000 h after a 12-hour fast. After 30 min with the patient in supine position, a basal sample was taken, and three consecutive tests were performed: the Valsalva, cold exposure and orthostatic tests.

The Valsalva maneuver was carried out as described for the diagnostic test, taking blood samples at 2, 5, 10 and 60 min. For the cold exposure test, the patients submerged a hand in cold water (4° C) for 30 s, and samples were taken at 2, 5, 10 and 60 min postmaneuver. The orthostatic test was similar to the diagnostic test, taking blood samples at 2, 5 and 10 min. The recovery period between each test was 60 min.

Blood samples were placed in polystyrene tubes containing 100 µl of a solution with heparin, acetyl-cysteine and ascorbic acid. After centrifugation at 3500 rpm for 10 min, plasma was separated and stored at –70° C until catecholamine measurement.

Laboratory methods

The following measurements were done in basal blood samples: glucose by a glucose oxidase method (GOD-PAP, Lakeside, Toluca, Mexico), creatinine, HbA_{1c} by ion-exchange chromatography, total cholesterol, HDL-cholesterol, and triglycerides. Plasma insulin was measured by a double antibody radioimmunoassay (Diagnostic Systems Laboratories, Webster, TX, USA).

Epinephrine and norepinephrine were measured by high performance liquid chromatography with spectrofluorometric detection. A Merck LiChrosorb RP18 column (10 µm, 200x4.6 mm) was used [9].

Statistical analysis

The epinephrine and norepinephrine blood levels as well as changes from basal (Δ values) were examined for differences among the groups by one way analysis of variance. Differences between pairs of groups were analyzed by the post-hoc least significant difference test. Significance was considered for p values < 0.05. For analysis we used the Statistic package (Statsoft, Tulsa).

The power analysis calculated was as follows: to detect a difference from 0.5 to 1.0, considering a standard deviation of 0.35, an $N = 6$ and $\alpha = 0.5$ gave a $\beta = 0.10$ (power = 0.90).

Results

The study group included 18 patients (15 women and 3 men): group 1, without neuropathy include 6 women; group

2 with early neuropathy included 5 women and a man; group 3 with definite neuropathy and group 4 with severe neuropathy included 2 women and one man each.

Table 1 shows the characteristics of patients for each study group. No significant differences were found for age, years since diagnosis, somatometric variables and metabolic control. Systolic ($F = 3.45$, $p = 0.046$) and diastolic blood pressures ($F = 4.59$, $p = 0.019$) were different among groups, with higher values in patients of group 4 as compared with groups one ($p = .007$) and three ($p = .018$).

Norepinephrine levels

Basal norepinephrine levels were significantly lower for patients with severe neuropathy (0.4 ± 0.2 nmol/l), as compared with the groups with no neuropathy (1.3 ± 0.5 nmol/l, $p = 0.034$), and with early neuropathy (1.3 ± 0.7 nmol/l, $p = 0.035$) (Fig. 1). The significantly lower levels for patients with severe neuropathy were maintained at 2 min ($p = 0.03$), 5 min ($p = 0.03$) and 10 min ($p = 0.02$). After the Valsalva maneuver, higher but non-significant increases were found

Table 1 Characteristics of patients. Values are mean ± SD

	No neuropathy	Early	Definite	Severe	F	p
Age (years)	55.7 ± 5.2	51.8 ± 9.8	48.3 ± 3.8	55.0 ± 4.6	.87	.48
Years since diagnosis	15.0 ± 7.8	10.8 ± 4.9	12.7 ± 3.1	19.0 ± 5.6	1.35	.30
Weight (kg)	59.3 ± 7.1	59.2 ± 13.2	62.8 ± 2.8	61.7 ± 5.8	.15	.93
Height (m)	1.53 ± .08	1.53 ± 0.14	1.57 ± 0.04	1.58 ± 0.07	.24	.87
BMI	25.4 ± 3.2	23.6 ± 3.6	25.7 ± 2.6	24.8 ± 2.4	.44	.72
Waist/hip ratio	1.027 ± 0.038	1.034 ± 0.047	1.058 ± 0.052	1.051 ± 0.064	.38	.77
SBP (mmHg)	127 ± 14	132 ± 10	135 ± 5	153 ± 15	3.45	.046
DBP (mmHg)	74 ± 5	80 ± 8	88 ± 2	85 ± 5	4.59	.019
Glucose (mmol/l)	8.94 ± 1.06	11.65 ± 4.82	12.29 ± 5.46	10.22 ± 4.44	.68	.58
HbA_{1c} (%)	10.9 ± 3.5	10.3 ± 3.1	11.7 ± 1.7	8.8 ± 2.0	.54	.66
Creatinine (μmol/l)	63.6 ± 10.6	70.6 ± 15.0	70.6 ± 8.8	79.5 ± 8.8	1.30	.31
Cholesterol (mmol/l)	5.2 ± 1.3	5.1 ± 0.83	6.6 ± 0.28	5.3 ± 0.75	1.97	.16
HDL-cholesterol (mmol/l)	1.3 ± 0.19	1.1 ± 0.19	0.96 ± 0.14	1.14 ± 0.34	2.65	.09
Triglycerides (mmol/l)	1.75 ± 0.31	2.40 ± 0.70	2.91 ± 1.3	1.68 ± 0.35	2.66	.09
Insulin (μU/ml)	17.6 ± 14.6	5.6 ± 2.2	6.4 ± 7.3	3.6 ± 2.6	2.36	.12

BMI, body mass index; *SBP*, systolic blood pressure; *DBP*, diastolic boold pressure

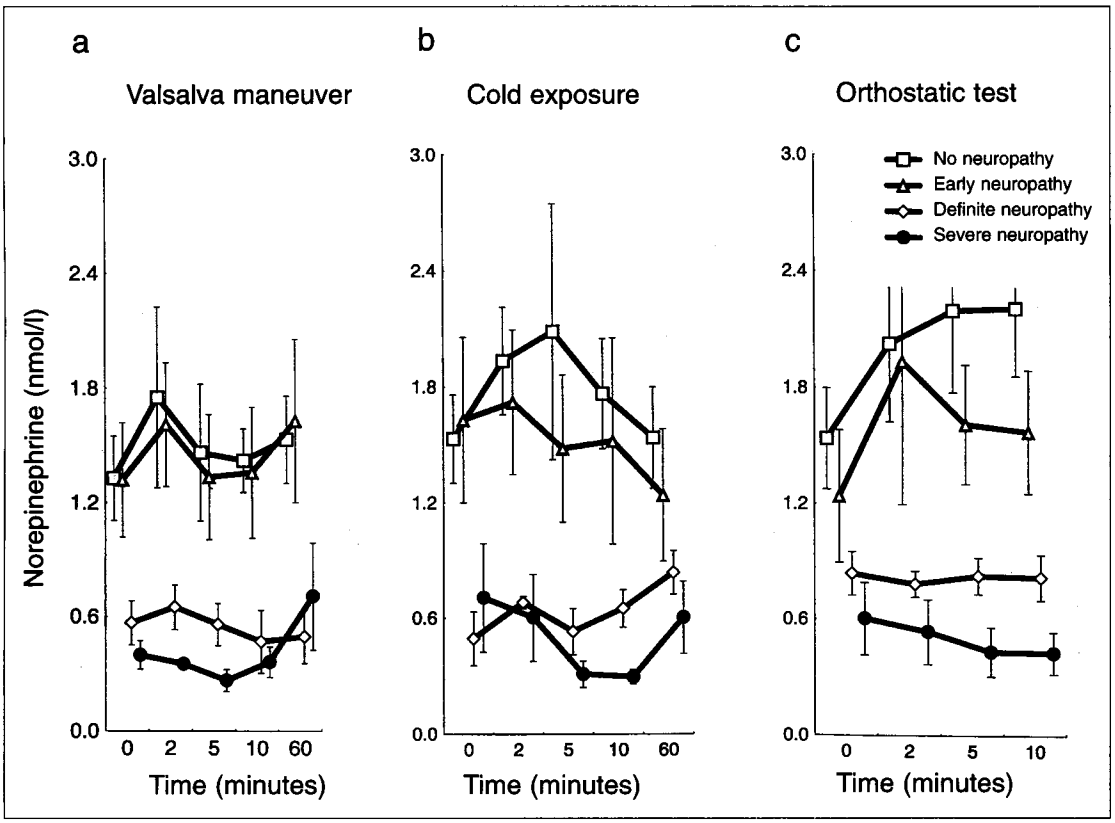

Fig. 1 Plasma norepinephrine response to the Valsalva maneuver, cold exposure and orthostatic tests in groups of patients with type 2 diabetes mellitus and different degrees of autonomic neuropathy. Vertical bars indicate standard error of the mean

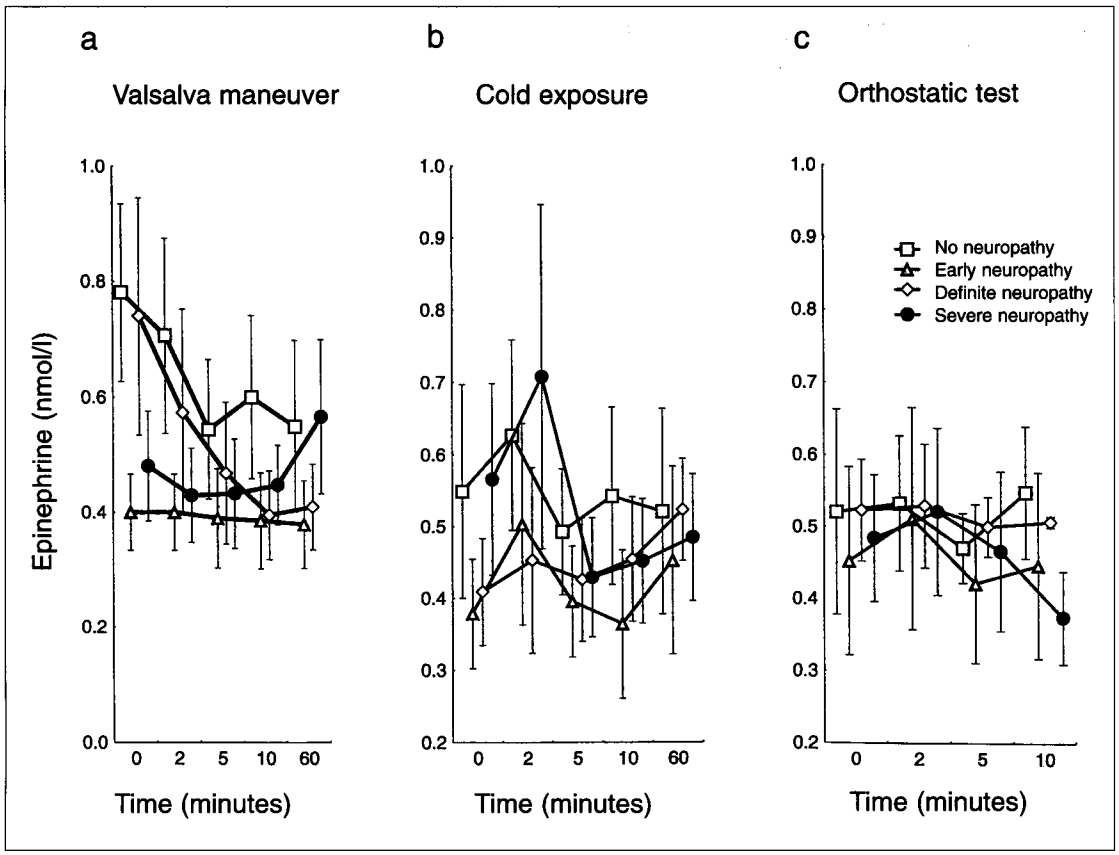

Fig. 2 Plasma epinephrine response to the Valsalva maneuver, cold exposure and orthostatic tests in groups of patients with type 2 diabetes mellitus and different degrees of autonomic neuropathy. Vertical bars indicate standard error of the mean

in the group without neuropathy ($\Delta = 0.4 \pm 0.8$ nmol/l) at 2 min ($F = 0.59$, $p = 0.63$) .

In the cold exposure test, norepinephrine basal levels were lower in the groups with definite (0.5 ± 0.3 nmol/l, $p = 0.026$) and severe neuropathy (0.7 ± 0.7 nmol/l, $p = 0.019$), as compared with the group without neuropathy (1.5 ± 0.6 nmol/l). The group with early neuropathy had also higher levels (1.6 ± 1.0 nmol/l) than patients in a severe stage ($p = 0.04$). During the course of the test the hormone levels showed the higher absolute increase at 5 min ($\Delta = 1.9 \pm 1.6$ nmol/l). The groups without and with early neuropathy showed higher increases in norepinephrine than the groups with definite and severe damage at 2 min ($t = 2.3$, $p = 0.037$), 5 min ($t = 3.27$, $p = 0.005$), and 10 min ($t = 3.50$, $p = 0.003$).

Significant responses to the orthostatic test were found in patients without or with early neuropathy, both at 5 min ($F = 4.28$, $p = 0.024$) and at 10 min ($F = 5.26$, $p = 0.012$), as compared to patients with definite and moderate neuropathy, in which minimal or no response was found (Fig. 1).

Epinephrine levels

During the Valsalva maneuver a moderate decrease in epinephrine levels was found in patients with no neuropathy and with early neuropathy (Fig. 2), but no difference was found among the four groups for absolute levels or for differences with respect to basal values.

The mean epinephrine levels showed a moderate, nonsignificant increase at 2 min of the cold exposure test. No difference was demonstrated among different groups. The epinephrine levels in the orthostatic test remained at similar values and no differences were shown among the groups.

Discussion

Autonomic neuropathy is a complication of diabetes mellitus largely underestimated in clinical practice. It is, however, a serious condition that threatens survival because it implies an impaired adaptation to stress. It should be pointed out that diabetic subjects may lose the parasympathetic innervation of the heart before they lose sympathetic innervation [10]. Besides current clinical diagnostic tests, a decrease in the catecholamine secretory capacity has been proposed to be of clinical significance to define the risk of morbidity and mortality in diabetic patients [11]. The evaluation of damage of the autonomic system by means of the catecholamine response to stressful stimuli may be of additional value [2].

Metabolic control is considered to be an important factor for the development of autonomic neuropathy [2]. However, the association of damage with recent metabolic control may not be shown [12]. In our study we did not find an association of blood glucose or glycated hemoglobin levels with the severity of neuropathy. This suggests that deficient glucose control may interact with other unidentified factors for the progression of this process.

Different factors may have an influence on catecholamine blood levels such as age [13], gender, and years since diagnosis. In our study, years since diagnosis was not significantly different in the four groups. Obesity has been related to parasympathetic neuropathy [2]. Hilsted [3] reported low plasma norepinephrine concentration in diabetic patients with severe autonomic neuropathy and a long duration of diabetes. Diabetic patients, especially those under insulin treatment, may suffer periods of transient hypoglycemia that may increase catechoamine blood levels. The influence of this factor seems to be unlikely in this study, considering that no patient was under insulin treatment, and that most were with deficient metabolic control.

In our group of patients, those with severe or definite neuropathy showed similar diminutions in norepinephrine plasma levels. The response to the Valsalva maneuver was also absent in patients with definite and severe neuropathy. Eckberg et al. [14] suggested that subnormal baseline plasma norepinephrine levels in diabetic patients represent advanced structural defects in sympathetic pathways. Hoeldtke and Cilmi [15] found that the production and secretion of norepinephrine is diminished in autonomic neuropathy, and Leveston et al. [16] proposed that this diminution resulted from axonal post-gangliar sympathetic lesions.

Adaptation to cold implies complex physiological mechanisms. Cold exposure induces an increase in the vascular contractile responsiveness to norepinephrine [17], and increases the cardiac output through a mechanism involving the sympathetic nervous system [18]. The norepinephrine thermogenic response is increased in cold-adapted subjects [19]. Sudomotor and vasomotor activities are regulated by temperature changes both in skin and in hypothalamic centers monitoring body core temperature. Sympathetic innervation of the skin regulates sweating and vascular tone [20]. During early stages of neuropathy, increased vasoconstriction results from hypersensitivity denervation to cold and catecholamine in arterioles [21].

In this study the most notable change to cold exposure was the diminished response of norepinephrine in patients with definite and severe neuropathy, notwithstanding the fact that duration of the stimuli was shorter than the time recommended by LeBlanc [22] and Herd [23]. This finding is of clinical significance, because it reveals an important functional deficit, usually not considered in the care of diabetic patients with autonomic neuropathy. This defective adaptation to cold exposure may be an important feature to explain the increased mortality and morbidity in patients with autonomic neuropathy.

Sympathetic response to postural changes is another important function for human adaptation. Low or occasionally high plasma norepinephrine concentrations have been reported in diabetic patients with orthostatic hypotension [24, 25]. In our study two patients with definite neuropathy had orthostatic hypotension and showed low levels of norepinephrine. The serum insulin concentrations were marginally higher in the group without neuropathy. This finding should be confirmed in further studies. It may mean that impaired insulin secretion, rather than insulin resistance, is a factor for the development of autonomic neuropathy. Töyry et al. [2], however, found higher insulin values in patients with parasympathetic neuropathy.

Plasma epinephrine levels were not different among the groups. The stimuli were ineffective in inducing changes in blood levels, except for an small early increase to cold exposure. This may indicate that in autonomic neuropathy functional changes of the adrenal medulla are not as prevalent as those found in sympathetic pathways.

In conclusion, our results do not show different epinephrine secretion in diabetic patients without or with autonomic neuropathy, a finding that may indicate that adrenal medullar function is not importantly altered. The diminution in norepinephrine secretion under the Valsalva maneuver, cold response and orthostatic tests provides further evidence of the decreased adaptative response to stress in patients with definite and severe neuropathy.

Acknowledgments This work was supported in part with the grant no. 3417-M from CONACYT. The graduate fellowship (G.G.) from the Instituto Mexicano del Seguro Social is also acknowledged.

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